Laurent
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COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Protocol Title: APPLAUD: **A** Double-Blind, Randomized, **P**lacebo-Controlled, **P**hase II Study of the Efficacy and Safety of **LAU**-7b in the Treatment of Cystic Fibrosis in **Ad**ults (most recent version): 31Jul2019

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Statistical Analysis Plan

Study Title:

APPLAUD: **A** DOUBLE-BLIND, RANDOMIZED, **P**LACEBO-CONTROLLED, **P**HASE II STUDY OF THE EFFICACY AND SAFETY OF **LAU**-7B IN THE TREATMENT OF CYSTIC FIBROSIS IN A**D**ULTS

Study Protocol #: LAU-14-01

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LIST OF ABBREVIATIONS

AA	Arachidonic Acid
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BID	Bis In Die (Latin), twice daily
BMI	Body Mass Index
BW	Body Weight
CBC	Complete Blood Count
CTCAE	Common Terminology Criteria for Adverse Events
CF	Cystic Fibrosis
CFQ-R	Cystic Fibrosis Questionnaire-Revised
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CFU	Colony Forming Units
Cmin	Minimal Concentration in Matrix (plasma, blood….etc)
Cmax	Maximal Concentration in Matrix (plasma, blood…etc)
CRF	Case Report Form
CRO	Contract Research Organization
CV	Curriculum Vitae
Css	Concentration at Steady State
DHA	Docosahexaenoic Acid
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
EPA	Eicosapentaenoic Acid
FEF	Forced Expiratory Flow
FEV1%	Forced Expiratory Volume in 1 second
FVC	Forced Expiratory Vital Capacity
FRD	Fenretinide, 4-HPR
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
HDL	High Density Lipoprotein
hsCRP	High sensitivity C-Reactive Protein
ICF	Study Informed Consent Form
IL-1ra	Interleukin 1 receptor antagonist
IL-6	Interleukin 6
IL-8	Interleukin 8

IL-10	Interleukin 10
IRB	Institutional Review Board
ITT	Intent-to-Treat
IV	Intravenous
IWRS	Interactive Web Response System
LDL	Low Density Lipoprotein
MDA	Malondialdehyde
MedDRA	Medical Dictionary for Regulatory Activities
NEAPC	Neutrophil Elastase Antiprotease Complexes
NVQ	Night Vision Questionnaire
PD	Pharmacodynamic
PEx	Pulmonary Exacerbation
PI	Principal Investigator
PK	Pharmacokinetic
PO	per os (Latin), by mouth, orally
PP	Per Protocol
PsA	Pseudomonas aeruginosa
QD	Quaque Die (Latin), every day/daily
QTc	QT corrected for heart rate
RBC	Red Blood Cells
RBP	Retinol Binding Protein
SAA	Serum Amyloid A
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SD	Standard Deviation
SUSAR	Suspected Unexpected Serious Adverse Reactions
TEAE	Treatment Emergent Adverse Event
TID	Ter In Die (Latin), three times daily
TMF	Trial Master File

1 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to provide a detailed description of the statistical analyses that will be performed according to the study protocol.

The SAP presents a summary of the study protocol and describes the populations that will be analyzed. Relevant subject characteristics and parameters to be evaluated are described along with the specific statistical methods assessing the study endpoints.

2 PROTOCOL SUMMARY

2.1 Background

Cystic Fibrosis (CF) is the most common fatal hereditary disease among Caucasians, affecting an estimated 70,000 people worldwide. CF is caused by mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (*CFTR*), a protein that acts as a chloride channel. Over 1,900 mutations have been described in the *CFTR* gene, the most common of which is F508del. The disruption of chloride and sodium transport is considered the basic defect in CF, resulting in viscous secretions in different exocrine tissues, mainly the respiratory tract, pancreas, gastrointestinal tract, and sweat glands. The most debilitating consequence is the viscous secretions blocking the airways and impairing mucociliary clearance, the primary defense mechanism that protects the lungs from deleterious effects of pathogens, leading to chronic lung infections, most commonly involving *Pseudomonas aeruginosa* (*PsA*).

CF is also characterized by an abnormally activated inflammatory response in the lung, which overreacts in the presence of pathogens and leads to irreversible lung damage[1, 2]. More recent evidences suggest that the excessive and persistent inflammation in the human CF airways is indicative of an inflammatory response that begins early in life, is of greater magnitude than is observed in patients without CF and persists beyond apparent eradication of infectious stimuli [3]. Multiple studies involving infants have demonstrated that the inflammatory response in CF lung may be upregulated even before evidence of infection or mucus plugging, including increased

levels of multiple cytokines and infiltration of neutrophils [4]. Paradoxically, this pro-inflammatory and predominantly neutrophilic environment is still unable to fight the opportunistic pathogens. On the contrary, constant inflammation stimulates more mucus secretion, and the inability to clear mucus from the lungs results in frequent bacterial infections, which triggers more inflammatory responses, resulting in an inflammation-infection vicious cycle that leads to chronic inflammation and infection, and to progressive loss in lung function over time. Severe pulmonary dysfunction is the usual cause of death in CF. As such, since both lung defense mechanisms are compromised by the mutant CFTR, there is a high need of disease-modifying therapies addressing not only the mucociliary clearance, but also the compromised immune-inflammatory response.

In addition to the disruption of inflammatory response mechanisms in the lung, CF patients have long been known to be associated with abnormal fatty acid metabolism, which is now believed to be directly linked to the defective CFTR function [5-8]. CF patients display an increased level of arachidonic acid (AA), which is an agonist of inflammatory pathways, and low level of docosahexaenoic acid (DHA) that has anti-inflammatory, protective and pro-resolving roles during the inflammatory process [9]. This lipid imbalance may explain why CF patients have an exaggerated and unresolved inflammatory response that, paradoxically, is unable to fight opportunistic infections [10-12]. Thus, the innate lipid imbalance found in all CF patients could play a major role in the initiation, maintenance and degree of progression of the infection-inflammation vicious cycle in CF patients, potentially being the "missing link" between the defective CFTR and the compromised host response [13, 14].

A recent and promising area of research has been the development of CFTR modulators, some being potentiators, some being correctors of the CFTR-protein function, the basic defect in CF. Because there are many gene mutations in CF, the CFTR-modulators developed are very specific and targeting only subpopulations of CF patients. For example, Kalydeco® (ivacaftor), Orkambi® (lumacaftor/ivacaftor) and Symdeko® (tezacaftor/ivacaftor), the only CFTR-modulators approved, target currently about 6% and greater than 30/30% of CF patients, respectively. Since its launch in 2012, Kalydeco® showed promising results in the improvement of the function of the

defective CFTR protein, leading to better lung function, weight gain and lower sweat chloride levels.

2.2 Rationale

Due to its abnormality and chronic manifestations, the regulation of inflammatory response remains one of the most important and unaddressed pathogenic mechanisms in CF. With ibuprofen being the only anti-inflammatory drug recommended in patients with CF, but only used in about 10% of patients, there is a significant need for novel therapies that can control inflammation in the lung tissue in a disease-modifying manner, addressing the link between the *CFTR* dysfunction and the aberrant immune-inflammatory response. Previous attempts to control inflammation in the inflamed CF lungs have been unsuccessful and resulted in frequent lung exacerbations and serious adverse events [15]. This is likely due to the fact that inhibition of inflammation can also inhibit the ability of patient's immune system to fight infections[16].

