Investigation of H01 in Adults With Pulmonary Hypertension Including Interstitial Lung Disease (The SATURN Study)

Study Protocol and Statistical Analysis Plan

NCT05128929

September 30, 2022

The SATURN Study

Short Title:
Investigation of H01 in **a**dults wi**t**h p**u**lmonary hype**r**te**n**sion including interstitial lung disease (The SATURN Study).

Study Description: A study of oral H01 in adults with pulmonary hypertension including interstitial lung disease

Study Phase: 2a
Product Name: H01
Study Document: Protocol (September 30, 2022)
Indication: Pulmonary Hypertension

IND Number: [redacted]

Investigator: Roham Zamanian, MD

CONTENTS

1 BACKGROUND

1.1 Introduction

This is a prospective, randomized, double-blind, study of H01 of 24 weeks in adults with pulmonary hypertension (PH) with an optional open-label extension (extension to 48 weeks).The primary objective of this double-blinded study is to evaluate the clinical efficacy of H01 over 24 weeks. Secondary objectives are to investigate the safety and tolerability of H01 and pharmacokinetic (PK) and pharmacodynamic (PD) markers in this population. Following IRB approval and written informed consent,a total of 16 participants will be enrolled, randomized approximately 2:1 (H01 1600 mg/day : placebo), stratified by PH type, and treated for 24 weeks. Data from this study will inform future larger studies. Participants will have the opportunity to extend their participation an additional 24 weeks by consenting to an open-label extension .

PH is characterized by remodeling of the pulmonary vasculature, resulting in narrowing and eventual obliteration of the lumen, leading to increased mean pulmonary arterial pressure (mPAP)[1].While multiple therapies have been developed to address PH, there are no disease modifying therapies and morbidity and mortality remains high.

Recent research has suggested that hyaluronan (HA), a key component of the extracellular matrix, plays an important role in the pathology of PH and has potential as a therapeutic target[2,3]. Hyaluronan is an extracellular matrix glycosaminoglycan capable of trapping large volumes of water and driving pro-inflammatory pathways and fibrosis[4]. The role of hyaluronan in pulmonary hypertension, lung inflammation, and fibrosis has been validated in multiple animal models[2,3,5,6]. Further, in these models, inhibition of hyaluronan synthesis with H01 led to significant reductions in hyaluronan and had positive effects on clinically relevant outcomes such as Right Ventricular Systolic Pressure (RVSP), the Fulton index and arterial oxygenation (Sp02).

Hymecromone (H01) is a known inhibitor of HA that has been studied in models of respiratory the context of both inflammation and fibrosis. The purpose of this clinical trial is to study the safety and utility of H01 in patients with pulmonary hypertension associated with chronic lung disease (selected from Groups 1 and 3 PH).

We hypothesize that oral H01, at doses of 1600 mg per day, will be a safe and well-tolerated agent in adults with pulmonary hypertension over 24 weeks. In addition, this study will investigate the potential benefit of oral H01 on clinical measures of PH disease severity including but not limited to: pulmonary vascular resistance, mean pulmonary arterial pressure, and the 6 Minute Walk Distance Test (6MWDT).

1.2 Study Overview

Protocol Title	Investigation of H01 in **a**dults wi**t**h p**u**lmonary hype**r**te**n**sion including InterstitialLung Disease (SATURN Study)
IND #	[redacted]
Study Design	Phase 2a, double-blinded, approximately 2:1 randomized and stratified placebo- controlled study to evaluate the safety, tolerability, and efficacy of H01 in adults with pulmonary hypertension (PH), stratified between selected Group 1 and Group 3 PH types with optional open-label extension .
Study Population	Adults with confirmed diagnosis of Group 3 PH secondary to Interstitial Lung Disease(ILD) and Group 1 PH secondary to connective tissue disease, idiopathic, drugs and toxins, or hereditaryPAH
Study Drug	400 mg H01 tablets [Isochol 400mg] and placebo
Objectives	1. Evaluate the change in clinical and functional measures in adults with PH treatedwith H01 2. Evaluate the safety and tolerability of oral H01 in PH 3. Evaluate the effect of H01 on serum biomarkers in PH
Clinical Phase	2a
Primary Endpoint	▪ Change in pulmonary vascular resistance (PVR) measured by right heart catheterization (RHC) from baseline to end of treatment (Week 24) of the blinded study
Secondary Endpoints	▪ The safety and tolerability of H01 in adults with pulmonary hypertension using the Common Terminology Criteria for Adverse Events (CTCAE) ▪ Change in mean pulmonary arterial pressure (mPAP) by RHC from baseline to endof treatment (Week 24) of the blinded study ▪ 6 Minute Walk Distance Test (6 MWDT) from screening to end of treatment (Week 24) of the blinded study and through Week 48 of the open-label extension ▪ Change in quality of life (QOL) score, EMPHASIS-10 score and St GeorgeRespiratory Questionnaire (SGRQ) score from baseline to end of treatment (Week 24)of the blinded study and through Week 48 of the open-label extension ▪ Change in serum HA concentration from baseline to end of treatment (Week 24) of the blinded study and through Week 48 of the open-label extension ▪ Change in NT-proBNP from baseline to end of treatment (Week 24) of the blinded study and through Week 48 of the open-label extension
Exploratory Endpoints	▪ Change in inflammatory markers (ESR, HSCRP) ▪ Change in pro-inflammatory cytokines ▪ Change in Forced Expiratory Volume in one second (FEV1) ▪ Change in Forced Vital Capacity (FVC) from pulmonary function test (PFT) ▪ Change in Total Lung Capacity (TLC) from pulmonary function test (PFT) ▪ Change in Lung diffusion capacity (DLCO) from pulmonary function test (PFT) ▪ Change in exhaled breath condensate (EBC) hyaluronan concentrations over thestudy period ▪ Describe the pharmacokinetics (H01 and metabolite serum concentrations) ▪ Describe HA fragment size

Planned sample size	16

Number of Sites	1
Duration of Participation	Total study period of the blinded study: 28 weeks Total study period including open-label extension: 48 weeks ▪ Screening Period: up to 4 weeks ▪ Treatment Period of the blinded study: 24 weeks ▪ Treatment Period including open-label extension: 48 weeks ▪ Follow-up Period: 4 weeks after treatment period ends (Week 24) if not enrolled in the open-label extension
Dose Schedule	▪ Participants randomized to treatment will receive two H01 400 mg tablets BID (1600 mg/day) for 24 weeks or through Week 48 if enrolled in the open-label extension ▪ Participants randomized to placebo will receive two placebo tablets BID for 24weeks

1.3 Scientific Rationale

1.3.1 Urgent need for pulmonary hypertension therapeutics

Pulmonary hypertension (PH) is defined by a pulmonary vascular resistance (PVR) ≥3 WU associated with an increased mean pulmonary arterial pressure (mPAP >20 mmHg) at rest[1]. It can lead to right ventricle hypertrophy (RVH), right-sided heart failure and eventually death[7] 010). Idiopathic pulmonary fibrosis (IPF) is a form of restrictive interstitial lung disease (ILD) associated with extremely poor outcomes. IPF is characterized by parenchymal fibrosis that leads to impaired gas exchange[8]. PH, with a prevalence of about 40% in advanced disease[9,10], is a common complication in IPF where it is strongly associated with increased morbidity and mortality[10,11]. It is classified as WHO Group 3 PH[12].

There are limited treatment options for patients with interstitial lung disease and PH, with lung transplantation being the only "curative" option. Despite the many pharmacological agents available to treat pulmonary arterial hypertension (PAH) (classified as Group 1 PH, where no lung parenchymal component is observed), these agents have been shown to be either ineffective or detrimental to patients with IPF + PH[13]. Consequently, it is essential to develop improved medical therapies for IPF+PH.

1.3.2 Discussion on pulmonary hypertension groups

This trial selects patients from Groups 1 and 3 PH based on the hypothesized utility of H01 in PH due to lung conditions associated with inflammation, vascular remodeling, and fibrosis. Brief discussion of these groups provides relevant context for selected inclusion/exclusion criteria.

PH is broadly classified into 5 groups based on etiology, although there can also be overlap between groups. Group 1 (also referred to as pulmonary arterial hypertension (PAH)) includes patients with PAHsecondary to connective tissue diseases, idiopathic PAH, hereditary PAH, and PAH secondary to selected other conditions (e.g. toxins, HIV, etc.). Although the prevalence of PAH is unknown in North America,several European registries have reported rates of 5 to 52 per million[14,15]. PAH is a proliferativevasculopathy, characterized by vasoconstriction, cell proliferation, fibrosis, and micro-thrombosis. Pathologic findings include hyperplasia and hypertrophy of all three layers of the vascular wall (intima, media, adventitia) in pulmonary arteries <50 microns (i.e., localizes to the small pulmonary muscular arterioles). In addition, fibrosis and in situ thrombi of the small pulmonary arteries and arterioles (plexiform lesions) can be seen[16,17]. The pathologic appearance of the small pulmonary arteries and arterioles is qualitatively similar in all patients with group 1 PAH.

