Laurent
PHARMACEUTICALS

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 A**d**ults (most recent version): 31Jul2019

NCT number: 03265288
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CLINICAL TRIAL PROTOCOL

LAURENT PHARMACEUTICALS INC.

PROTOCOL NO: LAU-14-01

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

Sponsor:	Laurent Pharmaceuticals Inc.
Sponsor address:	[redacted] Montréal (Québec) [redacted] Canada
Protocol Date:	Amendment No 3, July 31st, 2019
US IND #:	130312

Confidentiality Statement

Signature Page for Sponsor:

Product: LAU-7b
Study No. LAU-14-01

Title: APPLAUD: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, PHASE II STUDY OF THE EFFICACY AND SAFETY OF LAU-7B IN THE TREATMENT OF CYSTIC FIBROSIS IN ADULTS

Approved by the following:

_____ _____
Larry Lands, MD, PhD — Date
Chief Medical Adviser
Laurent Pharmaceuticals Inc.
[redacted]

_____ _____
Jean-Marie Houle, B.Sc.Pharm., M.Sc., PhD — Date
Vice President of Clinical Development,
Laurent Pharmaceuticals Inc.
and Study Director
[redacted]

_____ _____
Emmanouil Rampakakis, Ph.D. — Date
Study Statistician
JSS Medical Research
[redacted]

Signature Page for Investigator:

Product: LAU-7b
Study No. LAU-14-01

Title: APPLAUD: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, PHASE II STUDY OF THE EFFICACY AND SAFETY OF LAU-7B IN THE TREATMENT OF CYSTIC FIBROSIS IN ADULTS

I have read this protocol and agree to conduct this trial in accordance with all stipulations of the protocol and in accordance with all relevant local regulations, the current International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice (GCP), and with the principles of the most recent version of the Declaration of Helsinki.

Signed:

_____	_____	_____
Principal Investigator Name (print in block capital letters)	Signature	Date

_____
Institution Name

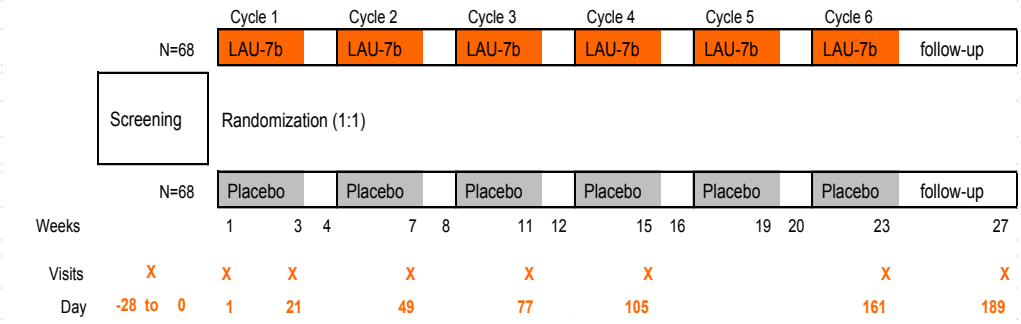
_____	_____
City and Province/State + Postcode/Zipcode	Country

1 SYNOPSIS

Note:	This synopsis does not contain all details and therefore cannot be used as a guide for operational conduct of the 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
Investigational Product	LAU-7b (fenretinide) oral capsules
Sponsor	Laurent Pharmaceuticals Inc.
Study Objectives	**Primary objectives:** 1- To assess the safety and tolerability of LAU-7b in Cystic Fibrosis (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 the Forced Expiratory Volume in 1 second (FEV_1) percent predicted, relative to placebo-treated patients. **Secondary objectives:** 1- To assess the normalizing effect of LAU-7b on the key lipidomic markers in plasma phospholipids, such as arachidonic acid (AA), docosahexaenoic acid (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 Pulmonary Exacerbations (PEx) and incidence of protocol-defined PEx, change in weight and Body Mass Index (BMI), usage of new antibiotics, and the change in CF Questionnaire Revised (CFQ-R); 3- To evaluate the change in selected systemic inflammation markers; 4- To determine if LAU-7b has an effect on lung colonization with *Pseudomonas aeruginosa (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, inflammation markers and FEV_1 between the start and the end of IV antibiotic treatment for a PEx (performed only in patients who receive IV antibiotics for what their treating physician considers a PEx); and 7- To explore clinical scoring using the Matouk Disease Score[i].
Study Rationale	LAU-7b is a novel oral formulation of fenretinide developed as a first-in-class, once-a-day, oral therapy with potential to be a master regulator of membrane lipid composition (essential fatty acids and sphingolipids) playing a dual role in i) the resolution of inflammation, and ii) stabilization of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) in the epithelial apical membrane during inflammatory stress. Patients with CF have increased AA and decreased DHA levels, and the abnormal metabolism of these lipids results in disruption in homeostasis of AA- and DHA-derived mediators eicosanoids and docosanoids, respectively, which are profoundly involved in the modulation of the inflammation process. LAU-7b was shown to trigger pro-resolving DHA pathway endogenously, a new therapeutic approach using the body's own ability to modulate inflammation without inducing immunosuppression. An imbalance is also

[i] Matouk Disease Score is a clinical score created to assess the severity of patient symptoms in a more longitudinal way in order to overcome the limitations of the current evaluation of the lung function. This score is a modified Huang Disease Score, with additional assessments to include important complications that may impact the disease (such as the number of pulmonary exacerbations in the past 6 months).

Study Rationale (cont'd)	observed in certain sphingolipids, which are key components of membrane self-defense mechanism. More recently, LAU-7b was shown to enhance the partitioning of CFTR into sphingolipid-rich microdomains during cellular stress, an effect that resulted in increased CFTR function. LAU-7b was recently tested in a dose-ascending Phase 1b clinical study in adult CF patients, showing good safety and tolerability, vastly improved pharmacokinetics at doses up to 300mg, and promising pharmacodynamic results.
Study Endpoints	**Primary Endpoints:** • 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, LAU-7b compared to placebo treated patients. **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 percent 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 difference in the 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. **Exploratory Endpoints:** • The change from pre-study in AA, DHA and eicosapentaenoic acid (EPA) in phospholipids and metabolipidomic markers (eicosanoids and docosanoids), LAU-7b compared to placebo;

Study Endpoints (cont'd)	• The change from pre-study in plasma oxidative stress markers (malondialdehyde, nitrotyrosine), LAU-7b compared to placebo; • The changes from pre-study 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.
Study Design Overview	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. A minimum of 136 patients will be recruited and randomized for the treatment phase of the study with the expectation that at least 120 patients complete the study as per protocol. Eligible patients will be randomized in a 1:1 double-blinded fashion to either LAU-7b (active) group or placebo (control) group, after stratification for 1- baseline FEV_1, 2- PEx number in prior year, and 3- Co-administration or not of Kalydeco® (ivacaftor), Orkambi® (ivacaftor/lumacaftor), Symdeko® (ivacaftor/tezacaftor) or another commercially available CFTR modulator product. Patients will be enrolled at approximately 35-40 centers in the United States of America, Canada and Australia, possibly in Europe. 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. A Data and Safety Monitoring Board (DSMB) will be created for this study. 
Study Population	**Study Inclusion Criteria:** • Signed Informed Consent; • Adult men and women (as per State or Province laws) and 18 years and older; • 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;

Study Population (cont'd)	• 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: • History of pulmonary exacerbations, defined as at least one (1) pulmonary exacerbation in the year prior to Screening which resulted in documented IV or oral antibiotics; • 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; • 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; • 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; • All patients, whether prescribed pancreatic enzyme replacement therapy or not, are eligible. However, modifications of the dosing regime during the study should be avoided; • 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. Allowed concomitant diseases and treatment are listed in specific section; • 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; • 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; • Patients deemed capable of adequate compliance including attending scheduled visits for the duration of the study; • The patient must be able to swallow the study drug capsules. **Study Exclusion Criteria:** • Pregnancy: due to the potential teratogenic effects of retinoids, pregnant women are NOT eligible; • Breast milk feeding by study patient is NOT allowed; • 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;

Study Population (cont'd)	• Clinically abnormal renal function: serum creatinine > 132 µM (>1.5 mg/dL); • 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; • Patients with plasma retinol levels below 0.7 µM; • 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 to be used in the study; • History of organ transplantation; • History of alcoholism or drug abuse within 2 years before screening; • 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; • Presence of nyctalopia (also called night-blindness, a condition making it difficult or impossible 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, xerophtalmia, uncontrolled glaucoma); • Presence of serious dermatological conditions at entry, including inflammatory or xerotic skin pathologies such as psoriasis or ichthyosis; • 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); • 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; • 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); • Patients with a confirmed diagnosis (as per the CFF diagnostic criteria) of Allergic Bronchopulmonary Aspergillosis "ABPA" and actively being treated with corticosteroids and/or antifungal agents; • 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; • 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.
Dose and Mode of Administration	• Three (3) capsules of LAU-7b (300 mg total dose) or matching placebo (randomization 1:1) will be administered in the fed state with the first meal of the day (morning if possible) for 6 consecutive cycles consisting of 21 days on treatment followed by a 7-day treatment-free period. • Study treatment will be administered on top of current Standard of Care therapies.
Statistical Analysis	**Sample size:** A minimum of 136 CF patients will be randomized in this study. Sample size calculation is based on the primary efficacy variable, FEV_1 % predicted (absolute change) and on the following assumptions:

	• A t-test of the difference of the mean absolute change in FEV_1 of each group (active versus placebo) will be used; • The FEV_1 (absolute change) data is normally distributed in each treatment group; • The FEV_1 variance, expressed as the Standard Deviation (SD) is 8%; • The FEV_1 clinical difference of interest (effect size) is 4%; • The minimum statistical power of the t-test is 80% (0.8); 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). **Primary Efficacy Endpoint Analysis:** In the analysis for the primary efficacy endpoint, change from baseline in FEV_1 % predicted (absolute change), including all measurements up to Week 28 (both during and after treatment measurements, including after treatment discontinuation) will be analyzed based on a mixed-effect repeated-measure model. Even though no imputations will be used in the primary analysis, a sensitivity analysis will be conducted where missing data points will be imputed using appropriate interpolation methods; extrapolation methods will be used for truncated FEV_1 % predicted values. The model will include the absolute change from baseline in FEV_1 % predicted as the dependent variable; treatment, visit, and treatment-by-visit interaction as fixed effects; and patient as a random effect with adjustments for a) FEV_1 % predicted determined at the Screening/Baseline Visits (average where possible), b) PEx number in the prior year, and c) Co-administration or not of Kalydeco® (ivacaftor), Orkambi® (ivacaftor/lumacaftor), Symdeko® (ivacaftor/tezacaftor) or another commercially available CFTR modulator product. 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 statistical analyses for the other study parameters are summarized in the Statistical Analysis, Section 12, and will be detailed in the SAP.

2 SCHEDULE OF EVENTS

	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		

	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**
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 C_{min}: 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.