While there are many therapies addressing the mucociliary clearance and related complications, little was done to understand and address the delicate balance between pro-inflammatory and anti-inflammatory signaling in CF, so essential for lung homeostasis. Thus, there is a high need for novel anti-inflammatory therapies that can safely control inflammation in the lung tissues of patients without suppressing their immune response. This is a particularly challenging unmet need, and a better understanding of the inflammatory and immune responses in CF lungs is needed. The development of such treatments could also prevent the initiation of the inflammation-infection vicious cycle and the consequent lung damage, which is the most frequent cause of mortality in CF. Therefore, drugs that interfere upstream with the cycle of infection and inflammation, and that can be administrated early in life, are the best hope for preventing the decline in lung function, representing one of the highest unmet needs for this disease.

All anti-inflammatory approaches explored thus far exclusively targeted the inhibition of pro-inflammatory signaling. The history of their failure showed that aggressively turning "off" the inflammatory process may result in inhibition of the ability of patient' immune system to fight infections and could result in more infections and pulmonary exacerbations [15, 16]. New approaches are needed for targeting the underlying mechanism between the CFTR dysfunction and the

compromised innate immune-inflammatory response. It is in this context that Laurent Pharmaceuticals is targeting a long time neglected approach in CF: the innate AA/DHA imbalance present in all CF patients, and which could play a major role in the initiation, maintenance and degree of progression of the infection-inflammation vicious cycle, holding the promise of a therapy that could benefit all CF patients.

2.3 Study Objectives

2.3.1 Primary Objective:

1. To assess the safety and tolerability of LAU-7b in CF patients by the incidence of treatment emergent adverse events as compared to placebo; and
2. To assess the efficacy of LAU-7b as depicted by the absolute change from Baseline in FEV1 percent predicted, relative to placebo-treated patients.

2.3.2 Secondary Objectives:

1. To assess the normalizing effect of LAU-7b on the key lipidomic markers in plasma phospholipids, such as AA, DHA and the AA/DHA ratio;
2. To evaluate the efficacy of LAU-7b on other clinically relevant parameters, such as Time-to-first protocol-defined PEx and incidence of protocol-defined PEx, change in weight and BMI, usage of new antibiotics, and the change in CFQ-R;
3. To evaluate the change in selected systemic inflammation markers;
4. Determine if LAU-7b has an effect on lung colonization with *PsA*, as measured by CFU in sputum;
5. To explore the pharmacodynamics of LAU-7b on select systemic metabolipidomic markers, oxidative stress markers, bone formation/resorption, and ceramides subclass concentration;
6. To explore the change in plasma AA/DHA ratio, EPA, inflammation markers and FEV_1 between the start and the end of IV antibiotic treatment for PEx (performed only in patients who receive IV antibiotics for what their treating physician considers a PEx);
7. To explore clinical scoring using the Matouk Disease Score.

2.4 Study Design

APPLAUD is an international, multicentre, randomized, double-blind (patients, Investigators and blinded study staff), placebo-controlled Phase II study of LAU-7b for the treatment of CF through its effect on the CF-linked AA/DHA imbalance.

To be eligible for the study, patients must have a diagnosis of CF, be aged 18 years and above, have undergone at least one IV antibiotic-treated PEx in the year prior and meet all other study inclusion and exclusion criteria at screening.

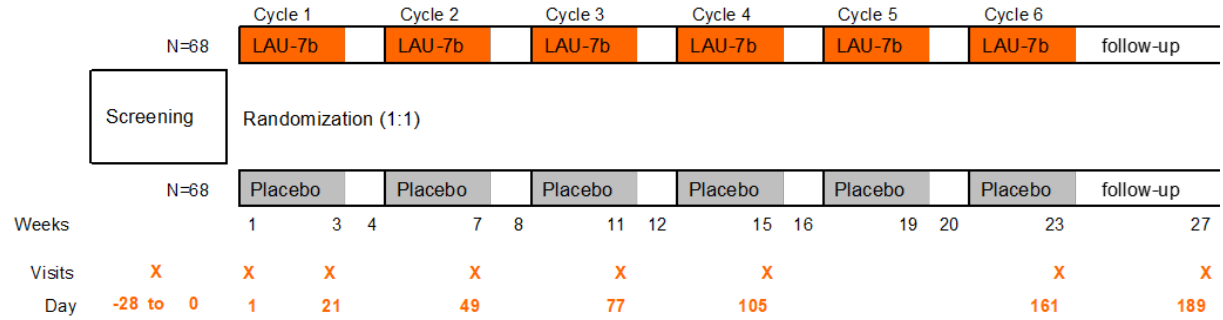
Eligible patients will be randomized 1:1, after stratification for 1- baseline FEV1, 2- PEx number in prior year, and 3- Co-administration or not of Kalydeco®, Orkambi®, Symdeko® or another commercially available CFTR modulator product, to either LAU-7b (active) group or placebo (control) group. After randomization, patients will enter the treatment phase of the study. There will be no active control treatment, but patients will undergo the CF Standard of Care at their institution.

A minimum of 136 patients will be recruited and randomized for the treatment phase of the study in the expectation that at least 120 patients complete the study as per protocol. Patients will be enrolled at approximately 25-30 centers in the United States of America, Canada, Australia and possibly in Europe. The study treatment (LAU-7b or matching placebo) will be administered in the fed state with the first meal of the day (morning if possible) for cycles consisting of 21 days on treatment followed by a 7-day treatment-free period.

Patients will be evaluated at pre-specified times during and after the study treatment period, for safety, efficacy, pharmacodynamics and pharmacokinetics, as well as Quality of Life. Patients undergoing PEx during the study will also be subjected to PEx-related assessments prior to- and after the therapeutic antibiotic course if the PEx severity meets the criteria for IV antibiotic treatment.

This design is appropriate for this phase of development and will ensure that the highest quality of data is collected with minimal bias and the patient's safety and wellbeing are preserved. A Data and Safety Monitoring Board (DSMB) will be created for this study.

The following figure presents the overall study design:



The study procedures to take place during the course of the study are summarized in the following flow-chart.

Figure 1: Study Design

Table 1: Study Flow Chart

	Screening	Baseline	Telephone contacts	End-Of-Cycle 1	End-Of-Cycle 2[1]	End-Of-Cycle 3	End-Of-Cycle 4[1]	End-Of-Cycle 6	Follow-up (End-Of-Study visit)	Start of PEx Visit [2]	End of PEx Visit [2]
Day number	Days -28 to -1	Day 1	Days 10, 38, 66, 94, 122, 150	Day 21	Day 49	Day 77	Day 105	Day 161	Day 189 (or early termination)	1st day of IV Antibiotic	Last day of IV Antibiotic
Visit number	Visit 1	Visit 2	N/A	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	N/A	N/A
Verification of ID and age	X										
Informed consent	X										
Confirmation of CF diagnosis	X										
Inclusion/Exclusion criteria	X	X									
Medical History including detailed PEx history and precise dates	X	X									
Concomitant Meds and verification of disallowed medications	X	X	X	X	X	X	X	X			
Weight, Height (screening only) and Body Mass Index	X	X		X		X		X	X	X	X
Complete Physical Examination	X								X		
Focused Physical Examination		X		X		X		X			
Ophthalmological examination [3]	X					X			X		
Night Vision Questionnaire	X			X		X		X	X		
Pulmonary Function Testing (at min. FEV1)	X	X		X	X	X	X	X	X	X	X
12-Lead ECG	X			X		X		X	X		
Vital Signs including respiratory rate, oxygen saturation and temperature	X	X		X		X		X	X		
Hematology [4]	X	X		X	X	X	X	X	X		
Chemistry [5]	X	X		X	X	X	X	X	X		
Pregnancy Test for women	X (serum)	X (urine)		X (urine)	X (urine)	X (urine)	X (urine)	X (urine)	X (urine)		
Urinalysis [6]	X	X		X	X	X	X	X	X		
Sputum [7]	X					X		X			
Cystic Fibrosis Questionnaire-Revised (CFQ-R)		X				X		X	X		
Matouk Disease Score (at selected sites)		X				X		X	X		
Day number	Days -28 to -1	Day 1	Days 10, 38, 66, 94, 122, 150	Day 21	Day 49	Day 77	Day 105	Day 161	Day 189 (or early termination)	1st day of IV Antibiotic	Last day of IV Antibiotic
Visit number	Visit 1	Visit 2	N/A	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	N/A	N/A