Over a dozen PAH specific therapies are available. They target components of five PAH relevant molecular pathways: voltage gated, L type calcium channels, nitric oxide cyclic guanosine monophosphate (cGMP), endothelin, and prostacyclin[18]. However, effective options for patients with group 1 PAH with *co-existing* lung disease are limited. These patients are primarily treated with

pulmonary vasodilators but outcomes remain poor.

Group 3 PH is the second most common form of PH and is associated with increased morbidity and mortality[19]. The most common lung diseases resulting in PH are chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD) and obstructive sleep apnea (OSA) but PH is also associated with other diseases, such as cystic fibrosis and high altitude exposure. Among the etiologies of group 3 PH, the strongest evidence favors hypoxic pulmonary vasoconstriction (HPVC) with re-modelling of the pulmonary vascular bed. Group 3 PH has a very high morbidity and mortality[19]. PH in the setting of fibrotic lung disease is observed in up to 80% of patients with interstitial lung disease (ILD). The presence of PH is considered the single most significant predictor of mortality in patients with chronic lung disease[10,11].

The treatment of Group 3 PH has traditionally been to optimize treatment of the underlying lung disease and give long-term oxygen therapy to those who are hypoxic. The efficacy of pulmonary vasodilators in this group of patients is unclear. There have been mixed results from meta-analysis assessing the effects of vasodilators on exercise tolerance and quality of life[20,21]. Only recently has an acute vasodilator therapy with inhaled treprostinil been evaluated and approved for Group II PH.[22]

In view of the poor prognosis associated with these conditions, and the lack of available specific therapy, further work is required to develop effective therapy. The only durable therapy for chronic lung diseases, such as ILD with PH, is lung transplantation. However, lung transplantation itself is associated with inferior outcomes in ILD+PH than for most other indications[23]. In addition, despite the many pharmacological agents available to treat pulmonary arterial hypertension (PAH), classified as Group 1 PH, where no lung parenchymal component is observed, these agents have been shown to be either ineffective or detrimental to patients with IPF + PH[13]. Consequently, it is essential to develop improved medical therapies for PH in the setting of chronic lung diseases such as ILD.

1.3.3 Hyaluronan is an important driver of respiratory disease and pulmonary hypertension

Hyaluronan (HA), also known as hyaluronate or hyaluronic acid, is a glycosaminoglycan and a major component of the lung ECM[24,25]. Elevated hyaluronan has been reported in lung diseases including IPF and PAH[26–30] and in PH associated with chronic obstructive pulmonary disease[31]. Furthermore, overexpression of hyaluronan synthase 2 (HAS2), an enzyme responsible for the synthesis of hyaluronan, has been implied in severe fibrosis[29]. In addition, increased hyaluronan has also been reported in autoimmune diseases. For example, in systemic sclerosis (SSc), elevated HA is present in both the skin[32] and associated with lung injury[33,34]. Elevated HA appears to be associated with progressive SSc affecting the lungs where it is associated with fibrosis[35] and in patients with connective tissue disease (CTD) associated ILD[36]. These are consistent with increased HA observed in patients with Group 1 PH [26]. Collectively, these studies point at a pathogenic role of HA in the PH associated with chronic lung diseases and suggest. that therapies targeting HA may serve as novel disease-modifying treatments for PH.

The inflammatory effects of HA are dependent on its molecular weight. High molecular weight HA (HMW-HA) of 1000 KDa is believed to have protective effects[30]. However, the breakdown of HA into low molecular weight (LMW) HA has been demonstrated to contribute to its pathobiological effects in the lung[30]. In line with this, increased LMW-HA fragments have been detected in an experimental model of PH associated with lung fibrosis[2].

1.3.4 H01 is a novel approach to addressing pulmonary hypertension secondary to interstitial lung disease

H01, also known as 4-methylumbelliferone (4MU), is a small molecule inhibitor of HA synthesis. H01 reduces HA levels primarily through two identified mechanisms. The first mechanism occurs through the extensive glucuronidation of H01 by UDP-glucuronyltransferases (UGTs) resulting in the depletion of UDP-glucuronic acid, one of the two substrates required for HA synthesis[37,38]. The second way that H01 reduces HA levels is by downregulating HAS mRNA levels[39]. 4MU has been shown to inhibit HA production in multiple cell lines and tissue types[40–44]. 4MU has also been used in multiple animal models of cancer, fibrosis and autoimmunity[39,45–51]. Several studies have demonstrated that inhibition of HA using H01 prophylactically and therapeutically attenuated features of experimental PH including vascular remodelling and elevated right ventricle systolic pressure (RVSP, a surrogate for mPAP in mice). Using a mouse model of lung fibrosis and PH studies showed that a 2-week oral treatment with H01 inhibited RVSP and vascular remodelling[2]. These results were replicated in a mouse model exhibiting lung fibrosis, PH and airspace enlargement, where H01 treatment for 4 weeks was able to attenuate vascular remodelling and RVSP values[3]. Interestingly, in these studies, lung fibrosis was also attenuated. In addition to these highly-relevant pre-clinical studies in models that recapitulate features of Group 3 PH, H01 has been shown to be effective in models of acute lung injury[5,6].

In addition, H01 has demonstrated anti-fibrotic activity in other settings of inflammation (e.g., liver fibrosis). Independent of H01, HA inhibition has also been linked directly with meaningful clinical outcomes such as Forced Expiratory Volume (FEV1) in respiratory disease[52]. Taken together, these results point at elevated HA as a key feature in patients and experimental models of pulmonary hypertension. Furthermore, preclinical studies using H01 both prophylactically therapeutically demonstrates a beneficial effect of H01 in treating PH associated with lung fibrosis. These results underscore the use of H01 as a novel agent to treat Group 3 PH, where limited treatment options are available.

Hymecromone (H01; 4-methylumbelliferone), a known inhibitor of HA synthesis, has been studied pre-clinically in inflammation and fibrosis[53–55]. In particular, H01 is of benefit in multiple models of airway inflammation[5,6,56]. Including in multiple pre-clinical mouse models of PH[2,13]. Hymecromone is an approved oral drug in Europe (European Union reference date [EURD] 07/27/1965) indicated in various diseases of the gallbladder and biliary tract (i.e. Biliary dyskinesia). It is hypothesized that its mechanismof action in biliary dyskinesia and autoimmunity may work, in part, via inhibition of hyaluronanbiosynthesis. In regards to inhibition of HA, H01 is extensively glucoronidated by UDP- glucuronyltransferases (UGTs) which results in the depletion of UDP-glucuronic acid, a precursornecessary for HA synthesis.[37,38] There is substantial evidence for the efficacy and safety of H01 based onits approved uses in multiple markets and

in multiple clinical trials[57]. A comprehensive review of H01 isavailable[39].

We propose a Phase 2a study of H01 in patients with Group 1 and Group 3 Pulmonary hypertension. This proof-of-concept study will generate data supporting the ultimate goal of investigating H01 as a safe, effective, oral treatment for patients with pulmonary hypertension.

2 H01 PHARMACOLOGY

2.1 Dose Rationale

H01 is an approved oral drug in Europe (European Union reference date [EURD] 07/27/1965) for disorders of the biliary tract. H01 has been administered chronically in humans for 50+ years at doses up to 1800 mg/day PO with few observed adverse safety events. Based on the European package insert, the standard dosing regimens for adults in hepatobiliary conditions are 300-600 mg oral up to 3 times / day (900-1800 mg/day).

Several clinical trials of H01 in humans have been published and all demonstrated safety during both short and long-term administration (Appendix B). The longest reported duration of administration was a study of oral administration for 6 months in patients (n=29)[58]. The highest dose administered in a clinical trialis 3600mg for 4 days.

To inhibit excess HA synthesis and alleviate respiratory distress, we propose to administer H01 800mg twice a day (1600 mg/day) PO for 24 weeks. Twice daily dosing was selected to facilitate outpatient adherence. If the participants are enrolled in the open-label extension, H01 is administered twice a day at dose of 800 mg (1600 mg/day) until Week 48.

The selected dose of 1600 mg/day is consistent with non-clinical models of lung injury where human equivalent doses (HED) of H01 ranging from 120 mg/day PO to 2600 mg/day intra-peritoneal (IP) demonstrate notable functional improvements and reduced HA in bronchoalveolar lavage fluid[2,5,6]. In the Phase 1 dose range study recently completed at Stanford University, doses of 1200mg, 2400mg and 3600mg per day doses over 4 days in healthy volunteers were well tolerated and led to decreases in sputum HA.