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3 GLOSSARY OF TERMS AND ABBREVIATIONS

AA: Arachidonic Acid
AE: Adverse Event
ALT: Alanine Aminotransferase
AMD: Age Related Macular Degeneration
AST: Aspartate Aminotransferase
ATRA: All-Trans Retinoic Acid
AUC: Area Under the Curve
BID: *Bis In Die* (Latin), twice daily
BHA: Butylated Hydroxyanisole
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
ERK1/2: Extracellular Signal-Regulated Kinase 1/2
FEF: Forced Expiratory Flow
FEV_1: 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

IP: Intra-Peritoneal
IRB: Institutional Review Board
ITT : Intent-to-Treat
IV : Intravenous
IWRS: Interactive Web Response System
K_2EDTA: Di-Potassium Ethylenediaminetetraacetic Acid
KO : Knock-Out
LDL: Low Density Lipoprotein
MDA: Malondialdehyde
MedDRA: Medical Dictionary for Regulatory Activities
MTD: Maximally Tolerated Dose
MUHC: McGill University Health Centre
NEAC: Neutrophil Elastase Antiprotease Complexes
NF-κB: Nuclear Factor-kappaB
NT3: Nitrotyrosine
NVQ: Night Vision Questionnaire
PD: Pharmacodynamic
PEx: Pulmonary Exacerbation
PI: Principal Investigator
PK: Pharmacokinetic
PO: *per os* (Latin), by mouth, orally
PP: Per Protocol
PPAR: Peroxisome Proliferator Activating Receptor
PsA: *Pseudomonas aeruginosa*
QD: *Quaque Die* (Latin), every day/daily
QTc: QT corrected for heart rate
RAR: Retinoic Acid Receptor
RBC: Red Blood Cells
RBP: Retinol Binding Protein
RXR: Retinoid-X-Receptor
SAA: Serum Amyloid A
SAE: Serious Adverse Event
SAP: Statistical Analysis Plan
SC: Subcutaneous
SD: Standard Deviation
SUSAR: Suspected Unexpected Serious Adverse Reactions
TEAE/TEAe: Treatment Emergent Adverse Event
TID: *Ter In Die* (Latin), three times daily
TMF: Trial Master File
TNF-α: Tissue Necrosis Factor Alpha
VLCC: Very Long Chain Ceramides

4 STUDY PERSONNEL

Sponsor:
Laurent Pharmaceuticals Inc.
[redacted]
Montreal, Quebec
[redacted]

Larry Lands, MD, Ph.D.,
Chief Medical Adviser
[redacted]

Jean-Marie Houle, B.Sc.Pharm., M.Sc., Ph.D.
Vice President of Clinical Development
and Study Director
[redacted]

Monitors:
The sponsor has contracted with a Contract Research Organization (CRO) to help manage the trial. Specific Contact information for the below CRO contact and each study monitor is maintained in the study monitoring plan and on file at each investigator's site.

CRO Senior Contact:
JSS Project Manager
JSS Medical Research
[redacted]

Manufacturer:
Corealis Pharma
[redacted]
Laval, Quebec
[redacted]

Serious Adverse Event (SAE) Reporting:
Dr Homaira Moqadar, Pharmacovigilance Lead
JSS Medical Research
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5 INTRODUCTION

5.1 Investigational product

LAU-7b is a solid oral dosage form of fenretinide (100mg capsules). Fenretinide, a small molecule synthetic retinoid derivative, is an investigational drug (new chemical entity) with well-documented history of safety in non-clinical and clinical studies ([redacted] US National Cancer Institute (NCI) since 1992). The active product ingredient is N-(4-hydroxyphenyl) retinamide, also referred to as 4-HPR or FRD, and is a synthetic amide of all-trans retinoic acid with a molecular formula $C_{26}H_{33}NO_2$. An earlier oral formulation of fenretinide (corn oil-based 100mg softgel) has been extensively studied in clinical studies, mostly for the prevention and treatment of cancer. Fenretinide has not yet been commercialized in any country.

LAU-7b is a novel and improved oral formulation of fenretinide. [redacted]

An orange opaque, size 00 hard gelatin capsule without active substance will be used as a control and matching placebo for blinding purposes and contains the same non-medicinal ingredients.

5.1.1 Rationale for LAU-7b, a novel and improved oral formulation of fenretinide

Fenretinide is poorly absorbed following oral administration, and it suffers from a wide inter-patient variation in bioavailability when delivered orally in an oily vehicle, such as the softgel capsules used in most oncology trials, which contains fenretinide suspended in corn oil. The poor bioavailability and the large size of the softgel capsules was a limiting factor in the clinical studies conducted so far, especially in pediatric population.

In the past, fenretinide has also been formulated in a lipid matrix, Lym-X-Sorb (LXS) [1], containing 2% of fenretinide and administrated as an oral powder delivered in non-milk fat-containing foods, and especially as a slurry in non-milk fat-containing, or soy-based nutritional supplements. However, this formulation has been shown to be associated with significant gastrointestinal side-effects, especially at higher doses[2], as well as to significant patient withdrawal due to the taste and texture of the medication.

There was thus a need for new pharmaceutical dosage form of fenretinide, especially for oral administration, capable of overcoming the poor oral bioavailability of corn-oil based formulation and the fair patient compliance. Dosage forms such as hard gelatin capsules, tablets, caplets, suspensions, or powders for suspensions were to be considered.

Laurent Pharmaceuticals has developed a novel oral dosage form of fenretinide with increased oral bioavailability and formulated as a powder that can be encapsulated or compressed in various sizes, thus with expected better compliance for both adult and pediatric patient populations, more specifically by reducing the size/number of unit dosage forms to ingest. [redacted] was successfully tested by Laurent Pharmaceuticals in a Phase 1b study involving adult CF patients [redacted]

5.2 Cystic Fibrosis Overview

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 [4, 5]. 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 [6]. 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 [7]. 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.

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 [8, 9, 10, 11]. 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 [12].

AA and DHA are essential fatty acids that cannot be synthesized by humans, and are derived from nutritional intake. Their metabolism is the source for the synthesis of eicosanoids (AA-derived, such as prostaglandins, leukotrienes and lipoxins), and docosanoids (DHA-derived, such as resolvins and protectins). Eicosanoids and docosanoids are key inflammatory mediators involved in the modulation of the innate immune-inflammatory response following bacterial challenge or other type of injury. Thus, the homeostasis of eicosanoids and docosanoids is crucial in maintaining an efficient and balanced immune-inflammatory response.

The high AA levels displayed in CF patients may increase the pro-inflammatory factors and stimulate mucus secretion, while the low DHA levels, important in the anti-inflammatory defense and the resolution of inflammation, may explain why CF patients have an exaggerated and unresolved inflammatory response that, paradoxically, is unable to fight opportunistic infections [13, 14, 15]. 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 [16, 17].

5.3 Treatment of Cystic Fibrosis

Despite the significant advances made in CF research in the recent years, Cystic Fibrosis remains a complex and heterogeneous genetic disorder with a large unmet medical need. There has been progress made on several fronts hence the longer (and still lengthening) life expectancy of CF patients. There is no permanent cure available for CF, and the current drug therapies goals are to reduce the symptoms of disease, such as preventing progression of lung infection by removal of the mucus plugging from the lungs, controlling the infection and inflammation, controlling the nutrition level, and ultimately increasing survival. Gene therapy, which means adding a normal copy of the *CFTR* gene, was unsuccessful to date because of the challenges in finding a good delivery vehicle that can bring the gene to the targeted organs.

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® (ivacaftor/tezacaftor), 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. The R&D effort continues in order to develop better generations of *CFTR*-modulators, targeting more mutations and re-establishing more of the defective protein function. This is exemplified by the recent discovery that it is possible to potentiate the function of *CTFR* by targeting key membrane lipids (essential fatty acids and sphingolipids).

Although considerable progress has been made in understanding the pathogenesis of CF, the connection between *CFTR* dysfunction and the chronic inflammation is still not completely understood, especially, being the least addressed component of CF airway disease at this time. Current anti-inflammatory treatments (high doses of ibuprofen and corticosteroids) have limited effectiveness in improving the symptoms of inflammation and the side effects associated with these drugs are severe thus they are rarely prescribed. Nevertheless, whether they are currently available or in development, anti-inflammatory, mucus regulation, anti-infective and nutritional therapeutic approaches only improve the quality of life of the patients and seek to ameliorate symptoms that are later stage manifestations of the disease.

It is in this context [redacted] 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. Furthermore, through recent advances, LAU-7b is also targeting the stabilization of CFTR in the epithelial apical membrane during inflammatory stress, thus potentially increasing CFTR function.

Figure 1: Pathogenesis of lung disease in CF and therapeutics targets

5.4 Study Rationale

Notwithstanding the significant advances made in CF research, the mechanism by which the mutation in the *CFTR* gene leads to the compromised immune-inflammatory response remains unclear. Although the dysfunction of the *CFTR*-protein explains the mechanisms leading to mucus plugging and the defective mucociliary clearance in the lungs, which creates a favorable environment for infection, it does not entirely explain the predisposition for exaggerated inflammatory response even in the absence of detectable infection. It is neither fully understood why the inflammation remains neutrophilic and does not resolve, nor the inability of the innate immune system to manage lung infections, especially when opportunistic pathogens are involved.

Indeed, a recent study involving Kalydeco® in G551D homozygote patients showed that the *CFTR*-modulation improves FEV_1, reduces exacerbations, improves Quality of Life and reduces *PsA* colonization, but does not impact the inflammation markers [18]. Moreover, *CFTR*-modulators only address specific CF phenotypes and they are not expected to completely reverse the existing organ damage, and patients still have exaggerated but ineffective airway inflammation failing to eradicate pulmonary opportunistic pathogens.

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 [19]. This is likely due to the fact that inhibition of inflammation can also inhibit the ability of patient' immune system to fight infections [20].

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 [19, 20]. 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 targeting the AA/DHA imbalance is emerging as a new treatment paradigm, with potential to modulate both "on" and "off" inflammatory signaling and re-establish the lung homeostasis in a disease-modifying manner. Likewise, more recent evidences show that the stabilization of membrane sphingolipid self-protection mechanism [21] can have effects on CFTR protein insertion and stability in the airway epithelial apical membrane, thus confirming the important link between inflammation and the basic defect in CF.

5.4.1 Inflammation and fatty acids metabolism in CF

Alterations in the metabolism of key fatty acids are known for decades as one of the hallmarks of CF and have been validated in patients and in multiple animal models of disease. CF patients have increased levels of AA and decreased DHA. AA and DHA are considered essential fatty acids because they cannot be synthesized by humans, and are received from nutrition. Their metabolism is the source for the synthesis of eicosanoids (AA-derived, such as prostaglandins, leukotrienes and lipoxins), and docosanoids (DHA-derived, such as resolvins and protectins). Eicosanoids and docosanoids are key inflammatory mediators involved in the modulation of the innate immune-inflammatory response following bacterial challenge or other type of injury.

The homeostasis of eicosanoids and docosanoids is crucial in maintaining an efficient and balanced immune-inflammatory response. The high AA levels displayed in CF patients may increase the pro-inflammatory factors and stimulate mucus secretion, while the low DHA levels, important in the anti-inflammatory defense and the resolution of inflammation, may explain why CF patients have an exaggerated and unresolved inflammatory response that, paradoxically, is unable to fight opportunistic infections such as *PsA*.

The abnormal AA/DHA metabolism was initially believed to result either from notorious bacterial infections occurring the lungs of CF patients, or being a consequence of malabsorption, hepatic dysfunction or secondary to infection. The intense basic research by several laboratories, have demonstrated that imbalance of AA and DHA represents a primary effects in CF, linked with CFTR gene deficiency and not secondary to other disease manifestations [9, 10, 11, 12, 13, 22, 23, 24]. The abnormal fatty acids metabolism observed in CF patients has major impact on the cellular and intracellular phospholipid membranes. They are important regulators of signaling channels, protein function, permeability, caveolae building and are involved in the regulation of several genes expression [9]. Lipid imbalance can be observed even in newborn mice with ablated CFTR gene, which are kept in pathogen free conditions [12, 25, 26]. Furthermore, a correlation was shown between the severity of CF lung disease and lipid deregulation [10, 13]. Interestingly, the lipid imbalance "signature" does not appear to be related to the type of CFTR mutation, *thus holding the promise of a therapy that could benefit all CF patients.*

In response to injury or infection, macrophages and epithelial cells secrete chemokines and cytokines, which stimulate AA release from membrane lipids, leading to production of pro- inflammatory eicosanoids (such as leukotrienes and prostaglandins). These pro-inflammatory mediators further stimulate mucus secretion by goblet cells and promote acute tissue inflammation, with neutrophil accumulation in infected/damaged sites to neutralize and eliminate potentially injurious stimuli. Once the stimuli are neutralized, the inflammation enters the resolution phase, where apoptotic neutrophils undergo surface changes enabling phagocytes to

recognize and ingest them [15, 27]. Resolution of inflammation not only depends on the removal of apoptotic cells but also on suppression of pro-inflammatory mediators' production, as well as the active involvement of anti-inflammatory and pro-resolving mediators generated from AA (such as lipoxins) and from DHA precursors (such as resolvins and protectins). The timely resolution of inflammation is as important as the initiation phase and a good balance between pro- inflammatory and anti-inflammatory (and pro-resolving) mediators is key to maintaining an efficient and harmless inflammatory response. Incomplete resolution leads to chronic inflammation and destruction of lung tissue, and ultimately to lung insufficiency and impairment [15, 27].