	Screening	Baseline	Telephone contacts	End-Of-Cycle 1	End-Of-Cycle 2[1]	End-Of-Cycle 3	End-Of-Cycle 4[1]	End-Of-Cycle 6	Follow-up (End-Of-Study visit)	Start of PEx Visit [2]	End of PEx Visit [2]
Lipidomics (AA, DHA, EPA)		X		X				X	X		
Inflammation and oxidative stress markers, ceramides and metabolipidomic markers		X		X				X			
Systemic bone formation/resorption biomarkers (all patients)		X						X			
Lumbar spine bone density (first 24 patients at selected sites)		X							X		
Randomization		X									
Supervised dosing		X									
Dispensing of study drug packs and drug compliance check where applicable		X		X		X					
Reporting of PEx by patients		X	X	X	X	X	X	X	X	X	X
PEx-related biomarkers in Plasma [8]										X	X
Plasma retinol and RBP [9]	X	X		X		X		X	X		
Plasma sample for fenretinide C_{min}				X[10]				X[10]			
Adverse Events		X	X	X	X	X	X	X	X	X	X

1 The Days 49 and 105 visits are simplified visits focused on safety

2 Only for patients experiencing episodes of PEx with need for IV antibiotic (as decided by the treating physician)

3 Complete ophthalmological examination: visual acuity, examination for amblyopia, examination of eyelids, conjunctiva, cornea, pupillary reactions to light and accommodation, specific dark adaptation tests, low contrast visual function, lens, vitreous humor, fundus examination of retina and retinal vessels, fundus visualization of optic nerve and macula. If clinically indicated by the ophthalmologist, an electroretinogram will also be performed

4 Hematology: CBC, hemoglobin, hematocrit, differentials (absolute and %) and platelets

5 Chemistry: creatinine, BUN, total bilirubin, alkaline phosphatase, AST, ALT, GGT, total protein, Na, K, Ca, bicarbonate, phosphate, albumin, glucose, total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides

6 Urinalysis: Includes appearance, pH, specific gravity, protein, hemoglobin, urobilinogen, ketones, bilirubin, nitrites, leucocyte esterase and leukocytes

7 Sputum: Sputum will be induced with hypertonic saline according to a standardized procedure across sites. Samples will serve for the assessment of presence and/or density of PsA by CFU determination

8 Blood sampling for select biomarkers in plasma (such as AA, DHA, EPA, interleukin-1 receptor antagonist (IL-1ra), IL-6, IL-8, IL-10, G-CSF, calprotectin, absolute neutrophils count, ceruloplasmin, haptoglobin, NEAPC, SAA, and hsCRP).

9 Plasma retinol and Retinol Binding Protein will be measured for exploratory correlation with the ophthalmological assessments

10 Blood sampling for Cmin: Blood samples will be obtained from all patients pre-dose on Days 21 and 161; Blood will be drawn using BD Vacutainer™ spray coated K2 EDTA tubes. Plasma will be harvested and assayed for fenretinide, its active metabolite (4-MPR) and 4-oxo-fenretinide by a validated LC/MS/MS method.

2.5 Study Duration

The duration of subject participation is approximately 24 weeks; an End-of-Study follow-up visit will occur 4 weeks subsequent to the last dose of the study drug, at approximately week 28

2.6 Sample Size

The primary measure of efficacy is the absolute change in percent predicted FEV_1 from baseline to Week 24, in fenretinide-treated compared to placebo-treated patients. The sample size has been estimated for this endpoint.

A minimum of 136 adult CF patients will be randomized in this study. The sample size was estimated according to the following assumptions:

- A t-test of the difference of the mean absolute change of FEV_1 of each group (active versus placebo) will be used;
- The FEV_1 data (absolute change) is normally distributed in each treatment group;
- The variance, expressed as SD is 8%;
- The clinical difference of interest (effect size) is 4%;
- The minimum statistical power of the t-test is 80% (0.8).

Below in Figure 2, are isotherms showing the required number of observations per treatment group (X-axis) as a function of the difference of interest (Y-axis) for different experimental assumptions of power (p) and SD ([redacted], direct communication).

Figure 2: Isotherms of detectable significant differences as a function of sample size per treatment group (assuming a 1:1 randomization) under different assumptions of statistical power (p) and variance of FEV_1 % predicted (absolute change).

For the assumptions listed above, the solid blue line represents the applicable isotherm. Therefore, a **minimum of 60 completed patients per group** will be required to detect as significant a difference of 4% between fenretinide and placebo for FEV_1 % predicted (absolute change). While there will be patients that drop out after starting dosing, it is not expected to exceed 10%, hence the need to randomize 136 patients, a multiple of 8, due to the use of 3 stratification factors (2x2x2). The desired number of completers is also a multiple of 8.

More than 136 patients will be recruited to ensure that at least 136 patients start dosing. Patients who are withdrawn or lost to follow-up prior to baseline will be replaced to ensure at least 136 eligible patients start dosing.

2.7 Subject Selection

Subjects meeting all the following inclusion criteria and none of the exclusion criteria are eligible for enrolment and participation in the study.

2.7.1 Inclusion Criteria

Patients may be enrolled in and randomized to the study only if they meet all of the following criteria at screening:

1. Signed Informed Consent;
2. Adult men and women (as per State or Province laws) and 18 years and older;
3. Diagnosis of cystic fibrosis (positive sweat chloride test) or confirmation (can be historical) of two genetic mutations (one mutation on each of the two alleles of the CFTR gene) causing CF;
4. Screening FEV_1 between 40% and 100% predicted value for age, gender and height, in patients capable of properly performing the test, and baseline FEV_1 within 15% of the screening value, indicative of stable pulmonary function at entry;
5. History of pulmonary exacerbation, defined as at least one (1) pulmonary exacerbation in the 12 months prior to Screening which resulted in a documented IV antibiotic treatment;
6. Clinically stable patients, i.e. patients that had no change in their standard antibiotic medication within 5 weeks prior to randomization, of which 2 weeks minimum are prior to Screening;
7. Patients are eligible independently of their history of pulmonary PsA infection and their PsA status at screening. Medical charts will be reviewed for the PsA history in the 12 months prior to screening;
8. If taking Kalydeco® (ivacaftor), Orkambi® (ivacaftor/lumacaftor), Symdeko® (ivacaftor/tezacaftor) or another commercially available CFTR modulator product, patients must be taking it for a minimum of 3 months prior to screening if naïve to CFTR modulators and 1 month if switched from another CFTR modulator product, and deemed to tolerate it;
9. All patients, whether prescribed pancreatic enzyme replacement therapy or not, are eligible. However, modifications of the dosing regimen during the study should be avoided;
10. No change in CF and allowed systemic chronic therapy for a minimum of 5 weeks prior to randomization, of which 2 weeks minimum are prior to screening. Changes in non-CF therapy

may be allowed during this period, on a case-by-case basis, after consultation with Laurent Pharmaceuticals;

11. Female patients of child bearing potential should be on a highly effective contraceptive method during the study, such as hormonal contraceptives, intrauterine device or tubal ligation. Women of childbearing potential are defined as any female who has experienced menarche and who is not permanently sterile or postmenopausal. Postmenopausal is defined as 12 consecutive months with no menses without an alternative medical cause. In addition, since the potential of fenretinide to reduce the effectiveness of oral contraceptives has not been established, such patients will be required to use a second contraceptive method such as a barrier method. The contraceptive methods will all be continued for a minimum of a month after the last dose of study treatment. Periodical abstinence, calendar-based methods and withdrawal are not considered effective methods of contraception;
12. Male patients with spouse or partner of child bearing potential, or pregnant, are eligible if they use an appropriate method of contraception, such as condoms and spermicide, and if their non-pregnant spouse or partner use an appropriate method of contraception such as oral contraceptives or intrauterine device. The contraceptive methods will all be continued for a minimum of a month after the last dose of study treatment. Male patients with documented sterilization are allowed to waive the contraceptive methods;
13. Patients deemed capable of adequate compliance including attending scheduled visits for the duration of the study;
14. The patient must be able to swallow the study drug capsules.