Given the existing body of safety data for H01, and the relevance of the models suggesting 2600mg/day IP, characteristics of H01's pharmacology, and the time necessary to see clinical efficacy in pulmonary hypertension patients, the decision was made to target 1600mg/day for 24 weeks. A BID regimen is selected over a QID regimen primarily to support patient compliance over a 24-week period. Participants enrolled in the open-label extension until week 48 will continue this BID regimen (800 mg twice a day).

The results at this dose will inform the H01 development plan and larger scale dose-range studies.

2.2 Clinical Efficacy

Dozens of previous studies of H01 have established efficacy in the context of approved and currently marketed indications (hepatobiliary conditions)(reviewed in Nagy 2015[39]) A recently completed Phase 1 dose-range study demonstrated inhibition of hyaluronan in the sputum of healthy adults over 4 days (pharmacodynamic marker).

The current study will be the first study to use the novel mechanism of HA inhibition to investigate the clinical efficacy in pulmonary hypertension.

2.3 Clinical Safety

In published clinical trials including more than 200 patients, H01 has demonstrated safety with over 28 patient years of exposure. In these studies, H01 was safe and well tolerated. The safety profile of H01 is supported further by decades of marketing authorization approval in Europe, which is characterized by broader pharmacovigilance data. A principal European manufacturer reports awareness of more than 60 clinical trials including 2638 patients (Grunenthal 2020). The main reported side effects for H01 in the product labels include diarrhea and allergic reactions. According to the WHO spontaneous reporting database[59], investigated on August 8th, 2021, only 63 cases of adverse drug events to H01 have been reported since 1967 worldwide. The majority of reported adverse events concerned skin disorders (42.9%, mainly urticaria and pruritus), GI disorders (20.6%, mainly diarrhea), and immune system disorders (20.6%, mainly anaphylactic shock, angioedema or face edema).

In fourteen randomized controlled trials, few side-effects were reported at doses up to 2400 mg/day PO[39,60]. In another study of H01 at 2400 mg/day in patients following surgical revision of biliary pathways, no serious adverse reactions were observed.

2.4 Non-clinical Efficacy

H01 has been studied in multiple animal models of lung injury. Across these animal models, inhibition of HA synthesis with H01 led to a significant reduction in HA content, as well as clinically relevant outcomes. Five relevant studies are summarized below:

- **Bleomycin model of pulmonary hypertension and lung fibrosis**. In 2017, Collum *et al.* published an article entitled "Inhibition of HA synthesis attenuates pulmonary hypertension associated with lung fibrosis"[2]. In an experimental model of lung fibrosis and pulmonary hypertension (PH), mice were treated i.p. with 0.035 U/g of bleomycin (BLM) or vehicle (PBS) twice a week for 4 weeks. This model of chronic injury presents with fibrotic deposition affecting the distal areas of the lung, which is more representative than the more common intra-tracheal (IT) instillation of bleomycin. On day 15, mice were provided with chow containing H01 at a dose of 20 mg/kg/day or regular chow for the remainder of the experiment. To deliver 20 mg/kg/day, H01 was added to the diet at a concentration of 125 mg/kg diet (assuming a mean body weight ~25 g and chow intake ~4 g per day). It was observed that blood oxygenation was reduced by 25% in the bleomycin-exposed mice on control chow, whereas only 5% oxygenation reduction was observed in the bleomycin-exposed and H01 treated group. This was consistent with improvised right ventricle systolic pressure (RVSP) values in BLM-exposed and H01 treated mice compared to BLM-exposed vehicle treated mice. RVSP is a surrogate value for mPAP in mice. Furthermore, hyaluronan (HA) levels observed by immunohistochemistry (IHC) showed increased HA deposition in bleomycin-exposed mice that were inhibited by H01 therapy. In line with this, bronchoalveolar lavage fluid (BALF) revealed a significant HA elevation in bleomycin-exposed mice that was attenuated from 1600 ng/mL HA to 1000 ng/mL HA by H01 treatment. These

observations were consistent with increased levels of the HA-synthases Has1, Has2 and Has3 expression in bleomycin-exposed mice. H01 attenuated Has2 and Has3 but not Has1 expression.

- **Adenosine deaminase null mouse ($Ada^{-/-}$) model of lung fibrosis and pulmonary hypertension in combined pulmonary fibrosis and emphysema (CPFE).** In 2019, Collum *et al.* published an article entitled "Adenosine and hyaluronan promote lung fibrosis and pulmonary hypertension in combined pulmonary fibrosis and emphysema"[3]. Genetically engineered mice lacking adenosine deaminase ($Ada^{-/-}$) were utilized as an experimental model of lung fibrosis and pulmonary hypertension (PH) in combined pulmonary fibrosis and emphysema (CPFE). $Ada^{-/-}$ mice received supplemental PEG-ADA, allowing them to live normally, from birth up to week 24. Starting on week 24, PEG-ADA was gradually reduced over 9 weeks and, starting on week 34, mice were provided with either control chow or medicated chow (H01 at a dose of 20 mg/kg/day) for 4 weeks. $Ada^{-/-}$ mice developed increased fibrosis in the lung as detected by Masson's trichrome staining for collagen which was decreased upon H01 treatment. Arterioles from $Ada^{-/-}$ mice exhibited increased aSMA staining indicative of increased muscularization of the arterioles which resulted in an increased smooth muscle area/vessel ratio. Arterioles from H01-treated $Ada^{-/-}$ mice had significantly decreased aSMA staining and a more normalized smooth muscle area/vessel ratio. $Ada^{-/-}$ mice also had significantly decreased levels of arterial oxygenation (SpO_2) which were normalized with H01 treatment. Importantly, bronchoalveolar lavage fluid (BALF) levels from Ada-/- mice contained HA levels that were significantly increased (> 10x) compared to normal mice while H01 treatment in Ada-/- mice were only ~3x above normal. In addition, in this study H01 was able to therapeutically reduce evidence of fibrotic matrix remodeling in these mice.

- **Hypoxia-induced Pulmonary Hypertension**. In an unpublished study using mice lacking HAS2, investigators demonstrate that these mice are protected from the development of pulmonary hypertension, induced following chronic exposure to hypoxia (10% O_2 for 28 days) with weekly Sugen (SU5416) injections. Similarly, in a model of hypoxia-sugen induced pulmonary hypertension, H01 administered starting on day 15 of 10% O_2 halted vascular remodeling, perivascular HA accumulation and attenuated RVSP after 13 days of treatment. The hypoxia-sugen model of PH is able to recapitulate some of the features of patients with PAH, including elevated RVSP and vascular wall thickening without parenchymal lung disease. These studies underscore the role of HA synthesis in PH and highlight the capacity of H01 to halt the development of disease.

- **Staphylococcus enterotoxin B (SEB)-induced model of acute lung inflammation.** In 2013, McKallip *et al.* published an article entitled "Treatment with the hyaluronic acid synthesis inhibitor 4-methylumbelliferone suppresses SEB-induced lung inflammation"[5]. The effects of H01 on staphylococcus (SEB)-induced acute lung inflammation were investigated *in vitro* and *in vivo*. Culturing staphylococcal enterotoxin B (SEB)-activated immune cells with H01 led to reduced proliferation, reduced cytokine production as well as an increase in apoptosis when compared to

untreated cells. In an *in vivo* model of lung inflammation, exposure to SEB led to significantly elevated levels of 1500 pg/mL hyaluronan (HA) in the bronchoalveolar lavage fluid (BALF). However, H01 treatment following SEB exposure significantly reduced BALF HA levels to 200 pg/mL. Concomitant with the decrease in BALF, HA levels, mRNA levels of HAS1, HAS2 and HAS3 were also all significantly decreased. Furthermore, exposure of vehicle-treated mice to SEB led to an increase in the expression of various cytokines, such as IL-1β, IL-2, IL-6, IFN-γ, and TNF-α, which are all reported to play a role in lung inflammation. In comparison, treatment of mice with H01 led to a significant reduction in the SEB-induced increase in all cytokines measured.

- **Lipopolysaccharide (LPS)-induced model of acute lung inflammation**. In 2015, McKallip *et al.* published an article entitled "Treatment with the hyaluronic acid synthesis inhibitor 4-Methylumbelliferone suppresses LPS-induced lung inflammation"[6]. The effects of H01 on lipopolysaccharide (LPS)-induced acute lung inflammation were investigated *in vitro* and *in vivo*. Culturing LPS-activated immune cells with H01 led to reduced proliferation, reduced cytokine production, and an increase in apoptosis when compared to untreated cells. Treatment of mice with H01 (single dose of 450 mg/kg i.p., approximately 18000 mg/kg assuming a body weight of 25 g) led to protection from LPS-induced lung injury. Specifically, H01 treatment led to a reduction in LPS-induced HA synthase (HAS) messenger RNA (mRNA) levels HAS1 decreased from 9 to 1.5, HAS2 from 8 to 0.5, HAS3 from 13 to 1. Vascular permeability induced by LPS was reduced nearly 4-fold with H01 treatment while pro-inflammatory cytokine levels (IL-6, IFN-α and IFN-γ) were reduced by >90% with treatment.