Inflammation signaling in the CF lung is a reflection of an imbalance between the initiation and resolution of the inflammatory response, with pro-inflammatory cytokines and mediators being over-expressed, while anti-inflammatory and pro-resolving mediators are suppressed (Figure 2). When counter-regulatory controls are abnormal, an imbalance occurs, resulting in prolonged and excessive inflammatory mediator production, fueling the destructive inflammatory cascade.

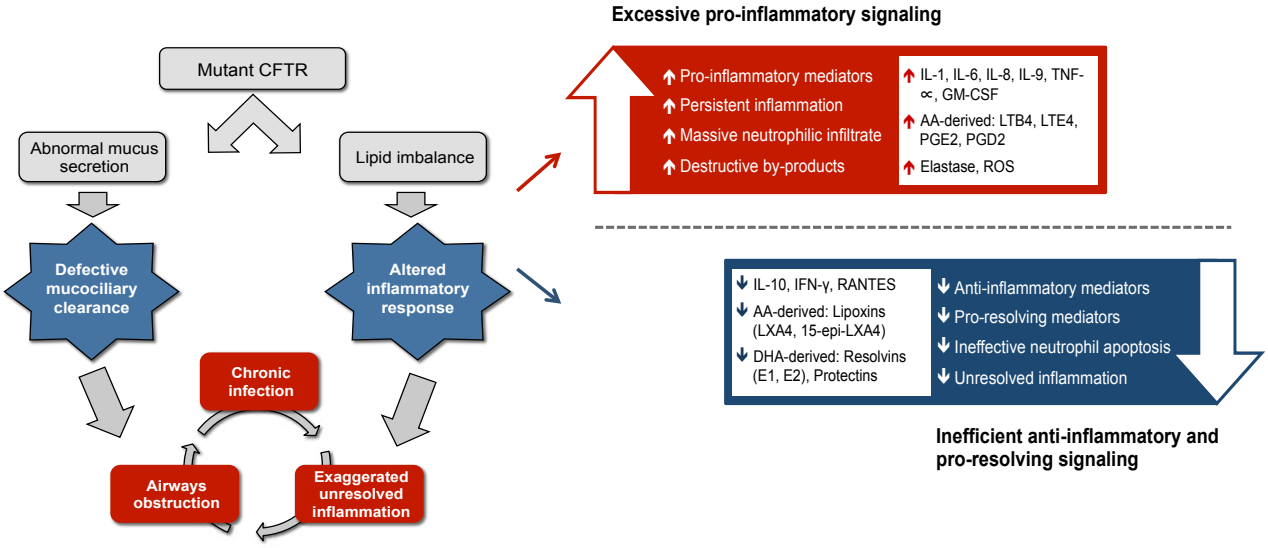

Figure 2: Imbalance in inflammatory signalling in CF lung

It is unlikely that a single defect in one pathway accounts for the entirety of the exaggerated inflammatory response. Airway surface fluid from CF patients contains large concentrations of pro-inflammatory mediators including the tissue necrosis factor alpha (TNF-α), IL-1β, IL-6, IL-8, IL-17, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [28]. The synthesis of these mediators is promoted by a few transcription factors including AP-1, nuclear factor (NF)-κB, and mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinase (ERK1/2). In addition to a heightened pro-inflammatory arm, there appears to be inappropriately decreased counter-regulatory pathways, particularly those involving IL-10 and nitric oxide.

Another mechanism of inhibiting NF-kB activity occurs via up-regulation of peroxisome proliferator activating receptor (PPAR). CF tissues appear to be deficient in PPAR [29, 30, 31, 32], leading to an imbalance between IκB and NF-κB and favors increased inflammation. Activation of PPAR may quell the CF inflammatory response. Interestingly, abnormalities in the fatty acid content of CF cells, with deficiencies of both DHA and linoleic acid, may contribute to impaired PPAR signaling. Indeed, the pro-inflammatory response and altered fatty acid metabolism in CF are linked to decreased expression of PPAR in epithelial cells and macrophages and corrected by DHA treatment in CFTR mice model [33, 34].

Multiple pathways are upregulated or down-regulated in response to the cell's attempt to correct the underlying physiological abnormality due to abnormal CFTR. The end result is that the delicate balance between the pro- and anti-inflammatory arms is disrupted, and pathways promoting the activation and perpetuation of the inflammatory response are favored.

Inhibiting the exaggerated pro-inflammatory signaling while augmenting the anti-inflammatory and pro-resolving signaling is a desired but never achieved approach to address the abnormal immune-inflammatory response in CF. The documented AA/DHA defective metabolism, resulting in an abundance and persistence of pro-inflammatory lipid mediators, together with a defective generation of anti-inflammatory and pro-resolving lipid mediators in the airways of CF patients, may be the link between the CFTR gene defect and the compromised immune-inflammatory response, thus emerging as an important therapeutic target.

Thus, AA/DHA modulation could address the aberrant immune-inflammatory response in a disease-modifying manner, with effect on the inflammation-infection vicious cycle, as showed by Dr. Radzioch's team at McGill University in the next section.

5.4.2 Fatty acids metabolism and fenretinide; Preclinical Evidence and Proposed Mechanism of Action

5.4.2.1 Preclinical Evidences

Evidence of early upregulation of inflammation and lipid imbalance was also shown in *Cftr*.KO (Knock-Out) mice by Dr. Radzioch team. This team, in collaboration with Dr. Lap-Chee Tsui team at University of Toronto, developed a specific mouse model (B6-Cftr.KO) that has most of the characteristics of human CF lung disease: inflammatory cell infiltration and mucus accumulation, ineffective mucociliary transport, interstitial basement membrane thickening and chronic inflammatory cell recruitment leading to progressive lung impairment, *even when they were not exposed to any lung pathogens* [25, 26, 35]. Based on these evidences it is postulated that inflammation in CF has two components:

- **Intrinsic inflammation**: An innate over-expression of pro-inflammatory genes related to the *CFTR* gene defect, resulting in an imbalanced and over-reactive inflammatory response that fails to defend against infection with opportunistic bacteria such as *PsA*. This effect is measurable by the presence of increased pro-inflammatory factors and AA levels, and decreased anti-inflammatory factors and DHA levels, known to be a hallmark of CF disease in patients, regardless of their type of mutation.
- **Secondary inflammation**: Triggered by the chronic lung infection, thus indirectly affecting the CFTR protein function and subsequent defective mucociliary clearance.

Using B6-Cftr.KO animal models, the McGill team demonstrated that oral treatment with fenretinide was able to increase the levels of DHA in *CFTR*-knockout mice, and it brought the levels of AA down to the levels observed in wild-type mice. Normalization of AA/DHA ratios translated into decreased expression of inflammatory genes normally overexpressed in *CFTR*-KO mice (IL-1β and S100A8) and also resulted in a dramatic improvement in fighting pulmonary infections of *PsA*. Results also showed that fenretinide caused a significant and rapid corrective effect on the AA/DHA imbalance in the liver, ileum and pancreas of *CFTR*-KO mice [24].

In the same vein fenretinide normalized the levels of a few species of ceramides, which levels correlated with the AA/DHA imbalance, an observation made in both the CF mouse model and CF patients, providing more insights on the importance of lipid homeostasis in CF [36].

Very relevant to CF is the recent discovery using the CFTR channel functional model (Ussing chamber) on human airway epithelial cells from CF donors under bacterial stress. Fenretinide has the ability to increase

CFTR protein recruitment, aggregation and confinement inside the lipid microdomains (rafts), and to enhance the formation of CFTR-containing platforms in response to cellular stress during inflammation. This effect is due to the rebalancing of very long chain ceramide levels by fenretinide in the cells membrane. This functional effect on CFTR is enhanced when cells are stressed with *PsA*, then treated with fenretinide. This effect is further enhanced in the presence of CFTR correction by lumacaftor (VX-809) [21, 37].

A study by the same group at McGill characterized the protective effect of fenretinide on the early onset of osteoporosis in *CFTR*-knockout mice [38]. Reduced bone mineral density, resulting in osteopenia and osteoporosis, is a common phenotype associated with CF. Treatment with fenretinide dramatically increased trabecular bone volume compared to controls in animal models, an effect correlated to down-regulation of phospholipid-bound AA in the *CFTR*-deficient mice.

In mouse model of spinal cord injury, fenretinide treatment resulted in significant enhancement of locomotor recovery and greater protection of spinal motor neuron fibers compared to untreated mice [39]. Fenretinide treatment lead to a reduction of AA and increase of DHA levels in the plasma and spinal cord as well as a reduction in the expression of pro-inflammatory mediators, such as TNF-α and iNOS, and attenuation of oxidative stress after the contusion injury of spinal cord. Fenretinide was also found to be an effective agent targeting inflammation, oxidation, and lung pathology observed in a mouse model of allergic asthma [40].

5.4.2.2 Proposed Mechanism of Action

Fenretinide is not a typical retinoid; it does not have a terminal carboxyl group believed to be an essential feature for active retinoids. However, it has been shown to have certain biological activities associated with the retinoid class. Fenretinide is a potent transactivator of retinoid acid receptor (RAR)γ and a moderate activator of RARβ, but is not an activator of RARα and retinoid-X-receptor (RXR)α [41].

Fenretinide corrects AA/DHA imbalance by a mode of action addressing the complex links between lipid metabolism and inflammatory signaling, which is distinct from the retinoid class mechanism of action. Although the complete mechanism of action in CF is not fully elucidated, it was shown that fenretinide corrects the AA/DHA imbalance and inhibits macrophage inflammatory mediators via the ERK 1/2 pathway [42]. Fenretinide was also shown to inhibit the activation of the pro-inflammatory transcriptional NF-κB [43], as well as inhibit the downregulation of PPARγ, which is known to have a role in lipid metabolism [44]. The pro-inflammatory response and altered fatty acid metabolism in CF are linked to decreased expression of PPARγ in epithelial cells and PPARα in macrophages. More recent evidences demonstrated fenretinide's ability to modulate ceramide biosynthesis by reducing the dihydroceramide desaturase (Des1) expression [45] and to increase the function of CFTR protein through its activity on the lipid microdomains (rafts) in the cell membrane. [redacted].

Figure 3: [redacted]

All three targets, ERK 1/2, NF-κB and PPARγ are known to be important components of the pro-inflammatory pathways in the CF lung, and are all influenced by fenretinide. The anti-inflammatory and pro-apoptotic role via modulation of ceramides is still in exploration, but there are evidences suggesting that their modulation is also important in balancing the pro- and anti-inflammatory responses, in neutrophils apoptotic mechanism, as well as in the internalization of *PsA* bacteria [24, 36, 46]. Dr. Radzioch group also shown that fenretinide is able to reduce the markers of oxidation, potentially protecting DHA from peroxidation [39], which is particularly important in periods of intense oxidative stress such as pulmonary exacerbations.

[redacted] unpublished data). The effect of fenretinide on [redacted] desaturases was already described in literature: modulation of ceramide biosynthesis by reducing the expression of dihydroceramide desaturase (Δ4-desaturase) [45], and inhibition of stearoyl-CoA desaturase (Δ9-desaturase) in retinal cell lines[47]. The protection of the DHA from peroxidation during the constant inflammatory state in the lungs would indirectly decrease the AA, as DHA is a natural down-regulator of AA. Overall, fenretinide appears to affect the inflammation process at multiple levels, involving both pro- and anti-inflammatory pathways, thus confirming a multi-target effect usually seen with disease-modifying drugs.

Of particular relevance to the current treatment trends in CF is the modulating effect of fenretinide on specific ceramides, down-regulating long chain ceramides and up-regulating very long chain ceramides (VLCC) through ceramide synthase 5 (Cers 5). VLCC are sphingolipids constituents of the lipid rafts at the surface of cell membrane and are essential to stabilize the CFTR protein [21]. These results in improved trafficking and expression of corrected CFTR [37]. While CFTR protein function can be improved by CFTR correctors (e.g. VX-809, lumacaftor), the CFTR protein still needs structured lipid rafts in order to migrate to the cell surface and express itself. Therefore, fenretinide could have a dual effect on CFTR: i) increasing CFTR trafficking and its functional expression at the surface of cell membrane by improving composition of the lipid rafts, and ii) protecting lipid rafts from oxidative depletion in an environment of constant inflammation.