2.7.2 Exclusion Criteria

Patients are to be excluded from the study at the time of screening for any of the following reasons:

1. Pregnancy: due to the potential teratogenic effects of retinoids, pregnant women are NOT eligible;
2. Breast milk feeding by study patient is NOT allowed;

3. Health condition deemed to possibly interfere with the study endpoints and/or the safety of the patients. In case of doubt, the Investigator should consult with the Sponsor's medical representative;
4. Clinically abnormal renal function: serum creatinine > 132 µM (>1.5 mg/dL);
5. Clinically abnormal liver function: Total bilirubin >1.5 x ULN (in the absence of demonstrated Gilbert's syndrome), ALT and/or AST > 2.5 x ULN;
6. Patients with plasma retinol levels below 0.7 µM;
7. Known history of a severe allergy or sensitivity to retinoids (e.g. vitamin A, isotretinoin, etretinate), or with known allergies to excipients in the oral capsule formulation proposed to be used in the study;
8. History of organ transplantation;
9. History of alcoholism or drug abuse within 2 years before screening;
10. Presence of a cancerous tumor, active or in remission, treated or not, except squamous or basal cell carcinomas of the skin that have been treated and deemed resolved;
11. Presence of nyctalopia (also called night-blindness, which is the inability to see well at night or in poor light) or hemeralopia (the inability to see in bright light) at enrolment, or any other serious retinal, ophthalmological condition (e.g., retinitis pigmentosa, choroidoretinitis, xerophthalmia, uncontrolled glaucoma);
12. Presence of serious dermatological conditions at entry, including inflammatory or xerotic pathologies such as psoriasis or ichthyosis;
13. Intake of chronic systemic steroids in the month prior to screening and during the study. Inhaled corticosteroids for treating asthma/rhinitis are allowed as well as systemic corticosteroids (≤5 mg/day of prednisone or equivalent, or a higher loading dose tapered down in subsequent days) administered temporarily to control asthma exacerbations or pulmonary infections associated with bronchospasm (topical corticosteroids for dermatologic conditions may be allowed, subject to Investigator and Sponsor's representative authorization);
14. History of acute infections (viral/bacterial/fungal) within 5 weeks prior to randomization, of which 2 weeks minimum are prior to screening, whether or not treated and resolved.

Exceptions are topical skin infections under treatment/treated with a local non-prescription antibiotic;

15. Presence of infection with Burkholderia cepacia (including all species within the Burkholderia cepacia complex group, and Burkholderia gladioli) in the 12 months prior to screening (resolution of previous episode confirmed by quarterly negative cultures for the 12 months prior to screening);
16. Patients with a confirmed diagnosis (as per the CFF diagnostic criteria) of Allergic Bronchopulmonary Aspergillosis "ABPA" and actively being treated with corticosteroids and/or antifungals agents;
17. Participation in another drug clinical trial within 30 days, meaning from the last study drug administration of the prior study (or a minimum of 5 elimination half-lives) prior to screening;
18. Any other clinically significant condition that is considered by the principal investigator as being susceptible to put the patient at greater safety risk, influence response to study product, or interfere with study assessments.

2.7.3 Replacement of Patients

Patients who are withdrawn or lost-to-follow-up prior to Baseline, or who do not meet the Baseline criteria, will be replaced to ensure 136 eligible patients are enrolled into the study. Patients who withdraw after Baseline will not be replaced.

2.8 Study Endpoints

The following sections describe the study endpoints used to address the study objectives.

2.8.1 Efficacy and Safety Measures

2.8.1.1 Primary Endpoints

The primary objectives will be assessed with:

- The safety and tolerability of LAU-7b will be assessed in all patients through the performance of physical examinations, vital signs, ECG, safety laboratory tests, and adverse events reporting, compared to placebo treated patients;
- The absolute change in FEV_1 percent predicted at the Day 161 visit (24 weeks) relative to pre-study values, in LAU-7b compared to placebo treated patients.

2.8.1.2 Secondary Endpoints

- The proportion of patients achieving normalization of AA, DHA and the AA/DHA ratio in plasma phospholipids, LAU-7b compared to placebo. In this study, the normalization means a value or ratio value reaching the mean observed in healthy controls ± 1 SD;
- The absolute and relative (%) change from pre-study in FEV_1 percent predicted on Days 21, 49, 77, 105 and 189 visits (3, 7, 11, and 15 weeks during treatment and approximately 4 weeks after treatment, respectively), as well as the relative (%) change in FEV_1 % predicted on Day 161 (24 weeks), LAU-7b compared to placebo;
- The effect on the time to first protocol-defined PEx, LAU-7b compared to placebo;
- The effect on the incidence of protocol-defined PEx, LAU-7b compared to placebo;
- The effect on the time to first antibiotic use (other than chronic inhaled antibiotics already started prior to trial or oral chronic azithromycin), LAU-7b compared to placebo;
- The effect on the number of antibiotic treatments (other than chronic inhaled antibiotics already started prior to trial or oral chronic azithromycin), LAU-7b compared to placebo;
- The effect on the number of days of antibiotic treatment (other than chronic inhaled antibiotics already started prior to trial or oral chronic azithromycin), LAU-7b compared to placebo;
- The change from pre-study in systemic markers of inflammation in whole blood (total white cell count and absolute neutrophil count), serum (hsCRP and serum amyloid A), and plasma (calprotectin, haptoglobin, IL-1ra, IL-6, IL-8 IL-10, G-CSF, ceruloplasmin and neutrophil elastase antiprotease complexes), LAU-7b compared to placebo;
- The changes from pre-study in body weight and BMI, LAU-7b compared to placebo;
- The overall change in the *PsA* density in the sputum, from baseline through 12 and 24 weeks of treatment, when measured by the Area Under the Curve (AUC) of the CFU, LAU-7b compared to placebo;
- The fenretinide steady-state pharmacokinetics, depicted by C_{min} plasma concentrations at the end of the first and sixth treatment cycle;

- To assess the impact on overall health, daily life, perceived well-being and symptoms measured with the CFQ-R (total and respiratory domain) from pre-study to 12 and 24 weeks, LAU-7b compared to placebo.