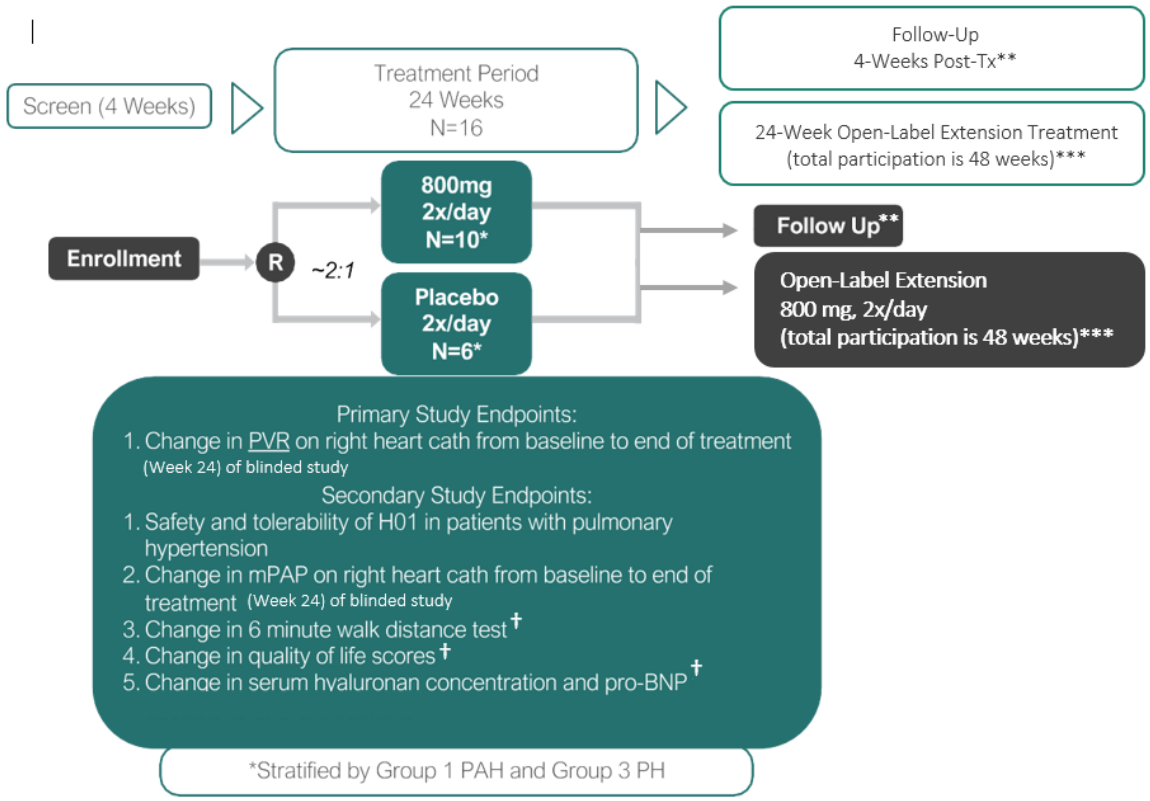
2.5 Non-clinical Safety

The acute toxicity of H01 is relatively low: the LD50 for oral administration is 7593 mg/kg in mice and 6220 mg/kg in rats (highest doses tested; HED 37,000 mg/day and 61,000 mg/day, respectively). Protracted oral administration of 800-2400 mg/kg/day for 3 months in a dog model (HED 79,000 mg/day) and of 400-1000 mg/kg/day for 4 months in a rat model (HED 9700 mg/day), showed no toxicities in physical or behavioral measures (Cantabilin Label Italy - Attachment). No teratogenicity was observed in rat or rabbit at oral doses up to 1200 mg/kg/day (HED 23,000 mg/day). Toxicology tests on animals have not shown any toxic phenomena, abnormalities of growth or behavior. No cases of teratogenicity or fetal toxicity have been encountered.

2.6 Hypothesis

We hypothesize that oral H01 at doses of 1600 mg per day for 24 weeks in the blinded study and for an additional 24 weeks as part of open-label extension will be safe and well-tolerated and show evidence of clinical efficacy. We will conduct exploratory analyses in both the blinded study and open-label extension to further establish the correlation of changes in serum HA with meaningful clinical outcomes.

3 STUDY DESIGN



** For participants not participating in the open-label extension
*** For participants participating in the open-label extension
† (Week 24) of the blinded study and through Week 48 of the open-label extension

3.1 Study Treatment

Participants will be randomized approximately 5:3 (Treatment:Placebo). Participants randomized to treatment will be given 800 mg oral H01 two times a day (total dose: 1600 mg/day). Participants randomized to placebo will receive oral tablet placebo (inactive ingredients) two times a day. Participants will be on treatment continuously for 24 weeks or 48 weeks if enrolled in the open-label extension.During the treatment period, participants will be monitored according to the schedule of assessments below. Participant a follow up will take place 30 days after the end of treatment at Week 24 if not enrolled in the open-label extension.

4 ENDPOINTS

4.1 Primary Endpoint

- Change in pulmonary vascular resistance (PVR) measured by right heart catheterization (RHC) from baseline to end of treatment (Week 24) of the blinded study

4.2 Secondary Endpoints

- The safety and tolerability of H01 in adults with pulmonary hypertension using the Common Terminology Criteria for Adverse Events (CTCAE)
- Change in mean pulmonary arterial pressure (mPAP) by RHC from baseline to end of treatment (Week 24) of the blinded study
- 6 Minute Walk Distance Test (6 MWDT) from screening to end of treatment (Week 24) of the blinded study or through Week 48 of the open-label extension
- Change in quality of life (QOL) score, EMPHASIS-10 score and St George Respiratory Questionnaire (SGRQ) score from baseline to end of treatment (Week 24) of the blinded study or through Week 48 of the open-label extension
- Change in serum HA concentration from baseline to end of treatment (Week 24) of the blinded study or through Week 48 of the open-label extension
- Change in NT-proBNP from baseline to end of treatment (Week 24) of the blinded study or through Week 48 of the open-label extension

4.3 Exploratory Endpoints

- Change in inflammatory markers and PH-specific biomarkers (ESR, HSCRP)
- Change in pro-inflammatory cytokines
- Change in Forced Expiratory Volume in one second (FEV1)
- Change in Forced Vital Capacity (FVC) from pulmonary function test (PFT)
- Change in Total Lung Capacity (TLC) from pulmonary function test (PFT)
- Change in Lung diffusion capacity (DLCO) from pulmonary function test (PFT)
- Change in exhaled breath condensate (EBC) hyaluronan concentrations over the study period
- Describe the pharmacokinetics (H01 and metabolite serum concentrations)
- Describe HA fragment size

5 STUDY POPULATION

Adults with Group 1 PAH secondary to connective tissue disease, idiopathic, hereditary, toxins, or drugs and adults with Group 3 PH secondary to interstitial lung disease. Individuals interested in participating in the study will be screened for inclusion and exclusion criteria. If the patient meets criteria and consents to participate, they will be enrolled and undergo randomization.