Thus, an efficacious lipid modulator can potentially address the inflammation-infection vicious cycle by acting upstream on the immune-inflammatory pathway, and being complementary to the mucociliary correction provided by the *CFTR*-modulators. If the benefits seen in *CFTR*-KO mice are duplicated in

humans, this would represent a new paradigm in the treatment of CF, addressing the entire CF population, irrespective of their type of mutation. Fenretinide has the potential of being one of the few therapies that could prevent early colonization in younger patients by improving the host response, protect from deterioration the lungs in chronic patients by promoting a pro-resolving effect on inflammation. Moreover, since the lipid imbalance is present in all patients and is targeting an alternative pathogenic pathway, this drug would not compete but rather complement all the other therapies, symptomatic or disease modifying. Furthermore, by its effect on CFTR lipids raft and trafficking LAU-7b can also have a potentiating effect on CFTR modulators.

[redacted]

Figure 4: Potential benefits of LAU-7b [redacted]

5.4.3 Supportive nonclinical experience with fenretinide

5.4.3.1 Mechanism of Action in Cancer and other Indications

Fenretinide appears to act by different pathways than those mediated by retinoid receptors. Studies in breast cancer cell lines have demonstrated that fenretinide has a low binding affinity for nuclear retinoic acid receptors (RARs) compared with trans-retinoic acid. Despite the similarities with other retinoids, comparative studies with the parent compound ATRA (all-trans-retinoic acid) suggest that fenretinide mediates its key apoptotic and anti-tumor cytotoxic activity through a novel mechanism, not completely understood [48, 49, 50, 51], potentially involving modulation of saturated and unsaturated ceramides in cell lines from various tumors. Furthermore, evidence shows that another of the drug's molecular targets is mammalian Target Of Rapamycin "mTOR", which is a central control of cell growth and division. The differential responsiveness of numerous cell lines, confirms that fenretinide has a mechanism of action distinct from the retinoid receptor pathway.

Fenretinide was also shown to form complexes with the retinol binding protein (RBP)4, reducing serum concentrations of retinol (vitamin A), which is potentially associated with the reversible delayed dark adaptation (nyctalopia, also called night blindness) in some patients. However, the effect on RBP4 is also a potential disease-modifier in dry age-related macular degeneration (AMD), an unaddressed chronic eye disease that causes vision loss in the center of the field of vision. Reducing the delivery of RBP4-retinol to the retinal pigment epithelium (RPE) was postulated to reduce the accumulation of the toxic metabolite retinylidene-N-retinylethanolamine "A2E" thus slowing geographic atrophy (GA) lesion growth. A two-year

Phase IIb proof-of-concept trial with FRD in dry AMD reported a trend for reduced GA lesion growth rates in patients who achieved serum levels of ≤1 μM [52].

Other groups showed that fenretinide inhibits obesity and insulin resistance in mice and is in early clinical trials for treatment of insulin resistance in obese humans. In another study, long-term fenretinide treatment prevents high-fat diet-induced obesity, insulin resistance, and hepatic steatosis. The mechanisms of action involved are the RBP and the PPARγ [44].

5.4.3.2 Fenretinide Nonclinical Toxicity

[redacted] conducted toxicity studies of fenretinide via per-os (PO), intra-peritoneal (IP), or intravenous (IV) routes of administration in mice, rats, rabbits, and dogs, and many of the studies carried out were published [53, 54, 55, 56]. Doses of up to 7000 mg/kg were given in acute studies, while in multiple-dose studies doses of up to 1800 mg/kg/day were administered. No mortality was observed in acute studies. Clinical observations following administration of high oral doses consisted primarily of diarrhea, loose stools, unkempt appearance, and reduced spontaneous activity. In multiple dose toxicity studies, up to one-year duration, mortality in fenretinide-treated animals was rare and occurred in less than 5% of animals tested.

[redacted]

[redacted]

Reproductive Toxicity Studies. Doses ranging from 20 to 800 mg/kg/day (120 - 4800 mg/m^2) had no effect on the fertility and general reproductive performance of female or male rats; however, the complete inhibition of uterine implantation has been reported in mice receiving 100 mg/kg/day of fenretinide. Studies in rats and rabbits indicated that 800 mg/kg/day dose of fenretinide was teratogenic. The key role of retinoic acid in embryonic development mediates the high teratogenicity of retinoid pharmaceuticals, such as isotretinoin used for treatment of cancer and acne. Oral megadoses of pre-formed vitamin A (retinyl palmitate), and retinoic acid itself, also have teratogenic potential by this same mechanism. A screen of retinoids for developmental toxicity following single-dose PO administration to hamsters on Day 8 of gestation suggested that fenretinide was less teratogenic than ATRA [58, 59]. Late gestation, parturition and pup survival were unaffected in a peri-postnatal toxicity study.

Genetic Toxicity Studies. In a variety of in vitro and in vivo test systems, fenretinide did not induce any genotoxic effects [60]. Its major metabolite, 4-MPR, was also negative in the mammalian cell mutation assay.

Metabolism and Excretion. Animal and human studies indicate that fenretinide undergoes significant phase 1 and phase 2 biotransformation *in-vivo*. The most abundant metabolite produced in human, dogs and rodents in *in-vivo* is N-(4-methoxyphenyl)retinamide (4-MPR) [61, 62], whereas additional minor metabolites, 4-oxo-N-(4 hydroxyphenyl)retinamide (4-oxo-fenretinide) and 4-hydroxy-N-(4 hydroxyphenyl) retinamide (4-OH-fenretinide), have been subsequently reported [63]. In contrast to rodents, 4-oxo-fenretinide is present at low concentrations in humans. Although 4-MPR is now generally considered to be inactive [64, 65], 4-oxo fenretinide was found to be an active metabolite that inhibits cell proliferation [63, 66].

The enzymes responsible for production of 4-MPR (phase 2, methylation reaction) were not clearly determined, however they include microsomal amine-N-methyltransferases, that are known to be involved in the metabolism of many drugs and carcinogens [67]. The oxidative metabolism (phase1) is predominantly carried out by cytochromes P450 (CYP)s 3A4, 3A5, 2C8 and 26A1. Glucuronidation was shown to not playing a major role in the metabolism and clearance of fenretinide and its metabolites at clinically relevant levels [68]. The metabolic profile of fenretinide in human indicates a relatively low potential for drug-drug interactions.

5.4.4 Clinical Evidences

5.4.4.1 Phase Ib with fenretinide in Adult CF Patients

Laurent Pharmaceuticals in collaboration with The Research Institute of the McGill University Health Centre recently completed a Phase Ib, First-In-CF-Patients study [3]. It was a single-site, double-blind, placebo-controlled escalating multiple oral dose study of fenretinide (LAU-7b formulation) in adult CF patients. It involved 15 patients randomized 3:1 (active:placebo) who received each dose level in a sequential fashion, for cycles of 21 days spaced by drug-free periods of a minimum of 7 days. The study drug or matching placebo was to be taken once a day, orally, along with the morning meal in addition to current Standard of Care therapies. Three (3) ascending dose levels of fenretinide as LAU-7b were administered (100mg, 200mg and 300mg/day). The rationale behind the dose selection was to achieve, on average, a mean plasma concentration at steady state (Css) between 1-2 µM. This target was based on pre-clinical evidences in the mouse model.

All tests relating to safety, PK and PD were performed at screening to obtain baseline values and then repeated at the end of each treatment cycle. The end of each treatment cycle was scheduled 21 days (± 2 days) from the first day of treatment. In total, these tests were performed four times throughout the study period. The tests included safety laboratory tests, PD laboratory tests, lung function tests and physical examination. For patients enrolled in the PK portion of the study (first 12 patients enrolled), the blood samples for safety and efficacy were obtained prior to taking the drug dose for that day, and on Day 21 this meant approximately 24 hours after their previous dose. The main objectives of the study were:

<u>Primary objective</u>

- To establish the safety and tolerability of fenretinide in adult CF patients, using a novel oral formulation designed to optimize fenretinide bioavailability.

<u>Secondary objectives</u>

- To evaluate the PK profile of fenretinide at multiple dose levels;
- To determine the recommended doses of fenretinide to be used in future Phase II trials;
- To evaluate the preliminary PD of fenretinide using the plasma AA/DHA ratio as a surrogate lipidomic marker;
- To evaluate selected plasma and urine inflammatory markers and explore potential correlation with lipidomic markers.

Study Patients

The key inclusion/exclusion criteria were as follows:

Inclusions: Men and women 18 years and older, having a diagnosis of CF, Chronic CF lung disease with baseline FEV1 equal or superior to 40% predicted, stable at time of enrollment, Chronic pulmonary *Pseudomonas aeruginosa* colonization and/or infection.

Exclusions: Pregnant or breastfeeding women, inadequate contraception, presence of nyctalopia, other serious ophthalmological condition, serious dermatological conditions at enrolment, clinically abnormal liver or renal functions, history of gastrointestinal surgery or underlying gastrointestinal disorders of motility, and unstable intake of retinoids or lipid supplements.

Results and conclusions of the study

SAFETY RESULTS:

Overall, fenretinide was shown to be safe at all three doses studied for 21 days. The vast majority of adverse events (AEs) reported were mild, reversible with no sequelae, and without any action needed. Interestingly, the number of AEs in the active group decreased with the increase in fenretinide dosing. Many of these events were expected within the CF population studied, such as pulmonary exacerbations, characteristic of CF evolution, as well as reversible nyctalopia and dry skin which have been reported for fenretinide in other patient populations.

A particular attention was given to symptoms of nyctalopia. Fenretinide was shown to form reversible complexes with the circulating RBP, thus reducing the serum concentrations of retinol, an effect that may be associated with delayed dark adaptation in some patients. This is a well-documented and generally mild AE of fenretinide, as shown in previous clinical trials [52, 54, 69, 70]. Moreover, it was shown to be fully reversible with treatment cessation and to be easily controlled by vitamin A supplementation and drug-free periods built within treatment regimens for chronic use.

However, because CF patients are known to be prone to vitamin A deficiency, their retinol levels and occurrence of ophthalmological AEs were closely monitored during the Phase Ib. The frequent probing of the patients about vision abnormalities resulted in a few referrals to the ophthalmologist but none of these reports resulted in objective measurements of nyctalopia or ophthalmologic abnormalities upon detailed examination. Also, the final ophthalmological examination did not reveal any abnormality or signs of retinal damage in these patients.

Biochemical and hematological safety parameters were not affected by fenretinide at all dose levels. The reduction of serum retinol levels as compared to baseline appeared to be smaller in CF patients than those reported in other patient populations. Two (2) SAEs occurred during this study, both classified as unrelated to fenretinide with one related to CF disease (exacerbation with hospitalization) and easily explained by the condition of the patient, and one related to a concomitant disease (varicella case).

[redacted]

In conclusion, the safety and tolerability of fenretinide in adult CF patients was comparable with the reported safety profile associated with the use of this drug in other patient populations over similar or longer periods of treatment, and with comparable systemic exposure levels. The study Safety Review Committee has acknowledged the safety of the drug in this patient population and has recommended 300mg QD as the dose to be tested in Phase II trial [3].

PHARMACOKINETIC RESULTS:

Following single and multiple doses of fenretinide as LAU-7b, plasma exposure (C_{max} and AUC) increased with dose, and this increase was judged dose proportional (p-value > 0.05). The plasma concentration profile indicated first-order elimination kinetics. [redacted]

[redacted]

Generally, inter-patient variability was modest for the fenretinide PK parameters, even with the small sample size. After a single dose, the mean $t_{1/2}$ was consistent across the dose range studied with values ranging from 6.99 h to 7.50 h. Mean $t_{1/2}$ values at PK steady-state ranged from 8.25 h to 16.65 h. [redacted]

EFFICACY RESULTS:

In this study, we showed an association between a fenretinide dose-related increase in the DHA levels, a decrease in the AA/DHA ratio in plasma phospholipids and a decrease in the markers of oxidative stress that may be indicative of a reduced peroxidation and better protection of DHA, especially during episodes of increased inflammation such as pulmonary exacerbations. The improvement of the CF-associated AA/DHA imbalance, as well as a decreased oxidative stress in a majority of patients, appears to produce a shift towards an anti-inflammatory pattern, particularly at the highest dose. While the changes were not significant statistically, the expected rise in certain inflammatory markers during PEx seemed blunted in participants while on LAU-7b, and the rise in IL-8 during the course of the study was numerically less when on LAU-7b.