2.8.1.3 *Exploratory Endpoints*

- The change from pre-study in AA, DHA and EPA in phospholipids and metabolipidomic markers (eicosanoids and docosanoids), LAU-7b compared to placebo;
- The change from pre-study in plasma oxidative stress makers (malondialdehyde, nitrotyrosine), LAU-7b compared to placebo;
- The changes from pre-study of in plasma ceramide subclass concentrations, LAU-7b compared to placebo;
- The change in plasma lipidomics (AA, DHA, AA/DHA ratio and EPA), inflammation markers, plasma oxidative stress markers, FEV_1, body weight and BMI measurements, between the start and the end of IV-treated PEx (performed only in patients who receive IV antibiotics for what their treating physician considers an exacerbation), LAU-7b compared to placebo;
- The change from pre-study in systemic bone formation/resorption markers, LAU-7b compared to placebo;
- At specific clinical sites, the change in bone density from baseline through 24 weeks of treatment, LAU-7b compared to placebo;
- At specific clinical sites, the changes in clinical scoring using the Matouk Disease Score contrasting the post-treatment score to the pre-study score, LAU-7b compared to placebo;
- Correlation of plasma retinol and retinol-binding protein levels measured during the study with ophthalmological assessments.

2.8.2 Other Endpoints

- Values for pharmacodynamic parameters EPA, inflammation markers, metabolipidomic markers, systemic bone formation/resorption markers, markers of oxidative stress, ceramides, and change from baseline.

3 STATISTICAL METHODS

3.1 Statistical Handling Policy

3.1.1 Analysis Conventions

This section details general guidelines to be used for the statistical analyses. Deviations from these general policies may be given in the specific detailed sections of this statistical analysis plan. When this situation occurs, the rules set forth in the specific section take precedence over the general conventions. The following policies will be applied to all data presentations and analyses:

- Two-tailed tests will be performed for all analyses that use statistical testing with a significance level of $\alpha = 0.05$
- All p-values will be rounded to 4 decimal places (SAS format p-value). All p-values that round to 0.000 will be presented as '<0.001' and p-values that round to 1.000 will be presented as '>0.999'.
- Summary descriptive statistics will consist of the number and percentage of responses in each category for discrete variables, and the mean, median, standard deviation (SD), minimum, maximum, and 95% confidence interval for continuous variables.
- All mean and median values will be formatted to one decimal place. Standard deviation values will be formatted to two decimal places.
- All percentages will be rounded to one decimal place. Where appropriate, the number and percentage of responses will be presented in the form XX (XX.X %), where the percentage is in the parentheses.
- All listings will be sorted for presentation in order of treatment group, site number, subject number, and date of procedure or event.
- When necessary for analysis purposes, partial dates will be completed (i.e., turned into complete dates) using the most conservative approach. Where appropriate, all analyses and summary tables will have the population sample size for each treatment group in the column heading.

- Tables will include titles with the corresponding analysis population and footnotes describing the analyses involved, and listings of covariates included in the analyses, where relevant.
- In order to maintain vertical alignment, all tables and listings will be incorporated into MS Word and an 8-point Courier New font will be used.
- Version 9.4 (or later) of SAS® will be the statistical software package used to produce all summaries, listings, statistical analyses, and graphs.
- Version 20.0 (or higher) of MedDRA will be used for adverse event and pre-treatment conditions coding.
- Version 2015 (or later) of the World Health Organization (WHO) Drug Classifications will be used for the coding of medications.

3.2 Control of Type 1 Error

The Type 1 error rate will be controlled to no more than 5% for the primary analysis of the primary efficacy endpoint. The study will be considered positive if the primary analysis reaches statistical significance.

The other statistical analyses / efficacy parameters will all be tested at a nominal alpha of 5%, but no control for multiplicity of testing will be applied since those additional analyses will be performed mainly for signal detection and will be confirmed in a subsequent Phase 3 trial.

3.3 Analysis Population

The Intent-to-treat (ITT) Population will include all patients randomized to a treatment group who complete the baseline evaluations, regardless of having received any study treatment or not. The ITT population will be used for the primary analyses of all study endpoints, taking into consideration the specific populations described below. The patients will be kept in their randomized treatment arm for the analyses.

The Per Protocol (PP) Population will include all patients randomized to a treatment group to whom at least 5 cycles of study treatment are administered in accordance with the protocol, defined as at least 80% compliance. The PP population will serve as the basis for secondary analyses of

all study endpoints, taking into consideration the specific populations described below. The patients will be kept in the treatment arm that they actually received for the analyses.

The FEV_1 Population is a subset of the PP population and will include all patients who have validated FEV_1 values (values judged to be obtained from adequate expiratory curves by central review) for at least 4 time points of the study comprising at the minimum the baseline and Day 161 assessments. This population will be used for the secondary analyses of all FEV_1-related endpoints. The patients will be kept in the treatment arm that they actually received for the analyses.

The Protocol-Defined PEx Subset Population will include all patients who experience a protocol - defined PEx episode (≥ 4/12 Fuch symptoms and use of IV antibiotic) during the study after completing at least one cycle of study drug treatment. The patients will be kept in the treatment arm that they actually received for the analyses.

The Overall PEx Subset Population will include all patients who experience an oral or IV antibiotic treated PEx episode during the study after completing at least one cycle of study drug treatment. The patients will be kept in the treatment arm that they actually received for the analyses.

The Safety Population will include all patients randomized to a treatment group who received at least one dose of study drug treatment. This population will serve as the basis for analyses of all safety endpoints. The patients will be kept in the treatment arm that they actually received for the analyses.

The Pharmacokinetic (PK) Population will include all patients randomized to the LAU-7b treatment group and provided the C_{min} blood samples. This population will serve as the basis for the between-group comparison of the steady-state fenretinide pharmacokinetics grouped according to the *a priori* stratification factors and to compare the results with those obtained during the prior Phase Ib study.

The Healthy Control Population will consist of 20 healthy controls. The data for these patients will be collected by Laurent Pharma, and provided to JSS Medical Research for assessment of

demographic and pharmacodynamic parameters (AA, DHA), the latter for use in the Pharmacodynamic analyses.

3.4 Subject Disposition and Discontinuations

Patient disposition will be summarized for the total enrolled population and by treatment group. The following data will be presented:

- The number of patients who were screened, enrolled and randomized
- The number and proportion of patients in each analysis population
- The number and percentage of patients who completed the study
- The number and percentage of patients who discontinued prematurely from the study and the associated reasons.
- The number and percentage of patients who attended each follow-up visit.

3.5 Treatment Exposure and Dosing

The duration of study drug exposure is defined as follows: last dose date minus first dose date plus 1 day, regardless of any interruptions in dosing. If the last dose date of study drug is missing, the patient's discontinuation or completion date will be used for analysis purpose. Duration of study drug exposure will be summarized descriptively as a continuous variable (number, mean, SD, median, minimum, and maximum), by treatment group, using the Safety population.

3.6 Subject Demographics and Baseline Characteristics

Demographic and baseline data will be summarized by treatment group using descriptive statistics. Demographics will include, among others, age, gender, race, ethnicity, weight, height, body mass index. Childbearing status and pregnancy test results will be summarized descriptively for each treatment group. Protocol deviations/violations will be provided as a patient data listing only.

The demographics and baseline characteristics summary will be presented for the ITT, PP, Protocol-Defined PEx and Overall PEx Populations.

In addition, baseline demographic data (age, gender, race, ethnicity), will be summarized in a separate table for the Healthy Control Population.

3.7 Medical History

Medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the number and percentage of subjects with medical history will be reported by System Organ Class (SOC) and Preferred Term (PT).

3.8 Vital Signs/Physical Examination/ECG

Vital signs (blood pressure, temperature, heart rate, oxygen saturation and respiration rate) will be presented at each time point using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change from baseline values for each variable will also be presented by treatment group.