5.1 Inclusion Criteria

- Adults age 18 to 75 years inclusive;
- WHO functional class II/III/IV despite treatment with maximally tolerated doses of 2 or more treatment modalities including PDE5 inhibitors, guanylate cyclase stimulators, endothelin receptor antagonists, and prostanoids when appropriate.
- Baseline 6MWT>100 meters and <550 meters
- Established diagnosis of Group 3 pulmonary hypertension secondary to interstitial lung disease OR established diagnosis of Group 1 pulmonary hypertension secondary to connective tissue disease, idiopathic, hereditary, drugs, or toxins.
- Right heart catheterization at randomization showing precapillary pulmonary hypertension (mPAP $\geq$ 25 mmHg and PVR $\geq$ 400 dynes * sec * cm $^{-5}$) and:
    - PCWP $\leq$ 20 mmHg for Group 3 PH patients and Group 1 PAH patients
- Participants on chronic medication for PAH, PH, or underlying lung disease must be on a stable and optimized dose for at least 90 days prior to the first dose of the study drug.
- Female participants who are heterosexually active must use an acceptable method of contraception: condoms (male or female) with or without a spermicidal agent, diaphragm or cervical cap with spermicide, IUD, or Hormone-based contraceptive
- Be able to provide written informed consent and comply with requirements of the study

5.2 Exclusion Criteria

- Participants with a diagnosis of PAH or PH for reasons other than the pulmonary hypertension *primarily* due to any of the following:
    - Group 2, 4, or 5
    - Group 1 due to HIV, veno-occlusive disease, porto-pulmonary hypertension, congenital heart disease
    - Group 3 due to severe chronic obstructive pulmonary disease (COPD) or obstructive sleep apnea (OSA)
    - Note: participants with overlapping syndromes will be evaluated on a case-by-case basis by the recruiting physician
- Total Lung Capacity (TLC) <60% predicted
- FEV1/FVC <50% predicted or FEV1 <55% predicted
- Inability to safely attempt completion of the 6MWD test
- Use of experimental PAH treatments within the past 3 months
- Current systemic treatment with hymecromone
- Left sided heart disease as defined by either a PCWP >20 mmHg and/or left ventricular ejection fraction <40%
    - Note: participants with abnormal left ventricular function attributable entirely to impaired left ventricular filling due to the effects of right ventricular overload (ie right ventricular hypertrophy and/or dilatation) are not excluded

- Participants must not have 3 or more of the following left ventricular disease / dysfunction risk factors:
    - Body mass index (BMI) $\geq$ 30kg/m^2
    - History of essential hypertension requiring medication
    - Diabetes mellitus
    - Historical evidence of significant coronary disease established by any of the following:
        - History of myocardial infarction
        - History of percutaneous coronary intervention or coronary artery bypass graft
        - Angiographic evidence of >50% stenosis in at least one coronary artery
        - Positive stress test with imaging
        - Stable angina
- Significant valvular heart disease as determined by more than moderate findings on echocardiogram or history of valve replacement
- Pregnant or actively breastfeeding
- Female participants with childbearing potential not willing to use a form of birth control (including abstinence) during the study
- Inability to undergo right heart catheterization
- Acute pulmonary embolism within 90 days of randomization
- Exacerbation of underlying lung disease or active pulmonary or upper respiratory infection within 30 days of randomizations
- Use of any inhaled tobacco products or significant history of drug abuse within 3 months prior to randomization
- Subject is receiving >10L/min of oxygen supplementation by any mode of delivery at rest at baseline
- Body mass index $\geq$ 40kg/m^2
- Participants with history of dysphagia, achalasia, or difficulty swallowing capsules, tablets or pills
- Participants with liver failure or AST or ALT greater than 2 times the upper limit of normal
- Participants with total bilirubin levels greater than 2 times the upper limit of normal
- Participants with CrCl <45
- Use of any investigational drug/device, or participation in any investigational study with therapeutic intent within 30 days prior to randomization
- Known allergy to hymecromone or any component of drug product (including rare hereditary problems of galactose intolerance, Lapp lactase deficiency, or glucose-galactose malabsorption)
- Known allergy to any component of placebo (including rare hereditary problems of galactose intolerance, Lapp lactase deficiency, or glucose-galactose malabsorption)
- Physician concern that participant may not adhere to the study protocol
- Significant psychiatric, addictive, or other disorder that compromises the subject's ability to provide informed consent

5.3 Concomitant Medication

To date, there are no known drug-drug interactions for H01. Metoclopramide, its derivatives and morphine are cited in one product label as potentially reducing the effect of H01.

Participants can remain on an FDA approved PAH/PH background therapy if they have been on a stable regimen for a minimum of 90 days prior to randomization.

Participants on a supportive therapy (e.g., anticoagulants, diuretics, oxygen) must have been on a stable and optimized dose for at least 30 days prior to the first dose of study drug. Exceptions are the discontinuation or dose changes of anticoagulants and / or dose change of diuretics. Participants on a chronic medication for underlying lung disease should be on a stable and optimized dose for > 30 days prior to the first dose of study drug.

Participants may not newly initiate PAH/PH treatment from the first dose of study drug (Baseline) through study termination. Participants may not initiate pulmonary rehabilitation (rehab) within 90 days prior to the first dose of study drug and until the end of the study.

All concomitant medications taken during the conduct of the study, including those taken for AEs or other medical events, should be recorded in the subject's source documents and transcribed into the eCRF as required.

6 SCHEDULE OF ASSESSMENTS

Schedule of Assessments for SATURN Trial

	Screen	Active								FU			
	Screening	Baseline	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 26**	Week 28+	Week 36**	Week 48 **
Visits	0	1		2	3	4			5		6		7
Day«	-30-0	0	14±3	28±3	56 ±3	84±7	140 ± 7	168±7	196±7	210±3	224±3	280±7	364±7
Telephone or Email Contact			X		X		X	X		X**	X ^	X**	
Informed Consent	X												
History and physical													
Medical history	X	X											
Symptom assessment/NYHA		X	X	X	X	X	X	X	X	X**	X+	X**	X**
Medications	X	X	X	X	X	X	X	X	X	X**	X+	X**	X**
Vital Signs & Weight		X		X		X			X		X**		X**
Physical Examination		X		X		X			X		X**		X**
EMPHASIS-10 / SGQ		X		X		X			X		X**		X**
General Testing													
Comprehensive Metabolic Panel		X		X		X			X		X**		X**
CBC		X		X		X			X		X**		X**
Pregnancy test (If Indicated)[a]		X		X	X*	X	X*	X*	X	X**	X**	X**	X**
12-Lead ECG		X											
Endpoint assessment													
Right Heart Catheterization		X							X				
Serum & EBC hyaluronan		X		X		X			X		X**		X**
6 minute walk distance	X			X		X			X		X**		X**
NT-pro-BNP		X		X		X			X		X**		X**
PFT with ABG	X								X				
Inflammatory markers		X		X		X			X		X**		X**
Serum and EBC drug levels		X		X		X			X		X**		X**
Study Procedures													
Medication Pill Count			X	X	X	X	X	X	X	X**	X**	X**	X**
Adverse Events		X	X	X	X	X	X	X	X	X**	X+	X**	X**
Study Termination											X^		X**

*Home pregnancy test permitted

^ Only for subjects not participating in OLE (Open-Label Extension)

**Only for subjects participating in OLE (Open-Label Extension)

+ For participants in OLE (Open-Label Extension) and those not participating in OLE

a. Pregnancy tests need to be done monthly in OLE (Open-Label Extension). Participants will be given home pregnancy test kits to take home.

7 ASSESSMENTS

7.1 Screening/Baseline Assessment

Prior to enrollment, each participant will undergo a medical screen, be introduced to study procedures,

and undergo informed consent. Screening will be conducted within 30 days of baseline assessment. If any of the screening labs were performed as part of standard of care within 4 weeks of the screening, these labs will be used for screening.

If the patient qualifies and consents to participate, they will undergo baseline assessments. Baseline labs that were done within the previous 4 weeks will be accepted as baseline labs; if there are no labs for >4 weeks, these will be repeated. If any of the following baseline procedures were performed and documented in the medical chart at part of standard of care within 2 months of the baseline visit, these will be accepted as the baseline assessment: RHC, PFTs, 6MWDT, 12-Lead ECG.

All participants will start taking the study drug on the morning of Day 1, the day after the baseline assessment. All participants will follow up in person for assessments at Weeks 4, 12, and 24. Participants will also be contacted electronically and/or by telephone on Weeks 2, 8, 16, 20, 28. Participants will continue the study drug for 24 weeks with the final in person assessment at Week 24. A final follow-up electronically or by telephone will be performed at week 28 (4 weeks after treatment completion). If participants do not complete the 24 weeks of treatment for any reason, every effort will be made to conduct the Week 24 final assessment at the time of treatment termination and the participant will be followed until at least 4 weeks after stopping the study drug (longer if necessary to follow AEs). If the participants are enrolled in the open-label extension, they will be contacted by telephone on Weeks 26 and 36. A final in-person assessment will occur at Week 48.

Biological samples:

- Blood:
  - 6 ml blood for serum concentration of H01 and metabolites
  - 6 ml blood for serum HA level and biomarkers
  - 6 ml blood for CBC
  - 6 ml blood for CMP
- Urine:
  - Pregnancy test for females of child-bearing potential (serum pregnancy test may be substituted for urine)
- Exhaled breath condensate:
  - Concentration of HA levels, H01 and metabolites

7.2 Laboratories for Central Study-specific Testing

Lab testing will be done at the trial site laboratory used in the provision of standard of care services will process routine bloodwork (as above). The laboratory manual and study operations manual will specify procedures for when tests in the study protocol are not completed as standard of care. The manual will also detail which tests may need to be processed for shipping to alternative laboratories at Stanford, two of which are referenced below.