This improvement in inflammatory markers is presumed to be caused by treatment with fenretinide which resulted in a dose-dependent normalization of the plasma AA, DHA and AA/DHA ratio at the onset of PEx. The effect on AA and DHA was superior to what was measured at onset and during the treatment of PEx with antibiotics in the previous, non-interventional, cohort characterization study conducted in the same type of CF patient cohort of the same clinical site in Montreal [70]. At the highest dose (300mg), the improvement of the anti-inflammatory profile at onset of PEx in the Phase 1b study was comparable (IL-6 and neutrophils) or better (IL-8 and IL-10) than at the end of the PEx treatment in the cohort characterization study. A better systemic anti-inflammatory profile at onset of PEx was shown to correlate with increased odds to better respond to antibiotics for PEx [73, 70]. It is hypothesized that the cumulated benefits of fenretinide, both during and outside the PEx episodes, will translate into clinical benefits such as pulmonary function preservation, reduced incidence and severity of PEx episodes, and ultimately a better Quality of Life for CF patients.

In conclusion, the Phase Ib study attained its objectives of demonstrating safety of fenretinide in CF patients, enhanced systemic exposure comparable to non-CF population after taking into consideration the higher bioavailability of fenretinide afforded by LAU-7b and observing a convergence of dose-related pharmacodynamic effects of fenretinide on markers of lipid imbalance, oxidative stress and inflammation. Moreover, the highest dosing regimen tested (300 mg/day for 21 consecutive days) was recommended as an optimal dose for future Phase II trials, since it was deemed safe and achieved the 1-2 µM fenretinide target plasma C_{ss8h} in all patients treated, as well as the C_{avg}.

5.4.4.2 Supportive clinical experience with fenretinide

Fenretinide has been extensively studied in humans, mostly for the prevention and treatment of cancer. There is a large body of safety data existing for fenretinide from previous low-dose/long-term clinical studies (1-2µM plasma concentration) and mid/high-dose clinical studies (2-14.5µM plasma concentrations) using oral administration of fenretinide, as well as very high doses (up to 28 µM plasma concentration) with IV administration. These Phase I to Phase III studies have been carried out in more than 3,000 subjects, including both adults and pediatric patient populations, some for as long as 5 years of treatment (with the low doses). Most recent clinical studies have been carried out by the US National Cancer Institute (NCI) using the original corn-oil based softgels formulation. [redacted]

[redacted]

Fenretinide was originally explored in adult patients primarily as a chemopreventive agent, and in that setting it was used at low doses to avoid side effects. Numerous clinical studies employing chronic oral doses of 200 to 800 mg fenretinide/day (≤ 3 µM plasma levels) using the corn-oil based formulation, have been well tolerated in previous trials, with the 200 mg/day dose tolerated for as long as five years in the case of chemoprevention trials [69, 74, 75, 76, 77]. Most of these studies utilized a once daily administration, some with a 3-day drug-free rest period every 4 weeks to prevent potential symptoms of nyctalopia.

Low-dose / long-term clinical trials

Toxicity of oral chronic doses achieving 1-2 µM plasma levels of fenretinide in chemoprevention clinical trials has been minimal [69, 74, 78].

Fenretinide has been safely administered in chemoprevention trials up to dose of 300 mg per day (1-1.5 µM plasma levels) for prolonged periods of time from 6 months to 5 years in large cancer populations with no significant toxicity [69, 77, 78, 79, 80, 81]. Mild grade of nyctalopia and dermatologic disorders (dry skin, pruritus) were the most common side-effects of fenretinide treatment; rate of occurrence of both types of events tended to decrease with time or to recover spontaneously during the treatment period or shortly after cessation of dosing. Fenretinide plasma concentration (12 h post dose) at steady state was estimated to be ~1µM at 200mg dose and ~1.5µM at 300 mg dose. Adverse effects typical of other retinoids, such as decreased bone density, ligament calcification, and skeletal hyperostosis were not observed in these studies. Even after five years of therapy, abnormalities in night vision improved significantly after 7 days off therapy, and completely resolved one month after stopping fenretinide; plasma retinol concentrations returned to normal in one month following discontinuation of fenretinide [80, 81].

Single and repeat daily dose PK studies with fenretinide softgel formulation in healthy subjects and patient population indicate that fenretinide plasma exposure (C_{max}, AUC) also increased upon repeat daily dosing to reach a steady state level at 4-5 days [82, 74, 83]. Presence of food increased significantly the plasma levels of the drug and has been used since as standard recommendation for fenretinide dosing[74]. The increase in exposure was dose proportional up to 800mg. Plasma exposure on Day 21 with 300mg/day dose of LAU-7b formulation in adult CF patients was approximately equivalent to the exposure at the dose of 800mg on Day 28 in healthy adults with the softgel formulation, indicating an increase in fenretinide bioavailability by a factor of >2 with LAU-7b.

High-dose clinical trials

Phase I trials of high-dose (up to 14.5 µM plasma levels) oral fenretinide in pediatric solid tumors have been conducted with the corn-oil softgel formulation [76, 79, 84].

In two Phase I studies in children with neuroblastoma, fenretinide was given up to the dose of 4000 mg/m^2/day over 28 days (3-6 patients/dose level), followed by a 7-day interruption, for a period of 6-25 courses without dose-limiting (DLT) toxicity [76, 84]. Fenretinide pharmacokinetics was linear in the dose range 100–1,700 mg/m^2. Steady state peak plasma concentrations between 1.3 µM at 100mg/m^2 and 14.5 µM at 4000mg/m^2 were observed in the first course of treatment on Day 28. Similar to what has been observed in adult patients, cutaneous toxicity (dry skin and lips) and nyctalopia (Grade 1-2) were the most common adverse effects observed at most dose levels which rapidly reversed during the 7-day drug-free intervals and did not appear to be dose related. Grade 2 toxicities included skin xerosis (6 cases), hepatic toxicity (1 case),

diarrhea (1 case), nyctalopia (3 cases), and headache (1 case). Nyctalopia of grade 3 occurred in one patient with the 1000mg/m^2 dose. None of the patients discontinued the drug because of toxicity. The maximum tolerated dose was not reached in these studies; however, they were terminated due to difficulties with patient compliance in consuming the required number of corn-oil based softgel capsules.

In a study in 54 children (2-20 years old) with high-risk solid tumors conducted by the Children's Cancer Group "CCG", a maximum tolerated dose (MTD) of oral fenretinide, divided BID - TID, given for 7 days, every 3 weeks, was defined as 2475 mg/m^2/day, which achieved fenretinide peak plasma levels of 9.9 ± 5 µM with minimal systemic toxicity [79]. Increased steady-state fenretinide levels were seen by Day 7 of therapy. Plasma retinol levels were decreased on Day 1 at all levels, and further decreased to an average of 33% of baseline by Day 7, with recovery to 55-106% of baseline by Day 21 (start of next course).

In all these high-dose pediatric studies, a wide range of peak plasma concentrations were observed at a given dose. It is possible that the differences of tolerability observed in these two studies (MTD achieved vs. no DLT observed) was dose-schedule dependent (i.e., TID vs. QD).

5.4.4.3 Conclusion

The results from both adult (Phases I-III) and pediatric Phase I trials of high-dose oral fenretinide suggest that peak plasma levels exceeding 10 µM can be achieved with tolerable toxicity.

Prolonged periods of fenretinide treatment (up to 5 years) in the cancer prevention studies were associated with fenretinide steady-state peak plasma concentrations of 1-2 µM at daily doses of 200 to 400mg and no significant toxicity/safety concerns. In the cancer treatment studies in children the peak plasma concentrations reached 14.5 µM with relatively mild adverse effects. In general, no significant toxicity was observed in children with peak plasma levels below 3µM. The incidence and severity of the most commonly observed toxicities related to fenretinide treatment such as nyctalopia, headache, dry eye, cutaneous, ungual, or mucosal toxicities observed in patients with 3–10 µM peak levels increased at peak levels >10µM. The adverse events were resolved after dose reduction or after discontinuation of the treatment. Maximum tolerated dose was not reached in children with neuroblastoma at daily doses of 4000mg/m^2 and fenretinide plasma peak levels of 14.5 µM.

Fenretinide is reducing plasma retinol levels and retinol binding protein potentially leading to development of symptoms of nyctalopia in some patients. This effect was rarely dose limiting, and quickly reversible following dose reduction or discontinuation of treatment. Moreover, this effect does not seem to be proportional to the dose. In previous fenretinide clinical studies a drug free period of 3-7 days following a 28-day oral exposure was proven to help prevent or alleviate nyctalopia and was integrated in all clinical studies.

[redacted]

There were no serious adverse events related to fenretinide recorded in the study, and the overall number of adverse events tended to decrease with the increase of the dose. The incidence of nyctalopia symptoms reported in this study is consistent with the incidence of symptoms of diminished dark adaptation reported in other clinical trials with fenretinide. Ad-hoc and scheduled ophthalmological examinations did not reveal any abnormality or signs of retinal damage in these patients. The reduction of serum retinol levels, as compared to baseline, appears to be smaller in CF patients than other patient populations for which lower doses of fenretinide were used. [redacted]

These results in adults and children are supporting the safety of the selected dose of 300mg with LAU-7b formulation in the planned Phase 2 study in adult CF patients and the dosing regimen of monthly cycles of 21 days of fenretinide dosing followed by a 7-days drug-free periods.

5.4.5 Specific Rationales for this Phase II study

5.4.5.1 Design/Structure

This is a randomized, double-blind (patients, Investigators and blinded study staff), placebo controlled, Phase II study (details are presented in Section 7.2). There will be no active control treatment but patients will undergo the Standard of Care at their institution. A randomized, double-blind study design will avoid observer bias and reduce symptoms or outcomes arising from the patients' knowledge of treatment. A parallel design will better detect treatment effects and is appropriate for this phase of development.

A Phase Ib study with fenretinide was conducted recently in adult CF patients. The safety results show that LAU-7b is well tolerated over 3 x 21-days cycles of progressively increasing oral doses up to 300 mg/day. The anticipated duration of treatment in the Phase II study is 6 cycles of 21 days, totalling around 24 weeks. Compared to the Phase Ib study, this duration of treatment will permit all safety and efficacy variables to be measured for an additional 3 months to obtain a more robust assessment that is less affected by short-term variability and influence of PEx.

The 24-weeks duration is anticipated to facilitate detection of significant changes in the primary efficacy endpoint FEV_1, and to also help determine the impact of fenretinide on secondary efficacy variables such as the PEx-related variables, weight and BMI, bone density, systemic bone formation/resorption markers as well as the Quality of Life assessments. While it is anticipated that the effect on the lipidomic and inflammation biomarkers endpoints will appear rapidly after the start of treatment, a 24-weeks duration will allow for an assessment of the durability of response for all parameters. The follow-up period of approximately one month after the last dose is deemed adequate for assessing safety, biomarkers and efficacy, because fenretinide and its metabolites are expected to reach undetectable levels within 7-10 days after the last dose. Thus, the follow-up period will be mostly on a drug free background. This period will also enable assessment of whether some effects on pharmacodynamic markers last longer than the drug systemic presence. The study also aims at gathering efficacy and additional safety information about LAU-7b in CF patients.