The number and percentage of patients that meet the abnormal criteria over time during the TEAE period will be presented by treatment group. The specific changes of interest are:

- Diastolic Blood Pressure ≤ 45 mmHg and decrease from baseline ≥ 10 mmHg, Diastolic Blood Pressure ≥ 110 mmHg and increase from baseline ≥ 10 mmHg;
- Systolic Blood Pressure ≥ 160 mmHg and increase from baseline ≥ 20 mmHg
- Temperature ≤ 35ºC and ≥ 38ºC;
- Heart rate ≤ 50 bpm and decrease from baseline ≥ 20 bpm, heart rate ≥ 120 bpm and increase from baseline ≥ 20 bpm;
- Oxygen saturation values and respiratory rate determined by the Investigator to be clinically abnormal.

The number and percentage of subjects reporting normal and abnormal (NCS and CS) for physical exam at each visit will be described by treatment group.

For ECG measurements, a summary of raw values and change from screening values will be provided by treatment group for each scheduled time point for the following standard digital ECG

measurements: PR, QT, and QT corrected for heart rate (QTc) intervals, QRS duration, and HR. The number and percentage of patients with shift changes from screening (normal/missing, not clinically significant, and clinically significant according to overall ECG evaluation) to the worst ECG evaluation during the TEAE period will be tabulated by treatment group.

In addition, the number and percentage of patients will be tabulated by maximum on-treatment value of QT/QTc intervals, categorized as ≤ 500 msec and > 500 msec, as well as maximum on-treatment change from baseline value of QT/QTc intervals, categorized as > 30 msec.

No statistical analyses of vital signs or ECG data are planned.

3.9 Clinical Laboratory Assessments

Safety laboratory variables (i.e. hematology, biochemistry, and urinalysis results) will be presented in SI units at each time point using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change from baseline values for each variable will also be presented by treatment group. The number and percentage of patients with shift changes from baseline (normal/missing, high, and low according to the reference range) to the highest/lowest laboratory evaluation during the TEAE period will be tabulated by treatment group.

3.10 Ophthalmologic Examinations and Night Vision Questionnaire

The answers to the NVQ questionnaires and the resulting levels will be summarized by treatment group. The results (normal/abnormal, new adverse findings) of the planned during- and post-study ophthalmologic examinations will be presented, alongside the baseline examination and summarized by treatment group. Ad-hoc examinations and findings will also be presented alongside the baseline examination. All reported symptoms of nyctalopia and hemeralopia will be tabulated by treatment group. Reported symptoms confirmed by objective measures will be tabulated separately by treatment group. Individual data will be presented in listings.
No statistical analyses of ophthalmologic data are planned

3.11 Plasma Retinol and Retinol Binding Protein

Plasma retinol and retinol binding protein values will be presented at each time point using descriptive statistics (mean, standard deviation, median, minimum, and maximum). The number and percentage of subjects reporting low, normal and high values at each visit will also be described by treatment group.

3.12 Adherence to Treatment (Compliance)

Study drug compliance will be performed on the Safety population. Study drug compliance will be assessed by calculating as follows: 100 × (1 − [total number of days of study drug interruption]/[(duration of study drug exposure) – (total duration of planned drug free intervals)]). The total number of days of study drug interruption is defined as the sum of (number of days of each study drug interruption), where number of days of each study drug interruption is defined as the interruption end date minus the corresponding interruption start date plus 1 day. Treatment compliance percentages will be summarized descriptively as continuous variables (number, mean, SD, median, minimum, and maximum). The percentage of patients whose compliance is < 80% or ≥ 80% will be summarized, by treatment group.

3.13 Prior and Concomitant Medications

Medications used in this study will be coded by using the World Health Organization Drug Dictionary Enhanced and categorized as the following:

- Prior medication: Any medication that started before the first dose of study drug, independently of when it ended.
- Concomitant medication: Medication continued or newly received at or after first dose of study drug to the last follow-up (4 weeks after the last dose of study drug).
- Post-treatment medication: Medication continued or newly received after the last follow-up (4 weeks after the last dose of study drug).

A given medication can be classified using the above, in one or more categories. If a medication has a missing or partial missing start/end date or time and cannot be determined whether was taken before the first dose of study drug, concomitantly, or after 4 weeks after the last dose of study drug,

it will be considered as prior, concomitant, and post-treatment. Prior medications and concomitant medications will be summarized descriptively based on the ITT population. Post-treatment medications will be listed for each patient.

3.14 Analysis of the Primary Efficacy Endpoints

The study will be considered successful if a statistically significant advantage for fenretinide over placebo is obtained for the absolute change in FEV_1 percent predicted from baseline to Week 24, as assessed in the ITT population. Confirmatory analyses will be conducted in the PP Population, Protocol-defined PEx Population and Overall PEx Population.

Values for percent predicted FEV_1 as well as their change relative to baseline will be tabulated by time point and descriptive statistics will be used. In the analysis of the primary efficacy endpoint, the absolute change from Baseline in FEV_1 percent predicted, including all measurements up to Week 28 (both during and after treatment measurements, including after treatment discontinuation) will be analyzed based on a mixed model for repeated measures, with the following specifications:

- The dependent variables will be all the absolute changes in FEV_1 percent predicted from Baseline to each time point, including Week 28;
- The fixed effects will be the treatment group, the visit and the treatment-by-visit interaction;
- The random effect will be the patient;
- An unstructured variance-covariance structure will be adopted as the default structure; alternate variance-covariance structures may be adopted as appropriate.
- The model will be adjusted for the following *à priori* stratification categories:
    - FEV_1 percent predicted determined at Baseline (<70% versus ≥70% predicted),
    - PEx frequency in the prior year (≤3 versus > 3 PEx episodes),
    - Co-administration or not of Kalydeco® (ivacaftor), Orkambi® (ivacaftor/lumacaftor), Symdeko® (ivacaftor/tezacaftor) or another commercially available CFTR modulator product (yes versus no);

- The difference between treatment means at each time point and its 95% confidence interval will be estimated.
- Stratum-specific estimates based on Least Square Means will be generated

The primary result obtained from the model will be the treatment effect at Week 24. The estimated mean treatment effect, a 95% confidence interval, and a 2-sided p-value will be generated.

The analysis will be repeated with the addition of the following covariates in the model:

- Baseline use of Trikafta (yes versus no);
- Effect of the pandemic confinement (pre versus post pandemic onset) (described in Section 3.14.1)

Alternative methods will be used if necessary due to data not meeting the standard requirements for the proposed analysis.

3.14.1 Subgroup Analyses

For the primary efficacy endpoint (change from baseline in FEV_1 percent predicted from baseline to week 24, for the overall cohort), descriptive subgroup analyses will be performed for the stratification category(ies) included in the repeated measures analysis, the baseline use of Trikafta (yes versus no) and the effect of the pandemic confinement (pre versus post pandemic onset), described above in Section 3.14.

The effect of the pandemic confinement will be assessed with a dichotomous variable defined as pre versus post pandemic onset. More specifically, the pre-pandemic group will include all patients who completed their Day 161 visit or discontinued from the study before in-person visits were jeopardized and confinement was initiated at the end of March 2020, while all other patients will be included in the post-pandemic onset group.

For the purposes of the analyses, the cut-off date will be set to March 31st, 2020. Patients completing their Day 161 visit or discontinuing from the study after March 31st, 2020 will be considered as having been affected by the pandemic confinement.

Additional post-hoc analyses may be performed in an exploratory manner depending on the frequency of other phenotypes or concomitant health conditions/drugs.

3.14.2 Sensitivity Analyses- Primary Efficacy Endpoint

In the analysis of the primary efficacy endpoint, the absolute change from Baseline in FEV_1 percent predicted will be repeated to include all measurements up to Week 24 and will be performed according to the specifications described in Section 3.14 above.