**HA Testing of Blood and/or EBC

Lab of Dr. Paul Bollyky, Division of Infectious Diseases, Stanford University, 279 Campus Drive, Beckman Center B237, Stanford, CA 94305

**H01 and metabolite Testing of Blood and/or EBC

Lab of Dr. Paul Bollyky, Division of Infectious Diseases, Stanford University, 279 Campus Drive, Beckman Center B237, Stanford, CA 94305

**Immune mediators (63-plex Luminex® human assay)

Stanford Human Immune Monitoring Center (HIMC), Fairchild Science Bldg., 299 Campus Drive, Stanford, CA 94305

7.3 Clinical Improvement

Clinical improvement in this study will utilize the RHC, 6MWD test, PFTs, and patient symptom score reports.

RHC at rest and with exercise will be performed in accordance with standard procedures by a trained pulmonologist in a cardiac catheterization laboratory and standard sedation medications may be used. This procedure typically takes less than one hour.

6MWD test and PFTs will be performed in the clinic at specified visits in accordance with standard guidelines and will be carried out by trained study staff.

Quality of life questionnaires will be performed a selected time points and will include questions to measure physical activity, respiratory symptoms, pain, general health, mental and emotional wellbeing.

8 STUDY DRUG PROCEDURES

8.1 Treatment Assignment

Up to 16 eligible participants will be randomly assigned to H01 or placebo in a 5:3 ratio using a computer-generated randomization scheme, stratified by PH type, developed by an unblinded biostatistician at Stanford University. The code used to generate the allocation, along with the seed used in the random number generation, will be stored on secure servers and maintained by the unblinded biostatistician.

8.2 Blinding

This is a double-blind study design. Participants, study investigators, and clinicians will be blinded. All doses of study drugs and placebo will be prepackaged by a central study pharmacy. The central pharmacy will coordinate randomization with the independent biostatistician for randomization codes. The allocation codes and labeled product will be used by dedicated study personnel who will administer

product to enrolled and randomized participants. An SOP will govern the blinding process. Any break in the blinding process will be reported to the DSMC and principal investigator.

8.3 Formulation of Test and Placebo Medications

H01 will be supplied by a manufacturer in Europe that holds current good manufacturing practice (GMP) certification. Specifically, the tablet formulation approved in the European Union called Isochol 400 mg will be used and provided by the marketing authorization holder, Zentiva. U Kabelovny, 529/16, 102 00 Praha-Dolní Měcholupy, Czechia.

Under normal conditions, unopened H01 has a reported shelf-life by the manufacturer of 3 years. The drug product will be stored at a temperature under 25 °C in bottles, and bottles will be tightly closed in order to protect from moisture, in accordance with the storage instructions on the Iscohol 400mg label.

The placebo tablets used in this trial are selected to match the general shape, size and color of H01 but are not identical. Storage and dispensing of medications H01 and placebo product will be performed at controlled room temperature, 59° to 77°F (15° to 25°C) and protected from light and moisture by study-designated pharmacy personnel. Study drug will be dispensed to enrolled participants based on site-level standard operating procedures. Study drug will remain in the original bottles until the time of release of the product to patients for home administration. A site Drug Accountability Log will be maintained by site personnel. This log is subject to audit and will be signed off by the site investigator atthe end of the trial. A temperature log will be utilized by the IDS to ensure compliance with temperature requirements.

8.4 Possible Reasons for Discontinuation of Treatment

All reasons for discontinuation from study drug will be captured in study CRFs enabling for the ability to differentiate treatment discontinuation from study withdrawal. These include the following reasons:

- Completed 24 weeks of treatment of the blinded study or 48 week of the open-label extension
- Grade ≥3 Adverse Drug Reactions
- Withdrawal of consent
- Physician request

Investigators and study staff are reminded of the importance of participant retention in the case of treatment discontinuation and should take steps to prevent missing data that will be critical to the interpretation of the study results. Investigators and study staff should remind participants of the importance of continued participation in laboratory and clinical monitoring. Participants who discontinue study drug should continue follow-up when possible. This concept will be reinforced in the Informed Consent Form (ICF).

In the case of missing data for participant disposition for a randomized participant, a vital records search will be undertaken.

9 MONITORING AND REPORTING OF ADVERSE EVENTS

9.1 Adverse Events Definition

An adverse event (AE) is any untoward or unfavorable medical occurrence in humans which occurs during the participant's participation of a clinical study, whether or not considered drug related. Any change in clinical status, routine labs, physical examinations, etc. that is considered clinically significant by the study investigator are considered AEs.

9.2 Suspected Adverse Reaction Definition

A Suspected Adverse Reaction is any adverse event for which there is a reasonable possibility that the investigational drug caused the AE. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

9.3 Serious Adverse Event or Serious Adverse Reaction Definition

An AE or suspected adverse reaction or adverse reaction is considered serious if, in the view of either the PI or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening AE: it is considered "life-threatening" if its occurrence, in the opinion of the investigator or sponsor, places the participant at immediate risk of death from the reaction as it occurred. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Inpatient hospitalization or prolongation of existing hospitalization
- Congenital abnormality or birth defect
- Necessary surgery or medical device intervention to prevent fatal or permanent adverse effects
- Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious adverse event

9.4 Unexpected Adverse Event or Reaction Definition

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the

Investigator Brochure or package insert or is not listed at the specificity, severity or rate of occurrence that has been observed. The below summary includes adverse reactions from product labels for H01 in Europe.

- Cantabiline® 400 mg (France)

Undesirable effects: Occasional cases of diarrhea and isolated reports of allergic skin reactions.

- Cantabilin® 300 mg (Italy)

Undesirable effects: Diarrhea, anaphylactic reactions, including urticaria, itching, dyspnea, Quincke's edema and hypotension that can evolve to anaphylactic shock.

- Cholspasmin® forte 400 mg (Germany)

Undesirable effects: With prolonged administration of higher doses, soft stool or diarrhea may occur; fullness, constipation and stomach pressures are also reported (<0.01%) hypersensitivity reactions may occur, which can manifest as skin changes (< 0.01%).

- Isochol® 400 mg (Czech Republic)

  Undesirable effects: Rare frequency of headache and rash (<0.001)

9.5 New Fact

A new fact is defined as any new data that may lead to a reassessment of the risk-to-benefit ratio of the research or of the product being researched, changes in the use of this product, in the conduct of the research, or to the documents relating to the research, or data that may lead to the suspension, discontinuation or modification of the research protocol or similar research.

9.6 Identification of Adverse Events

As all participants in this study will have pre-existing medical conditions, those pre-existing conditions will not be considered adverse events. New events that occur or worsening (through frequency or intensity) of pre-existing conditions will be considered as adverse events.

Adverse events (including SAEs) may be discovered through any of these methods:

1. Observing or interviewing the participant.
2. Procedures / laboratories as performed as part of participating in the study.
3. Receiving an unsolicited complaint from the participant.

The investigator will provide the following information about an AE:

- Date of onset and resolution
- Severity, graded using the NIH Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0
- Action taken with study drug product
- Changes in study drug dosing
- Causal relationship to study drug

- Outcome

All reportable drug adverse events as defined above will be recorded on the appropriate AE/SAE CRF form starting after consent has been obtained until Day 196 (ie at least 30 days after the last dose of study drug).

9.7 Follow-up of Adverse Events

If a participant experiences any clinical or laboratory abnormality attributed to the study drug, they will be monitored until the abnormality returns to baseline (as defined at enrollment) or stabilizes. This procedure is implemented in the study database and case report forms and includes both adverse events and serious adverse events. Participants can take medications for symptomatic relief (e.g. anti-emetics, anti-diarrhea, analgesics).

9.8 Guidelines for Assessing Severity of an Adverse Event

Adverse events will be graded on a scale from 1 to 5 according to the NIH Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0 (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf). A summary is below.

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL).
- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4 Life-threatening consequences; urgent intervention indicated.
- Grade 5 Death related to AE.

9.9 Guidelines for Determining Causality

Attribution of all grade 2 and higher AE's as treatment related or not will be done in two ways. First, attribution will be done by the treating clinician at the time of clinical evaluation using the formal assignment detailed below. Later, attribution we be done again by investigators in aggregate data review and reporting.

Attribution Standards:

1. Unrelated: The AE is *clearly not related* to the investigational agent
2. Unlikely: The AE is *doubtfully related* to the investigational agent
3. Possible: The AE *may be related* to the investigational agent
4. Probable: The AE *is likely related* to the investigational agent

5. Definite: The AE *is clearly related* to the investigational agent

9.10 Serious Adverse Events

Investigators will report to the Primary Investigator (PI) all serious adverse events within 24 hours of becoming aware of the event, regardless of relationship or expectedness.

For serious adverse events, all requested information on the AE/SAE CRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE CRF will be updated and submitted.

9.11 Unexpected Non-serious Adverse Events

An unexpected, non-serious adverse event that is of Grade 2 severity or higher and study related will be recorded and reported to the PI under the serious adverse event reporting procedure above (i.e., within 24 hours).