5.4.5.2 Selected Dose and Regimen

In the previous Phase Ib study, three doses of LAU-7b were investigated and all showed good tolerability. Doses for that study were chosen based on the enhanced bioavailability that LAU-7b capsule formulation conferred to fenretinide, the active product ingredient, and the target concentration range derived from pre-clinical pharmacology experiments conducted in mouse models. In essence, the Phase Ib was a concentration-driven study, while aiming at finding a maximum tolerated dose. On the exposure standpoint, the Phase Ib dose of 300 mg (highest dose) achieved or slightly surpassed the circulating level (Css8h) objective set a priori, in all patients and as of Day 1, which was not the case for certain patients receiving the 200 mg/day dose. There was no evidence of saturation of exposure with 300 mg when compared to the two lower doses. From the efficacy standpoint, the improvement of the CF-associated AA/DHA imbalance, as well as a decreased oxidative stress in a majority of patients, appeared to produce a shift towards an anti-inflammatory pattern, particularly at the highest dose of 300 mg. Therefore, this study will aim at testing a daily dose of 300 mg of fenretinide as LAU-7b, using a similar approach as in the Phase Ib, which consisted

of dosing cycles of 21 days on study drug treatment spaced by drug-free periods averaging 57 days between Cycles 1 and 2 and 27 days between Cycles 2 and 3.

In this Phase II study of 6 cycles of 21 days, the intermittent dosing scheme will also be employed but the drug-free periods will have a set duration of 7 days. The drug-free periods aim to prevent as much as possible any risks of clinically significant nyctalopia. Symptomatic reversible nyctalopia is an expected and well-documented adverse effect of fenretinide, due to its affinity for RBP resulting in decreased plasma retinol levels. The drug-free periods, allowing for restoration of retinol levels, have been in use in oncology-related fenretinide development and have resulted in low incidence/severity (CTC Grade 1 in most cases) of nyctalopia, even for long periods of administration, as described in Section 5.4.4.2. Likewise, in the Phase Ib in adult CF patients, reported nyctalopia was not confirmed by objective ophthalmological examination, despite frequent symptom probing with specific questionnaires. Post-study full ophthalmological examination did not reveal any significant changes from the baseline examination [3]. The 7-days drug-free periods are not expected to influence the treatment continuity, as the PK evaluation in CF patients showed a slight degree of fenretinide plasma accumulation after 21 days of continued administration.

This study is intended to be a definitive Proof-of-Concept and therefore it is appropriate to assess the potential of fenretinide at the highest dose tested in the previous Phase Ib, considering its good tolerability. This dose also gives assurance that the target therapeutic systemic concentrations will be reached in all patients while remaining in a concentration range associated with a very good safety profile in non-CF patient populations. Assuming positive outcome for this study, it is intended to thoroughly assess the minimally effective dose during Phase III confirmatory studies, through an adaptive approach.

5.4.5.3 Rationale for Study Assessments

The safety and PK assessments are standard parameters for clinical studies in drug development. The efficacy assessments are widely accepted and generally recognized as reliable, accurate, and relevant to the study of patients with CF.

Spirometry: Because lung disease is the major cause of morbidity and mortality for patients with CF, CF lung disease is the desired primary target of fenretinide therapy. FEV_1 as measured by spirometry is the most widely implemented standardized assessment to evaluate lung function in CF and the absolute change from baseline of FEV_1 percent predicted is the primary efficacy endpoint of this study.

PEx-related measures: These assessments (time to first protocol-defined pulmonary exacerbation, incidence of protocol-defined pulmonary exacerbation, time to first antibiotic use, number of antibiotic treatments over the study treatment period, number of days of antibiotic treatment) are outcomes used to assess efficacy in therapies targeting improvement in CF disease. CF PEx are a compilation of patient signs and symptoms that often result in the need for aggressive treatment, including the use of IV antibiotics that may require hospitalization. To date, there is no generally accepted objective definition of a PEx. Despite the lack of a standard definition, reduction in pulmonary exacerbation rate has served as a key clinical efficacy measure in definitive CF clinical studies, supporting the registration of 2 chronic CF pulmonary therapies (inhaled recombinant human DNase and inhaled tobramycin). To evaluate the potential effect of fenretinide on the PEx, the variables enumerated above will be derived, with distinction between oral and IV antibiotic treatments for PEx. For data analysis purpose, this protocol utilizes a definition of pulmonary exacerbation, which is based on the definition of Fuch, presented in Section 9.3.1.

Weight and BMI: Malnutrition is common in patients with CF because of increased energy expenditures due to lung disease and fat malabsorption. Given that fenretinide is a systemic therapy, it has the potential to improve extra-pulmonary manifestations of CF, including those in the gastrointestinal system. Improved nutritional status, defined as an increase in weight and/or BMI, is considered an appropriate endpoint for

therapies targeting pancreatic insufficiency and was used in the Phase Ib. To evaluate the effect of fenretinide on growth/health, depicted by change in weight and BMI, will be determined.

Bone density and systemic bone formation/resorption markers: Early onset osteoporosis is becoming more prevalent in the CF population as the median survival increases. The effect of new drugs on bone homeostasis should be evaluated as a safety measure in patients with CF, many who are treated with corticosteroids. Furthermore, since it was shown that fenretinide eloquently increased bone volume and density in the knockout mice model of CF, it is appropriate to explore in this study if this protective effect can be reproduced in CF patients. For this purpose, all patients will be sampled before and after the study treatment for systemic bone formation/resorption markers, and a subset of 24 patients at select sites with the capability for doing bone densitometry according to specific criteria, will contribute pre- and post-study bone density data. The selected systemic bone formation/resorption markers are serum osteocalcin, serum C-telopeptide (CTx) and serum P1NP. Bone density will be assessed on the lumbar spine as it is the most appropriate site for monitoring the effect of a treatment for osteoporosis [85].

Lipidomic, inflammation and metabolipidomic biomarkers: There is ample demonstration that key lipid imbalance is linked to CF and it was demonstrated in non-clinical pharmacology studies at McGill that fenretinide corrects the AA and DHA imbalance in a specific animal model of CF (Sections 5.4.1 and 5.4.2.1). Supported by the findings of the recent Phase Ib study, it is planned that lipidomics (AA, DHA) and in a more exploratory manner, metabolipidomics (eicosapentaenoic acid (EPA), selective eicosanoids, docosanoids and ceramides, etc) will be assessed serially in this study. As a drug with a potential to modulate pro-inflammatory and pro-resolving inflammation cascades, the effect of fenretinide on a series of inflammation markers will be measured serially in this study, building on the knowledge from the Phase Ib study [3] and others working in the field [73].

CFQ-R and Matouk Disease Score: The CFQ-R is a validated CF-specific instrument that measures the health-related quality of life of patients with CF. The instrument measures quality-of-life domains including respiratory symptoms, digestive symptoms, emotion, and health perception. Furthermore, the CFQ-R has been evaluated in clinical studies involving therapies for CF lung disease. Linguistically validated versions of the CFQ-R are available, thereby allowing consistent interpretation of the results in this international study. As an exploratory outcome, the Matouk Disease Score will be assessed for its potential to complement more established parameters such as FEV_1. Given the lack of recognized biomarkers for CF inflammation clinical studies, and the long time required for FEV_1 to show changes, we believe that exploring additional alternatives, such as this scoring system, will be welcomed by the CF community.

Markers of oxidative stress: CF disease is characterized by high levels of oxidation due to the abnormal inflammatory response, chronic bacterial infections and the reduced anti-oxidant mechanisms [86, 87]. Polyunsaturated fatty acids (PUFAs), such as AA and DHA, are particularly susceptible to oxidation due to their molecular structure with few double bonds between carbons. Malondialdehyde (MDA), a marker of lipid peroxidation, was found to be abnormally high in CF patients [88]. Nitrotyrosine (NT3) is a marker of protein oxidation. The levels of NT3 were also found to be high in CF patients [89, 90]. MDA and NT3 plasma levels will be measured in order to explore the oxidation of lipids and proteins. We have previously seen correlations between MDA levels and DHA whereby lower levels of the oxidation marker were observed in conjunction with higher DHA levels [3]. Thus, in this study, it will be explored whether fenretinide would reduce these oxidative stress markers in patients following fenretinide treatment.

6 STUDY OBJECTIVES

Primary objectives:

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 FEV_1 percent predicted, relative to placebo-treated patients.

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[ii].

Rationale for the selection of objectives:

This is a Phase II study that aims at gathering efficacy data as well as expanding and confirming the safety and pharmacodynamic information obtained during the Phase Ib study in adult CF patients.

In that latter study [3], LAU-7b administered at doses up to 300 mg per day for 21 days at each dose level, on a background of normal standard of care showed good tolerability and no treatment-emergent laboratory abnormalities. The pharmacokinetic profile of LAU-7b fully achieved the targeted blood levels and dose selection rationale. The treatment also normalized the lipid imbalance and decreased oxidative stress in a vast majority of patients, causing a shift towards an anti-inflammatory pattern particularly at the highest dose tested, and potential association with stability of some clinical parameters. The most promising effects were observed after dividing the patients into those that did not experience episodes of PEx and those who did, the latter subgroup showing stronger positive pharmacodynamic responses. This requires further confirmation and correlation of the findings with clinically relevant outcomes. However, the sample size was small and for most of these measures, variability prevented reaching statistical significance or robust conclusion on efficacy.

This proposed Phase II study design follows the regulatory guidance from the European Medicines Agency (EMA), as well as recommendations for the conduct of clinical trials with agents addressing inflammation in CF, recently published by the CF Foundation Anti-inflammatory Therapy Working Group [91].

It is hypothesized that 6-month treatment with 300 mg of LAU-7b, administered along with the Standard of Care, orally once-a-day in cycles of 21 consecutive days, spaced by 7-day drug-free intervals, will be safe and well tolerated by the study population. It is also hypothesized that LAU-7b treatment in CF will have

[ii] Matouk Disease Score is a clinical score created to assess the severity of patient symptoms in a more longitudinal way in order to overcome the limitations of the current evaluation of the lung function. This score is a modified Huang Disease Score, with additional assessments to include important complications that may impact the disease (such as the number of pulmonary exacerbations in the past 6 months).

positive effects on the immune-inflammatory response, and consequentially on the pulmonary function as depicted by the absolute change in FEV_1% predicted, the primary efficacy endpoint of the study. The secondary line of efficacy assessments will include the PEx-related variables, the measures of systemic pro- and anti-inflammatory markers, as well as with the evolution of other clinical parameters. To help establish the benefits to the patients, health economists and payers, the Quality-Of-Life assessments such as the CFQ-R and measures of long-term benefit such as the *PsA* colonization in sputum will be assessed, compared to placebo, and correlated with primary and other secondary endpoints, to develop a better understanding of the inter-relations of the study variables and their interactions with the disease. As an exploratory outcome, the Matouk Disease Score will be assessed for its potential to complement more established parameters such as FEV_1.

The study will also evaluate the impact of LAU-7b therapy on the subset of patients with IV antibiotic-treated PEx, in the light of recent clinical evidence showing a good correlation of systemic inflammation biomarkers with FEV_1 and response to antibiotics during PEx events [3, 73]. The robust dataset of efficacy endpoints collected will allow the selection of clinically relevant endpoints for the pivotal studies, and the identification of parameters susceptible to become validated surrogate endpoints, assuming a good correlation is demonstrated.

With pulmonary function as the primary efficacy variable, this study is aimed at the population at risk of losing lung function if left on the current standard of care. Consequently, the lower limit of inclusion for the baseline FEV_1 will be set at 40%, and the upper limit at 100% predicted, depicting that patients have a fairly decent pulmonary function at entry. These are values that do not represent mutually exclusive entry criteria with the other inclusions destined to enrich the study population in patients susceptible to experience a PEx episode during the study (see below). PEx episodes and the ensuing lack of proper resolution of inflammatory defense mechanism are responsible for the lung structure losses and associated non-return to pre-PEx lung function in a sizeable proportion of patients. The entry criteria of the study have therefore been set to enrich the study population with patients more susceptible to develop a PEx episode. Based on the newly discovered regulator effect of LAU-7b on key membrane lipids, such as the stabilization of CFTR, the more mildly affected CF patients may benefit as well from LAU-7b therapy. We plan to enroll patients with at least one pulmonary exacerbation in the previous 12 months that required a documented change of antibiotic, whether oral or IV. It is known that patients who required IV antibiotics to treat a PEx episode in the prior year have about a 30% chance of having an IV-treated exacerbation during a 24-week study [92]. In the case of no IV administered antibiotics for PEx, the odds drop by half [71].