In addition, though no imputations will be used in the primary analysis, a sensitivity analysis will be conducted for the analysis of the primary endpoint (change from baseline in FEV_1 percent predicted from baseline to week 24, for the ITT cohort), when > 5% of data points in each group have missing information for the change in FEV_1 predicted from baseline to week 24 (<57 patients per group have information). Missing data points will be imputed using a multiple imputation method. This method will generate *m* completed data sets (*m*=5-20), each with the observed values and different plausible values imputed for the missing observations. Each data set is analyzed using usual methods and the results are then combined across the analyses.

3.15 Analysis of the Secondary Endpoints

For all secondary efficacy endpoints, all the analysis will be based on data obtained at Week 24 or cumulated up to Week 24

Pre-defined Clinical Parameters:

- Count data variables: the number of antibiotic treatments, the number of days of antibiotic treatment (other than chronic inhaled antibiotics already started prior to trial or oral chronic azithromycin, in summary, any antibiotic change required to address worsening of CF symptoms) and the number of PEx and protocol-defined PEx will be tabulated by treatment group and compared using an appropriate count data model, e.g., Mixed ANOVA model (if assumptions of normality and homogeneity of variance appear acceptable), Poisson regression, negative binomial regression, zero-inflated regression, etc., depending on their distribution. The analysis of the number of protocol-defined PEx will also consider the type of antibiotic

prescribed (I.V. vs. oral) and the time spent on study as well as other covariates described below for the time to event data.

- Time to event data variables: the time to first antibiotic use, the time to first PEx and protocol-defined PEx, the time interval between IV antibiotic-treated PEx episodes will be tabulated by treatment group and compared using a Cox proportional hazards model. The model will include treatment group as main effect with covariates for FEV_1 percent predicted category (<70% versus ≥70% predicted), PEx number category in prior year (≤3 versus > 3 PEx episodes), and co-administration or not of Kalydeco® (ivacaftor), Orkambi® (ivacaftor/lumacaftor), Symdeko® (ivacaftor/tezacaftor) or another commercially available CFTR modulator product (all as per entry stratification). A patient without exacerbation before withdrawal or completion of the study is considered censored at the time of withdrawal or completion of the study. If the proportional hazards assumption is violated, a stratified analysis will be conducted using stratified Cox regression. Additionally, Kaplan-Meier methods will be used to produce graphical presentations of the survival (exacerbation-free survival) by treatment group and to estimate cumulative exacerbation-free survival rates by treatment group.
- Relative change (in %) in FEV_1 percent predicted from Baseline to Week 24: analysis of this variable will be similar to that of the primary analysis of the primary efficacy endpoint, i.e. repeated measures mixed model.
- Body weight and BMI changes from Baseline/Screening will be summarized using descriptive statistics by treatment group and compared between treatments using a linear mixed model similar to what is described under Section 3.14, with the addition of the baseline parameter (Weight or BMI at baseline) as covariate in the model.

Quality of Life Endpoints:

- Quality-of-life data will be derived from the questionnaires according to the corresponding scoring manuals and will be summarized by treatment group. Patients' health state will be derived from the CFQ-R questionnaire, including the extracted CFQ-R respiratory domain, a separate endpoint. Additional exploratory health condition will be evaluated by the Matouk Disease Score. Data will be summarized by treatment group using descriptive statistics.

Analysis of the absolute change from baseline for the CFQ-R (overall and respiratory domain) as well as the Matouk Disease Score will be performed similarly to that of the primary efficacy endpoint with the addition of the CFQ-R score at baseline as a covariate for the analysis of the CFQ-R variables.

3.16 Analyses of the Exploratory Endpoints

The analyses of the exploratory endpoints will be performed on both ITT and PP populations unless stated otherwise.

- On the subset of patients that exacerbates and are treated with IV antibiotics (PEx subset population), the changes of select biomarkers in blood, serum and plasma (absolute neutrophil count, white blood cell count, lipidomics, inflammation markers, and oxidative stress markers), as well as FEV1, weight and BMI, from prior to the start of IV antibiotics to after the IV antibiotic treatment will be summarized by treatment group and analyzed using descriptive statistics. The mean changes will be compared between fenretinide and placebo using parametric and non-parametric statistics, where appropriate.
- The overall change in the PsA density in the sputum, from Baseline to Week 12 and Week 24, and the AUC of the CFU from Baseline to Week 24 will be the variables studied for this investigation. Data will be summarized by treatment group and analyzed using descriptive statistics. The mean changes and the AUC data will be compared between fenretinide and placebo using parametric statistics assuming the data meet standard pre-requisites for such analysis (normal or log-normal distribution and homogeneity of variance). Area under the curve from baseline to Week 24, will be calculated with a linear trapezoidal method.
- The overall change in lumbar spine bone mineral density, from Baseline to last follow-up on Week 28 will be summarized by treatment group and analyzed using descriptive statistics. The mean changes will be compared between fenretinide and placebo using parametric statistics assuming the data meet standard pre-requisites for such analysis (normal or log-normal distribution and homogeneity of variance).

3.17 Analysis of the Pharmacokinetic/Pharmacodynamics Endpoints

Pharmacodynamic Endpoints:

Values for AA, DHA and AA/DHA ratio as well as their change relative to baseline will be tabulated by time point and descriptive statistics will be used. The rate (frequency) of normalization (normalization meaning a parameter value reaching the mean parameter value observed in healthy controls± 1 Standard Deviation) for AA, DHA, and the AA/DHA ratio will be calculated for the end-of-treatment cycle values, and the treatments will be compared using two-sided multivariate logistic regression with the respective baseline value as a covariate. Alternative methods will be used if necessary due to the data distribution. Healthy controls will consist of a group of approximately 20 healthy, non-CF adult subjects of both genders, not specifically matched to the APPLAUD CF patients.

Values for pharmacodynamic parameters (AA, DHA and AA/DHA ratio), EPA, inflammation markers, metabolipidomic markers, systemic bone formation/resorption markers, markers of oxidative stress, ceramides), as measured in plasma (or serum, or blood, as required), as well as their change relative to baseline, will be tabulated by time point and descriptive statistics will be used. An analysis of variance will be used to compare treatments assuming datasets meet standard pre-requisites for such analysis (normal or log-normal distribution and homogeneity of variance). Alternative methods will be used if necessary.

Pharmacokinetic Endpoints:

Blood samples for fenretinide C_{min} analysis will be obtained from all patients randomized to fenretinide. Patients randomized to placebo will provide samples (to maintain the study blind) but will not contribute to the C_{min} data.

Plasma fenretinide C_{min} concentrations will be presented in a tabular summary, by sampling day and compared between groups of CF patients receiving fenretinide grouped according to the a-priori stratification factors, and assuming stratum size will allow such comparison.

3.18 Analysis of the Safety Endpoints

For the purpose of analyses and tabulations, AEs will be classified as pretreatment AEs, TEAEs, or posttreatment AEs. More specifically: Pretreatment AE are those that started before the first dose of study drug. TEAE are those that increased in severity or appeared at or after the first dose of study drug and before or at the last follow-up, planned for 4 weeks after the last dose of study

drug. Post-treatment AEs are those that increased in severity or that appeared after the last follow-up, planned for 4 weeks after the last dose of study drug; these AEs will be reported by the Investigator to the sponsor and the appropriate regulatory agency, as well as treated and followed according to local regulations. Post-treatment AE's will be reported separately from the study's clinical study report.

For AEs with missing or incomplete start dates, if there is no clear evidence that the AEs started before or after the first dose of study drug, then the AEs will be classified as TEAEs.

TEAEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the following information will be summarized:

- Overall TEAEs will also be described according to:
  - Seriousness (yes, no),
  - Severity (mild, moderate, severe, life-threatening),
  - Causality (definitely related, probably related, possibly related, not related),
  - Action taken with study drug (dose not changed, dose interrupted, drug withdrawn, not applicable),
  - Outcome (recovered / resolved, recovered / resolved with sequelae, not recovered / resolved, fatal, unknown).
- The total number of TEAEs, the total number and proportion of patients experiencing at least one TEAE during the treatment period will be summarized by system organ class (SOC) and preferred term (PT). TEAEs occurring with an incidence ≥ 10% will be tabulated in a separate table. To count the number of patients who experience each TEAE, patients experiencing the same TEAE multiple times will only be counted once for the corresponding PT. TEAEs will be tabulated presenting the SOCs alphabetically. All TEAEs, and TEAEs occurring with an incidence ≥ 10%, will be presented overall as well as stratified by causality (Definitely Related, Probably Related, Possibly Related, Not Related).
- AE summary tables will be presented for TEAEs only and will include the following:
  - All TEAEs;

- TEAEs by relationship;
- TEAEs by maximal severity;
- TEAEs leading to treatment discontinuation;
- Serious TEAEs;
- Fatal TEAEs.

All safety analyses will be performed on the Safety population.

Additional Safety Evaluations

The following safety evaluations, listed below, will be performed per the methodology stipulated in each of the respective sections provided:

- Clinically relevant changes from baseline in vital signs (for more details, please see Section 3.7);
- Clinically relevant changes from screening in 12-lead ECG (for more details, please see Section 3.7);
- Clinically relevant changes from baseline in physical examinations (for more details, please see Section 3.7);
- Clinically relevant changes from baseline in safety laboratory assessments (hematology with differential count, biochemistry, and urinalysis) (for more details, please see Section 3.8);
- Plasma retinol and retinol-binding protein values, changes from screening/baseline, correlated with ophthalmological symptoms, specifically symptoms of nyctalopia. More precisely, Spearman correlation between plasma retinol and retinol-binding protein concentrations (change-from-baseline to Week 24) and the cumulative number of AEs for "nyctalopia" will be presented in scatter plot figures along with the regression line. Nyctalopia will be identified according to AEs of the following SOC/PT/LLT: Eye disorders/ Night blindness/ Nyctalopia.
- Change in PEx incidence and antibiotic treatment requirement (for more details, please see Section 3.14).

3.19 Interim Analysis

The following specific times have been selected a priori for DMSB meetings:

- When a minimum of half of the enrolled patients have completed at least one dosing cycle and corresponding safety information is available, along with Day 21 plasma retinol and retinol binding protein concentration data. This will not be a full unblinding; it will be an unblinded safety analysis of Active versus Placebo;
- When a minimum of half of the enrolled patients have completed the first 3 dosing cycles and corresponding safety data is available for review. This will not be a full unblinding; it will be an unblinded safety analysis of Active versus Placebo;
- Outside of the above, every 6 months;
- Anytime as needed for review of clinically significant safety information brought to the attention of the DSMB.

For more information, please see the DMC Charter as well as the DSMB Mock Tables document.

4 LIST OF REFERENCES

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5 METHODOLOGICAL CHANGES FROM PROTOCOL

SAP Version (date)	Relevant Section in Protocol (Amendment 3 – dated: 31JUL2019)	Section (s) and Change (s) in SAP	Rationale for Change
Final v5.0 (dated: 08OCT2019)	Protocol Section 12.2 (Analysis Population): PEx Analysis Subset Population	SAP Section 3.3. (Analysis Population): 2 separate PEx Analysis Populations have been defined: 1) Protocol-Defined PEx Population (patients who experience a PEx with ≥ 4/12 positive Fuch symptoms and treated with an IV antibiotic); 2) Overall PEx Population (patients who experience a PEx treated with an oral or IV antibiotic). This differs from the Protocol, wherein only one PEx Population, defined by patients treated with an IV antibiotic, is defined	To distinguish between PEx defined by Investigators based on clinical judgment and PEx defined by a standard criteria.
Final v5.0 (dated: 08OCT2019)	Protocol Section 12.2 (Analysis Population)	SAP Section 3.3. (Analysis Population): Addition of a definition of the Healthy Control Population used for the first secondary parameter.	To define the Healthy Control Population to be used in the assessment of pharmacodynamic parameters (AA, DHA and AA/DHA ratio)
Final v5.0 (dated: 08OCT2019)	Protocol Section 6 (Study Objectives-Secondary Objectives); Protocol Section 7.1.3 (Secondary Endpoints)	SAP Section 3.15 (Analysis of Secondary Endpoints): assessment of Overall PEx has been added, in addition to the assessment of Protocol-Defined PEx	To distinguish between PEx defined by Investigators based on clinical judgment and PEx defined by a standard criteria.
Final v5.0 (dated: 08OCT2019)	Protocol Section 12.5.1 (Adverse Events), 12.5.2 (Clinical Laboratory Assessments)	SAP Section 3.18 (Analysis of the Safety Endpoints): removal of the methodology related to the Fisher's Exact test to assess between-group differences in treatment emergent and clinically significant AEs	This phase 2 trial was not designed to test specified hypotheses about safety. Therefore, the Fisher's exact test will not be performed. AEs will be presented descriptively, and a qualitative evaluation will be performed.
Final v6.0 (dated: 03SEP2021)		SAP Section 3.14 (Analysis of the primary efficacy endpoints): Success of the study will be based on the primary efficacy endpoint assessment in the ITT Population. The PP population, Protocol-defined PEx Population and Overall PEx Population will serve for confirmatory analyses	To clarify that the success of the study will be based on the primary analysis, not the primary and all the secondary analyses.

Final v6.0 (dated: 03SEP2021)	Protocol Section 12.4.1 (Analysis of primary endpoint)	SAP Section 3.14 (Analysis of the primary efficacy endpoints): the analysis of the primary efficacy endpoint will be repeated with the addition of the following covariates in the model: baseline use of Trikafta (yes versus no) and for the effect of the pandemic confinement (pre versus post pandemic onset)	To assess the impact on study results of the concomitant use of Trikafta and COVID-19 pandemic which occurred during the course of the trial.
Final v6.0 (dated: 03SEP2021)	Protocol Section 12.2 (Analysis Population)	SAP Section 3.14.1 (Subgroup Analyses): addition of subgroup analyses for baseline use of Trikafta (yes versus no) and for the effect of the pandemic confinement (pre versus post pandemic onset)	To assess the impact on study results of the concomitant use of Trikafta and COVID-19 pandemic which occurred during the course of the trial.

6 HISTORICAL CHANGE

SAP Version (Date)	Section(s) Changed	Reason(s) for change
Final v5.0 (dated: 08OCT2019)	N/A	New document
Final v6.0 (dated: 03SEP2021)	Section 3.14 Analysis of the Primary Efficacy Endpoints	Success of the study will be based on the primary efficacy endpoint assessment in the ITT population. The PP, Protocol-defined PEx and Overall PEx populations will serve for confirmatory analyses New model adding baseline use of Trikafta (yes versus no) and effect of pandemic confinement (pre versus post pandemic onset) as covariates
	Section 3.14.1 Subgroup Analyses	Addition of baseline use of Trikafta (yes versus no) and effect of pandemic confinement (pre versus post pandemic onset) subgroups.
	Section 7 Mock Tables	Update of mock tables and figures in order to include the new model and subgroup analyses for baseline use of Trikafta (yes versus no) and effect of pandemic confinement (pre versus post pandemic onset)