9.12 Reporting to Health Authority

The Principal Investigator sponsor for the IND will report all AEs and SAEs to the FDA within the reporting time limits set forth by the FDA.

9.12.1 Standard Reporting (IND Annual Report)

This option applies if the AE is classified as one of the following:

- Serious, expected, suspected adverse reactions
- Serious and not a suspected adverse reaction
- Pregnancies

*Note that all adverse events (not just those requiring 24-hour reporting) will be reported in the Annual IND Report.

9.12.2 Expedited Reporting

The investigators must report in an IND safety report any suspected adverse reaction to study drug that is both serious and unexpected. In particular, any cases of anaphylaxis potentially due to the study drug must be reported. The time frame for submitting an IND safety report to FDA and all participating investigators is no later than 15 calendar days after the PI determines that the suspected adverse reaction or other information qualifies for reporting.

Unexpected fatal or life-threatening suspected adverse reactions represent especially important safety information and, therefore, must be reported more rapidly to FDA. The requirement for reporting any unexpected fatal or life-threatening suspected adverse reaction to FDA is no later than 7 calendar days after the sponsor's initial receipt of the information.

Investigators must submit IND and/or CTA safety reports as required by IRB. Documentation of the submission and receipt by the IRB must be retained for each IND safety report.

9.13 Protocol Violations

All protocol deviations will be recorded in a protocol deviation log by qualified study personnel. Based on IRB policies, protocol violations will be reported to the Institutional Review Board within 5 days of the protocol violation. As warranted, the PI will develop a corrective action plan to present to the IRB to address deviations and/or violations.

9.14 Reasons for Early Termination

Study drug administration must be discontinued if any of the following occurs:

- Participant develops grade 3 adverse drug reaction.
- Participant desires to discontinue participation in the study (withdrawal of consent)
- Participant is unwilling or unable to comply with the protocol.
- The investigator feels that is in the participant's best interest to discontinue treatment with study drug.

Participants who halt study drug due to an AE will continue to be followed until AE resolution or stabilization.

9.14.1 Halting the Study

The study will be halted if:

- At any point in the study the Investigators feel that there are safety concerns in this study that put the participants at increased risk as compared to the original information on risk in the consenting process; enrollment will be paused and a safety review will be performed. The investigators in discussion with the FDA can choose to stop the study.

  OR

- The independent Data and Safety Monitoring Committee for the study, after reviewing data on a rolling basis concludes that it is in the best interests of the participants to discontinue the study.

Investigators and DSMC will consider the following to guidelines for adverse drug reactions (ARs) to guide decisions regarding study suspension. Given the severity of PH-ILD disease, the DSMC will need to assess the safety data to make final decisions and recommendations, including consultation with IRB and FDA as they deem important.

Grade 2 serious ARs

- If a total of 3 or more grade 2 ARs occur, enrollment will be paused and a safety review performed.

Grade 3 or 4 serious ARs

- If 1 grade 3 or 4 serious AR occurs, enrollment will be paused and a safety review performed.

Grade 5 (fatal)

- Any incidence will lead to pausing enrollment and a safety review.

A final decision to halt the study will be made by the investigators after consultation with the DSMC. Such a decision with its supporting documentation and possible future plans including possible protocol amendments for the study will be submitted to, and discussed with, the FDA.

9.15 Data Safety Monitoring

An independent Data and Safety Monitoring Committee (DSMC) will be established. The DSMC will consist of 3 individuals representing statistical and clinical expertise needed to perform the role of safety oversight and interpretation of data for this trial. Members will be selected to ensure no conflict of interest exists relative to study outcomes and will not be directly engaged in study conduct.

The DSMC will monitor the trial, review safety data, and assess the performance of trial operations and will provide recommendations on whether the trial should continue as planned, be modified, or be stopped. Any action taken by the Board, and the reasons for the action, will be recorded. These documents will include any data summaries or analyses provided to the DSMC and will remain confidential until the study is concluded. The DSMC charter includes additional details on roles and responsibilities.

10 STATISTICAL METHODS AND CONSIDERATIONS

10.1 Analysis Population

There will be two analysis populations for the trial: the intent-to-treat (ITT) and per-protocol (PP) populations. The following description provides how the populations will be derived and analyzed:

- ITT population
  - Composed of all randomized participants
  - Analyzed according to their assigned treatment arm
- PP population
  - Composed of all randomized participants who completed treatment and didn't have any major protocol deviations
  - Analyzed according to their assigned treatment arm

10.2 Descriptive Statistics

Descriptive statistics will be reported for all key participant variables, including, but not limited to,

baseline clinical and demographic characteristics, use of medications, laboratory values, vital signs, clinical outcomes, adverse events, compliance, and study completion status. Proportions will be presented for categorical variables. Continuous variables will be presented using means, standard deviations, and number of observations if the data are normally distributed. If the data are not normally distributed, then continuous variables will be presented using medians and interquartile ranges. In all cases, between-group comparisons will be assessed using absolute standardized differences.

10.3 Analysis of Primary Endpoints

The primary endpoint is the change in PVR on RHC from baseline to the end of treatment period. No hypothesis testing will be performed in analyzing the primary endpoint. The primary endpoint will be analyzed in the ITT population.

The PVR on RHC will be presented as means, standard deviations, number of observations and 95% confidence intervals for each randomization arm. Differences in PVR may be calculated between the treatment group, placebo group, and different types of PH (e.g. Group 1 versus Group 3) to assess treatment differences. The analysis will be performed after all participants in the ITT population have reached the end of treatment or have been withdrawn from the trial.

10.4 Analysis of Secondary Endpoints

All secondary endpoints will be analyzed in the ITT population.

The proportion of participants experiencing adverse events (AE) events will be summarized by AE type and treatment arm. The proportion of participants experiencing an AE will be calculated as the number of participants who have experienced an AE divided by the number of participants in the ITT population. AEs will be summarized by each arm separately. AEs may be compared by arm using a Fisher's exact test.

The proportion of participants experiencing serious adverse events (SAE) events will be summarized by SAE type and treatment arm. The proportion of participants experiencing an SAE will be calculated as the number of participants who have experienced a SAE divided by the number of participants in the ITT population. SAEs will be summarized by each arm separately. SAEs may be compared by arm using a Fisher's exact test.

The baseline, follow-up, and change from baseline in mPAP, 6MWDT, EMPHAIS-10 score, SGRQ score, serum HA concentration, and NT-proBNP will be summarized using means, standard deviations numbers of observations, and 95% confidence intervals for each randomization arm. Differences in these measures may be calculated between the treatment and placebo group overall and within different types of PH (e.g. Group 1 versus Group 3) to assess treatment differences.

10.5 Analysis of Exploratory Endpoints

All exploratory endpoints will be analyzed in the ITT population and will be analyzed after all participants have completed treatment or have withdrawn from the trial.

The baseline, follow-up, and change from baseline in inflammatory markers, pro-inflammatory cytokines, PFTs (including FEV1, FVC, TLC, and DLCO), and EBC HA concentration, and HA fragment size will be summarized using means, standard deviations numbers of observations, and 95% confidence intervals for each randomization arm. Differences in these measures may be calculated between the treatment group, placebo group, and different types of PH (e.g. Group 1 versus Group 3) to assess treatment differences. The pharmacokinetics and fragment size will be described. There will also be a sensitivity analysis to investigate whether drug product used (Isochol vs Cantabiline) led to differential primary endpoint outcomes, using a stratified analysis by drug product.

10.6 Missing Data Handling

While we expect minimal missing data, it is inevitable in clinical trials. We will thoroughly and transparently present the amount of missing data for each variable where missing data is present. Given the small sample size, we will use complete case analyses.

10.7 Sample Size and Randomization

A total of 16 participants with PH will be enrolled in the study. Participants will be randomized 5:3 to treatment:placebo. Randomization will be stratified by PH type. As no formal hypothesis testing will be performed on the primary endpoint, this sample size is not based on a statistical powering. However, data on hand regarding clinical improvement and HA changes in preclinical and clinical settings suggests we will be able to draw meaningful insights from a study of this size that informs future trials.

11 DATA COLLECTION, RETENTION AND MONITORING

11.1 Data Collection Instruments

Data entry, database management, and data quality assurance will be led by a study coordinator at the study site. Source documentation will include site assessment forms, laboratory results, telephone surveys, and internet surveys. Where possible, electronic transfer of electronic source data to the study CRFs through a secure electronic data capture (EDC) system will be automated. Any paper-based forms that are used will be maintained in a secure locked facility at the study site and maintained by the site clinical research coordinator (CRC). An eCRF will ensure capture of information including subject identification, study identification, provision and date of informed consent form (ICF), visit dates, clinical results (safety and efficacy) as specified in the protocol, adverse events, concomitant medications, investigational medicine receipt/use/destruction, production administration, data of study completion, and reason for withdrawal if needed. The individual author of all source documents will be identifiable. Any adverse related reports will be signed off by the investigator. All study related documentation will employ the minimum of identifying information.