Since our planned inclusion criteria are aligned to enroll patients susceptible to lose pulmonary function because of their baseline FEV_1, and in light of the potential for LAU-7b to stabilize and augment the function of CFTR protein in the cell membrane, an effect shown to more evident with CF patients with higher pulmonary function, as depicted by FEV_1, the prior requirement for a minimum of one (1) IV antibiotic-treated PEx episode in the prior 12 months, deemed to likely limit the necessary number of observations required to reach significance for the primary efficacy comparison is presumed to not be weakened by the enrollment of less severely affected patients. For this study, a difference of interest in absolute change of FEV_1 over a period of 6 months is set to 4%; this target difference is similar to what was planned and found to be clinically meaningful in some previous confirmatory clinical trials, such as those of lumacaftor/ivacaftor combination therapy [93]. In addition, considering that fenretinide reduced *PsA* lung burden compared to no treatment in the Cftr.KO mouse model, there is a distinct possibility that in the planned study, fenretinide also exerts an anti-infective activity, susceptible to cause a net improvement (not just a stabilization) of FEV_1 in CF patients, distancing itself from the FEV_1 decay expected in placebo-treated patients.

7 STUDY DESIGN

7.1 Endpoints

7.1.1 Primary Endpoints:

- 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, LAU-7b compared to placebo treated patients.

7.1.2 Justification for the Primary Endpoints

Primary Efficacy Endpoint: Fenretinide is proposed as an anti-inflammatory therapy with the ability to normalize plasma AA/DHA ratio, and in pre-clinical work, reduce *Pseudomonas* burden. Chronic lung inflammation and infection are at core of the lung destruction over time. Along with safety, it is therefore most relevant to assess the effect of correction of the lipidomic abnormality on FEV_1, a clinically meaningful outcome in CF and the most characterized index for assessing lung function in CF. If indeed fenretinide has positive effects on the immune-inflammatory response, on the function of CFTR and exert anti-infective effect against *PsA*, this will result in a positive absolute difference in the lung function relative to placebo-treated patients, an effect that should be observed over the 6-months duration of the study. Other variables susceptible to be positively affected by an anti-inflammatory and pro-resolving therapy will be evaluated as secondary endpoints.

Safety as a Primary Endpoint: In the proposed study, due to the longer duration of treatment in CF patient, relative to the previous Phase Ib and the increased sample size, these are relevant reasons for nominating safety as a co-primary endpoint, along with assessing efficacy. Similar to the previous study, safety will be assessed during and after the study, taking the screening and baseline status of the patients as a reference. In addition, particular attention will be put on objectively investigating reported symptoms of nyctalopia and/or dermatological disorders, the latter being known class effect of retinoids (including vitamin A). Fenretinide has a very good safety record in other indications at doses superior to the one evaluated in this study, and was shown to be well tolerated in the Phase Ib study with only few adverse events deemed related to the study drug. Still, by administering the study drug over a longer period of time, it is necessary to err on the safe side and continue to build a comprehensive safety database on fenretinide in CF.

7.1.3 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 difference in 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.

7.1.4 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.

7.2 Study Overview

7.2.1 Description

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 FEV_1, 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 35-40 centers in the United States of America, Canada and Australia, 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 overall study structure is presented below in Figure 5.

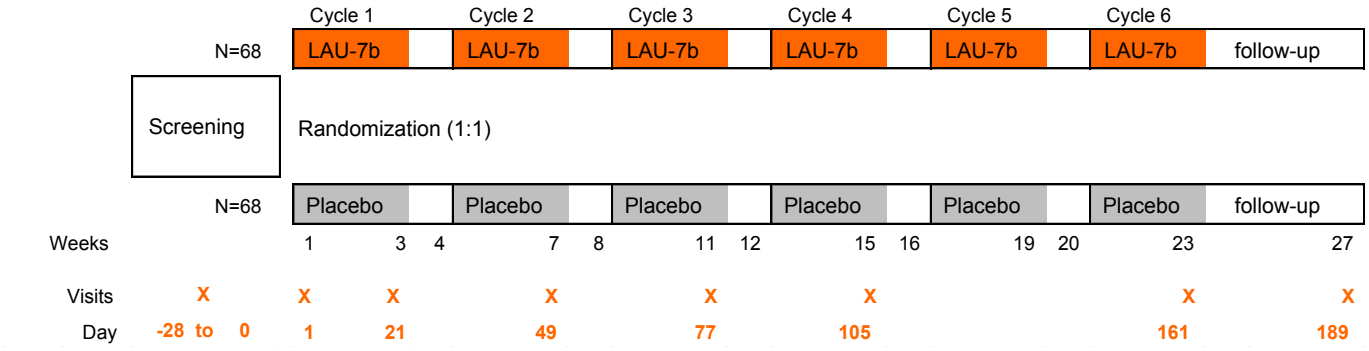

Figure 5: Overall structure of the study

7.2.2 DSMB Review

Throughout the study, the DSMB will monitor blinded safety measures and demographics at regular intervals as specified in the DSMB charter, or as requested by the DSMB chair. The DSMB will receive reports of adverse events by organ class, with emphasis on SAE's, clinically significant adverse events with a high causality potential to the study treatment, PEx episodes, and adverse events in the ophthalmological and dermatological class. Members of the DSMB will not be directly involved in the conduct of the study and cannot serve as Investigators in this study.

The DSMB will be composed of:

- Two physicians involved in the care of CF patients, experienced in clinical research, independent of Laurent Pharmaceuticals and not involved as an Investigator on the study.
- One Ophthalmologist experienced in clinical research, independent of Laurent Pharmaceuticals.
- One study biostatistician that can be unblinded with regards to patient randomization, meaning that it is not involved in the statistical design, analysis and reporting of the study.
- Ad-Hoc members recruited to provide specific expertise and meet the Board's mandate.

Each DSMB member will sign a Conflict of Interest Statement which includes current affiliations, if any, with pharmaceutical and biotechnology companies (e.g., stockholder, consultant), and any other relationship that could be perceived as a conflict of interest related to the study and / or associated with commercial interests pertinent to study objectives.

Data will be presented in a blinded manner during the open sessions of the DSMB unless noted otherwise below. At DSMB meetings data and discussion are confidential. Participant identities will not be known to the DSMB members.

Mandate of the DSMB (to be defined in greater detail in the DSMB Charter):

- Review safety information emerging from the study (and any other study of fenretinide). Particular attention will be paid to PEx incidence and due to the profile of fenretinide, ocular and dermatological AEs. Moreover, the DSMB will have access to the randomization code, if justified.
- Meet at specific times of the study to review the safety data cumulated to date. The following specific times have been selected a priori:
    - 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.
- Provide guidance to the study team with regards to safety trends. Recommendation to implement a systematic dose reduction or continue the study at the planned dose level will be made by the DSMB and reviewed by the Laurent Pharmaceutical's Team, who will take the final decision to pursue dosing or not (see Sections 7.2.3, 7.2.4 and 8.4.1 for Study/Treatment Arm Stopping Rule Guideline, Treatment Arm Dose Reduction Guideline, and Individual Patient Stopping Rules, respectively).

7.2.3 Study / Treatment Arm Stopping Rule Guideline

The following should serve as a guide for the DSMB to recommend stopping the enrolment and/or further drug administration in a given treatment arm. An agreement will be reached with the DSMB on the guideline prior to the study. Other considerations may be used to arrive at such decisions, which must involve Laurent Pharmaceuticals Executive Management.

- Occurrence of any case of death that may be attributed to fenretinide (in such a case, the blind will have to be broken and allocated treatment identified);
- Occurrence of an increased number, relative to placebo, of unexpected SAEs that could be attributable to the drug (at least *probable* causality relationship) that raise Investigators/DSMB concerns about patients' safety;
- Increased frequency and/or severity of drug-related adverse events, relative to placebo, that in the judgment of the Investigators/DSMB, will result in the advice to discontinue fenretinide treatment.

7.2.4 Treatment Arm Dose Reduction Guideline

The following should serve as a guide for the DSMB to recommend the reduction of the dose in the fenretinide arm from 300 to 200 mg/day. An agreement will be reached with the DSMB on the guideline prior to the study. Other considerations may be used to arrive at such decisions, which must involve Laurent Pharmaceuticals Executive Management.

- Increased frequency and/or severity of drug-related adverse events that in the judgment of the Investigators/DSMB, will result in the advice to reduce the dose of fenretinide treatment.

7.3 Blinding and Randomization

Given the potential for bias in the interpretation of safety and Quality of Life endpoints, as well as the potential for treatment bias that would affect PEx severity and related antibiotic treatment route of administration and duration, among other factors that could potentially bias the trial, the APPLAUD study must be placebo-controlled, double-blinded, and randomized in order to obtain sufficiently robust data.

7.3.1 Rationale for Placebo Control

There is no permanent cure for CF, and the current drug therapies goals are to reduce the symptoms of disease, such as preventing progression of lung infection by removal of the mucus plugging from the lungs, controlling the infection and inflammation, controlling the nutrition level, and ultimately increasing survival. As they are achieving their goals it is therefore appropriate to assess the safety and potential benefit of LAU-7b added to the standard of care for CF patients. A placebo control is suitable and will permit to have unbiased assessment of the several variables. It is essential to maintain the patients on their standard of care, assuming this meets all inclusion/exclusion criteria, as well as allowed vitamin supplements.

7.3.2 Blinding and Breaking the Blind

This is a double-blind study. Patients, Investigators and Site Staff, sponsor and CRO personnel, and data managers will be kept blinded to treatment assignments until the end of the study, except for emergency unblinding as described below. More specifically, to ensure the blinding is maintained when there are no safety concerns requiring unblinding, pharmacokinetic data, pharmacodynamic biomarker results, including post-screening plasma retinol and retinol-binding protein, will not be communicated back to blinded staff.

Only the Clinical Trial Material manufacturer's and Packaging/Distribution provider's unblinded staff and CRO staff administering the interactive web response system (IWRS) will be knowledgeable of individual treatment assignments. As well, in the case of emergency unblinding as described below, some additional people will become aware of treatment assignment for specific patient(s).

The study blind may be broken for an individual patient or several patients in the event of an emergency in which knowledge of the treatment assignment is needed for the safety of the patient(s) and/or for medical decision-making or as required by local regulatory authority. Unless the event for which the blind needs breaking is life threatening, the investigator should first contact the CRO's medical monitor (or designee) prior to breaking the blind. The investigator will obtain unblinding information from the staff administering the IWRS in the form of IWRS access and sealed randomization envelopes. The reason and justification for breaking the blind must be fully documented in the source documentation and captured on the patient(s) electronic case report form (e-CRF). Additionally, all SAE, as they are received but in respect of the reporting requirements enunciated in the DSMB charter, will be referred to the chair of the DSMB, who may request unblinding of the patient, and may call an *ad hoc* meeting of the DSMB to review the data relevant to the study stopping/dose reduction guidelines.

7.3.3 Randomization Scheme and Stratification

Computer-generated randomization sequences will be programmed into the IWRS. At the time of Randomization during Visit 2, not before, the IWRS will assign a randomization number to each patient.

Randomization will be stratified by
1- FEV_1 severity collected at the Screening Period (<70% versus ≥70% predicted);
2- Number of PEx (all PEx episodes, whether treated by oral or IV antibiotics): ≤3 versus > 3 PEx episodes in prior year; and
3- Co-administration or not of Kalydeco® (ivacaftor), Orkambi® (ivacaftor/lumacaftor), Symdeko® (ivacaftor/tezacaftor) or another commercially available CFTR modulator product.

Once stratified, patients will be randomized in a 1:1 double-blinded fashion to either LAU-7b (active) group or the placebo (control) group. Stratification will enable an even distribution of severity and key baseline characteristics among the two treatment groups.

Detailed instructions for randomization will be provided separately.

8 SELECTION AND WITHDRAWAL OF PATIENTS

8.1 Screening 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 documented IV or oral 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 regime 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. Allowed concomitant diseases and treatment are listed in specific section;
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.

8.2 Screening 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 (eg: retinitis pigmentosa, choroidoretinitis, xerophtalmia, 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.