11.2 Data Management Procedures

In compliance with ICH/GCP guidelines, the study site will maintain all source documentation, CRFs, and study-related documents. All participants will be assigned a study ID. All study tools will use only the subject's study ID. Specimen containers and blood tubes for assignment to site laboratories or shipping to central laboratories will be labeled by the site CRC with the minimum amount of identifyinginformation (e.g., participant identifier, study identifier, and date of collection).

A secure trial master file (TMF) maintained by qualified study staff and will include all study-related documents including the protocol, investigator brochure, study operations manual, statistical analysis plan, laboratory manual, email communications, SOPs, evidence of validated processes for ECD systems. Trial site-specific files will also be maintained on the TMF including a delegation of authority log, site roster for study personnel, qualified person certification, local SOPs affecting study conduct, ICF, and IRB approvals. The trial master file system will include audit trails for document versions and archiving capabilities.

11.3 Data Quality Control and Reporting

The lead investigator is responsible for ensuring that a roster of active study personnel is maintained, a delegation of authority log assigns tasks to individual team members, and that access to the secure study EDC and TMF systems are controlled in alignment with the roster and log.

In order to ensure data quality, quality audits will be performed. The timing of audits will be developed based on a risk-based approach, reflecting the nature of the study design, known profile of the drug being used, and patient population. Study monitors will follow an SOP. Elements of study monitoring will include:

1. Implementation of patient consent processes
2. Source data verification (SDV) (e.g., for questionnaires, hospital medical records, and biospecimen collections)
3. Pharmacy monitoring (drug accountability processes, IMP storage, labeling and expiry dates, randomization and blinding)
4. Laboratory monitoring (Adherence to Lab Manual and specimen handling requirements)
5. Adverse event reporting (ensuring reporting from source documents to trial team, reporting to local authorities)

The results of site audits will be shared with the clinical trial investigator(s) and IRB as appropriate.

11.4 Archival of Data

Electronic data including all study databases and supporting electronic documentation will be archived to

cloud-based servers on a daily basis.

11.5 Availability and Retention of Investigational Records

All electronic data will be kept in secured servers. All paper-based forms that are used will be maintained in a secure locked facility at the study site and maintained by the site CRC. Only the research team will have access to the data.

11.6 Subject Confidentiality

Participant medical and study information will be kept confidential. The following health information related to this study may be used or disclosed in connection with this research study, including, but not limited to, name and initials, address, email, phone number(s), date of birth, age, sex, race, ethnicity, medical record number, information related to COVID-19 disease, symptoms, physical exams, symptoms that might relate to medication side effects, vital signs including temperature and oxygen saturation levels, laboratory tests, pregnancy test, and medications received including study drug.

All participants will be given a sequential study ID. The code for this ID with personal identifiers will be maintained in a locked research file accessible only to study personnel. Only research personnel will have access to the research records. The data will be keyed into a secure study website in a coded fashion by the study coordinator. Paper research charts will be kept in a locked file cabinet with limited access. Laboratory personnel will have access to study specimens. The data is transferred by computer via password protected electronic network. When transferring via electronic networks, a password protected encrypted computer will be used.

12 ETHICAL CONSIDERATIONS

12.1 IRB and HIPAA

This study protocol, all procedures and informed consent forms (ICF), and any subsequent protocol amendments must be reviewed and approved by the Institutional Review Board (IRB). Prior to any study-related procedures, the investigator or designee will obtain from the participant a signed and dated written ICF consistent with FDA/ICH regulations and the HIPAA Privacy Rule. A HIPAA Privacy Rule Authorization language will be included in the ICF and must be IRB approved prior to study implementation. In addition, the document of Bill of Rights will be attached to the ICF so that the participants can read and understand the same. All versions will be maintained in the TMF.

12.2 Potential Participant Risks

H01: H01 is a natural active ingredient that is synthesized for pharmaceutical use. H01 is currently not FDA approved for use in the United States. However, H01 has been marketed and used as approved drug in Europe for >50 years (European Union reference date [EURD] 07/27/1965). Overall, H01 appears to be a safe and well-tolerated medication. The French product of H01 named Cantabiline will be used in

this trial. Cantabiline is authorized by the French Medicines Agency (Agence nationale de sécurité du médicament et des produits de santé (ANSM)) under CIP : 6 197 431 7. The recommended dose is 400 mg with main meals. The main side effect listed in the product label is the gastrointestinal pathology of diarrhea, which is listed as common 0.1% to 10%. Potential anaphylactic or allergic reactions are also listed as possible side effects with an unknown rate. The label recommends to not use in cases of biliary obstruction or severe hepatic impairment.

To minimize risks of potential severe allergic reactions, participants with a known allergy for H01 will be excluded. A standard operating procedure for managing allergic reactions, including anaphylaxis, will also be in place. These risks as well as guidance on the potential signs and symptom of a severe allergic reaction will be included in the participant consent. Additionally, if participants develop a grade 3 or higher allergic reaction thought to be due to the study drug, treatment with the study drug will be discontinued immediately. To minimize risks of gastrointestinal side effects, patients with acute biliary obstruction of severe hepatic impairment will be excluded from the study. Additionally, if participants develop diarrhea, we have developed guidelines based on diarrhea severity and participant preferences for 1) treating symptoms, 2) avoiding dose increases, 3) reducing doses, or 4) discontinuing treatment.

Right heart catherization: RHC can cause bruising of the skin or infection at the site where the catheter (sterile tube) is inserted into a vein, injury to blood vessels, excessive bleeding because of puncture or damage to the vein during catheter insertion, or partial collapse of a lung if a neck or chest vein is used to insert the catheter. Rare but serious risks include abnormal heart rhythm or electrical blockage of heart beats, perforation of the heart muscle with bleeding into the sack around the heart, blood clots, stroke, heart attack, or death. Medications for pain or sedation can cause allergic reaction, an unconscious state, and depressed breathing. During the RHC, you maybe asked to perform an exercise test. With your feet on a supine ergometer you may exercise for 3 to 6 mins at a gentle pace. You may feel short of breath during this portion of the study. You can stop at any point.

Pulmonary function tests and expired breath condensate: PFTs and EBC are usually safe for most people. However, PFTs may require participants to breathe in and out quickly so they may feel dizzy and may faint.

Venipuncture & arterial blood gas: The risks of venipuncture and ABG include temporary discomfort, pain, and anxiety from the needle stick, bruising, and, rarely, infection. To minimize risk, all blood drawswill follow hospital/clinical procedures and be drawn by experienced providers. There is minimal risk regarding the blood loss related to study tests.

Privacy: There is a risk of loss of privacy around health information. To minimize this risk, information about participants will be handled with a goal of maintain confidentiality in compliance with privacy regulations (e.g. HIPPA and local government laws). Participant names and medical record numbers will be recorded for study management purposes. This information will not be included in the hard copy data case report forms or the electronic database used for data analysis and will be maintained in a locked filein a lockable office. It will not be disclosed to others. Study data will be identified by a unique study codefor each participant. Hard copy study data will be maintained in a separate locked research file in a lockable

office. The electronic data set, which will be identified by the unique study codes, will be keyedinto a computer that is password protected and encrypted. Access to study data will be limited to researchpersonnel who have completed protocol-specific training.

12.3 Potential Benefits

Participation in this trial gives participants the potential benefits of a new treatment for PH that may be a disease-modifying agent.

13 Abbreviations

6MWDT = six minute walk distance test

AE = adverse event

ALT = alanine aminotransferase

AST = aspartate aminotransferase

BL = baseline

BMI = body mass index

BUN = blood urea nitrogen

CBC = complete blood count

CRC = clinical research coordinator

CRP = C-reactive protein

CTCAE = common terminology criteria for adverse events

EBC = exhaled breath condensate

ESR = erythrocyte sedimentation

rateHA = hyaluronan, hyaluronic

acid HED = human equivalent dose

HFNC = high flow nasal cannula

H01 = 4-Methylumbelliferone, Hymecromone

ICF = Informed Consent Form

INR = international normalized ratio

ITT = intention to treat

LDH = lactate dehydrogenase

mPAP = mean pulmonary arterial pressure (mPAP)

NIPPV = non-invasive positive pressure ventilation

PFT = pulmonary function test

PK = pharmacokinetics

PP = per protocol

PT = prothrombin time

PTT = partial thromboplastin time

PVR = pulmonary vascular resistance

RHC = right heart catheterization

SC = subject contact

TMF=trial master file

V = visit

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