8.3 Withdrawal of Patients

Patients should be withdrawn from the study in the event of any of the following:

1. If a patient does not continue to meet eligibility criteria at baseline, the patient will be withdrawn prior to randomization;

2. Occurrence of a SAE deemed definitely or probably related to drug that is a life-threatening adverse reaction or one resulting in hospitalization;
3. If a patient withdraws consent during the treatment period, and more specifically consent to contribute additional outcome information, the patient shall be withdrawn from the study (although it is recommended that the investigator attempt to perform a Treatment Discontinuation Visit Evaluation). If the patient withdrawing consent to further treatment agrees to contribute additional outcome information, then the regular schedule of visits could be maintained, for safety and relevant outcome measures of efficacy.
4. If a patient is serially and persistently noncompliant with study procedures and/or visit schedules, the investigator or the sponsor may withdraw the patient from the study
5. A patient's treatment is unblinded by the Investigator.
6. If a female patient or a female partner of a male patient has a confirmed pregnancy (with the exception of a pregnancy resulting from *in-vitro* fertilization), this should be immediately reported to the Medical Monitor and Laurent Pharmaceuticals (within 1 working day of discovery) and the patient shall be withdrawn. Pregnancy, whether in a patient or in a partner of a patient should be followed up until conclusion, with an assessment of child health at birth.

The reason for patient withdrawal will be noted on the e-CRF. The investigator should attempt to follow patients withdrawn after first dosing until resolution of any adverse events, or at least 4 weeks after the last dose of study agent, or until completion of pregnancy. Upon withdrawal, no further dosing should be performed for these patients. Investigators shall make reasonable attempts to contact lost-to-follow-up patients for evaluation.

8.4 Treatment Discontinuations

Patients should discontinue treatment with the study treatment, but remain on the study for adequate follow-up, for any of the following reasons:

- Patients may discontinue study drug treatment at any time if the patient, Investigator, or Laurent determines that it is not in the best interest of the subject to continue treatment.
- Unacceptable toxicity associated with treatment (see below stopping rule, section 8.4.1)
- Clinically significant lab abnormalities or adverse events that, in the Investigator's judgment, would preclude continued treatment.

The reason for treatment discontinuation will be noted on the e-CRF.
If a patient discontinues treatment due to unacceptable toxicity or any other safety reason, that patient should either continue to be assessed according to the planned visit schedule for safety and for relevant efficacy parameters, or at the minimum, attend a Treatment Discontinuation Visit four (4) weeks after having received the last dose of study drug.

8.4.1 Individual Patient Stopping Rule

The following should be used as a guide for the Investigator to determine if the study drug administration is to be interrupted based on toxicity (see Section 11.1.1.3 on Study Drug Action Taken, for more detail on documentation):

- A subject develops a medical condition that requires prolonged concomitant therapy with a prohibited medication or prolonged interruption of the study drug;

- Occurrence of a SAE that could be attributable to the drug (at least *probably* causality relationship), and causes discontinuation of study drug for more than 3 weeks;
- High and unexpected frequency and/or severity of "at least probably related" adverse events that in the judgment of the Investigator, requires withdrawal from the study.

8.5 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.

9 STUDY CONDUCT

9.1 Timing of Assessments

All questionnaires must be performed before any other assessment at the clinic visits with the CFQ-R questionnaire performed first, followed by the Night Vision Questionnaire (NVQ) and the Patient self-assessment form of the Matouk Disease Score (at selected sites only). For the remaining assessments, the following assessments must be performed (if possible) in the following order when more than 1 assessment is planned at a particular time point:

1. 12-lead ECG recordings
2. Vital signs
3. Pulmonary function tests (FEV_1) and Sputum Induction
4. Safety laboratory assessments (i.e., blood draws)
5. PK sampling

9.2 Study Visits and Procedures

9.2.1 Screening and Eligibility Assessments (Visit 1, Day -28 to -1):

Within 28 Days of randomization, interested CF patients will undergo a screening session, Visit 1:

1. Verification of identity and age;
2. Prior to any study specific screening activities, all potential patients will sign the Study Informed Consent Form (ICF). The consent forms will comply with all applicable regulations governing the protection of human subjects. An ICF Form, approved by Laurent Pharmaceuticals and the site's institutional review board (IRB) or ethics committee (EC), must be used. At this point, the site assigns a "subject/patient number" to each patient, in order of screening. This will serve to identify the patient for the entire study participation. This is <u>not</u> the randomization number, which is generated by the IWRS at Visit 2, at time of randomization;
3. Confirmation of the CF diagnosis will be obtained, with proper genetic data if available;
4. Verification of all inclusion and exclusion criteria;
5. Obtain medical history including history of prior pulmonary infections, pulmonary exacerbations and their causative pathogens, all recent and current medications and therapies, non-prescription drug intake, pancreatic enzyme quantities (where applicable) as well as vitamin supplementation (types and quantities). Particular attention will be put on obtaining the history of presence or absence of *PsA* in throat or sputum cultures, the dates of start of oral/IV antibiotics taken to treat PEx episodes, both in the 12 months prior to screening. This will help establishing a background exacerbation *PsA* and PEx pattern;
6. Verification of the medications against list of disallowed medications and associated ban periods;

7. Body weight, height, with BMI calculated;
8. Complete Physical examination;
9. A 12-lead ECG;
10. Vital signs including respiratory rate, oxygen saturation and body temperature;
11. A pulmonary function test comprising at a minimum FEV_1, to be performed under the care of a trained study personnel. Three FEV_1 measurements will be collected and the best of three FEV_1 values (according to American Thoracic Society standards) will be recorded. Central review of the expiratory loops will be performed to ensure accurate baseline FEV_1;
12. Eligibility-defining laboratory tests including at a minimum, urinalysis (dipstick), hematology, serum chemistry, plasma retinol and retinol-binding protein, and pregnancy test if applicable;
13. A Night Vision Questionnaire will be self-administered;
14. Complete ophthalmological examination (includes examination of visual acuity, examination for amblyopia, examination of eyelids, conjunctiva, cornea, pupillary reactions to light and accommodation, low contrast visual function (Contrast Sensitivity), specific dark adaptation tests (see note in Section 11.5), lens, vitreous humor, fundus examination of retina and retinal vessels, fundus visualization of optic nerve and macula), will also serve as the baseline exam;
15. Sputum will be induced using hypertonic saline for the assessment of the CFU for *PsA*. Patients with known intolerance to hypertonic saline will not undergo the procedure, as judged by their treating physician. A precise set of instructions will be provided and applied across sites to minimize sampling variations and ensure sputum sample quality;
16. Screening may be split into more than one visit and all results must be available prior to randomization so that the study Investigator can determine eligibility and schedule the baseline visit.

9.2.1.1 Repeat performance of screening assessment(s)

Repetition of individual screening assessment(s) that did not meet eligibility criteria is not permitted with the following exceptions:

- If there is clear and documented evidence of a laboratory error (e.g., hemolyzed sample) or equipment malfunction, collection of a repeat sample for the appropriate laboratory test or assessment may be permitted;
- Due to the unstable nature of CF, any clinically significant laboratory test abnormality, but deemed transient by the Investigator, may be retested within the screening window.

If repeat values of the individual assessment(s) are within the eligibility criteria and completed within the screening window, then the patient is eligible for the study.

9.2.1.2 Rescreening

Patients may only be rescreened with the approval of the medical monitor. If a patient is rescreened, all screening assessments will be repeated except CF genotyping (if not already in the patient's medical history), and the ophthalmologic examination (if performed within the last 3 months). If a patient is rescreened, the screening window will begin once the first rescreening assessment has been initiated.

9.2.2 Baseline and First Dosing Visit (Visit 2, Day 1)

1. Eligible CF patients will be invited to the study site for the baseline procedures and first dosing. This visit must be within 28 days of screening otherwise all or a part of expired screening procedures will need to be repeated/updated (see Section 9.2.1.2);
2. Confirmation of continued eligibility by verification of the inclusion/exclusion criteria, review of the results of the screening safety laboratory tests including plasma retinol and retinol-binding protein, performance of baseline vital signs, urine pregnancy test for women, and focused physical examination;

3. Pulmonary function tests (FEV_1). The actual baseline FEV_1 value for the study will be the average of the screening's best value and the best value obtained during the baseline visit as long as the screening and baseline FEV_1 values are within 15% of each other. A higher difference in FEV_1 % predicted indicates a lack of acceptable pulmonary function stability and the patient should not be randomized on study;
4. Body weight, with BMI calculated;
5. At sites that participates in the bone density exploratory assessment, the first 24 patients who consent to the additional procedure of measuring bone density prior to and after the study treatment, will undergo their baseline assessment; being not exclusionary, this test may be scheduled within a few days of first dose but not after;
6. Baseline Quality of Life questionnaire will be filled out (CFQ-R);
7. At select sites participating in the investigation of the Matouk Disease Score, the Baseline Score will be determined;
8. Pre-dose blood sampling and urine collection for baseline safety laboratory tests, pregnancy test if applicable, plasma retinol and retinol-binding protein, lipidomic tests, ceramides, metabolipidomic tests, systemic bone formation/resorption markers, inflammation and oxidative stress markers;
9. All eligible patients will be stratified according to 1- Baseline FEV_1, 2- PEx number in the prior year, and 3- Co-administration of Kalydeco® (ivacaftor), Orkambi® (ivacaftor/lumacaftor), Symdeko® (ivacaftor/tezacaftor) or another commercially available CFTR modulator product, and equally allocated/randomized to either a daily dose of 300mg of LAU-7b, (as 3 x 100 mg oral hard gelatin capsules), or matching placebo.
10. Randomized patients will receive their first dose of study treatment under supervision, under fed condition and will receive a 21-day supply of study treatment. Patients will be discharged after tolerability of the first dose has been observed (minimum one hour post-dose).

9.2.3 Report of a pulmonary exacerbation (any time during the study)

Patients will be instructed to adhere to the standard of care in use at the institution, that is to contact their treating physician and team when they feel that they are experiencing a change in symptoms for at least 3 days (ease of breathing, cough, quantity and nature of sputum, activity tolerance). If indicated based on the initial interaction, the study team will invite the patients to the clinic to do a proper assessment and determine a course of action such as antibiotic treatments. Being an efficacy variable, particular attention will be put on obtaining precisely the start of any antibiotics, whether oral or IV. If an IV antibiotic is prescribed and the patient can attend the study site prior to starting the IV antibiotics and after the planned end of antibiotics, this will trigger a set of pre- and post-antibiotic treatment additional blood sampling for study-related markers, a pulmonary function test (FEV_1), as well as measurements of weight, with BMI calculated. Details are presented in Section 9.3 "Pulmonary Exacerbation Reporting and Evaluation".

9.2.4 Telephone contacts (around Days 10, 38, 66, 94, 122 and 150)

All patients will be contacted by phone on or about 10 days post-first dose of each cycle, to inquire about potential adverse events. Specific attention will be put on probing about adverse events related to visual disturbances or dermatological problems as follows: "How is your vision since the last visit/phone call?" and "How is your skin since the last visit/phone call?" Actions may be taken if necessary.

9.2.5 End-of-Cycle Visits (Visits 4, 5, 6 and 7, on Days 21(window: Days 21-22), 49(window: Days 49-51), 77(window: Days 77-79), 105 (window: Days 105-107) and 161(window: Days 161-163)

Special attention will be put on reports of symptoms/signs of pulmonary exacerbation by patients as this may trigger a set of additional assessments. Details are presented in Section 9.3 "Pulmonary Exacerbation Reporting and Evaluation". Adverse events will be monitored and captured at all visits.

1. **On Days 21, 77 and 161 visits**, the following assessments will also be done:
    a. Focused physical examination;
    b. Ophthalmological evaluation: The NVQ will be self-administered, and if Grade 2 severity criteria is met or exceeded (On Day 77 visit, the ophthalmological examination is planned, independently of the NVQ result), a full ophthalmological examination will be performed (includes dark adaptation tests), and if clinically indicated by the ophthalmologist, an electroretinogram will also be performed, in order to evaluate any potential ophthalmological condition developed during the treatment cycle);
    c. Vital signs and 12-lead ECG;
    d. Pulmonary function tests (FEV_1);
    e. Pre-dose blood sampling and urine collection for safety laboratory tests, pregnancy test if applicable, plasma retinol and retinol-binding protein;
    f. Study drug compliance check and concomitant medications;
    g. Body weight, with BMI calculated.
2. **On Days 49 and 105 visits**, the following assessments will also be done;
    a. Safety laboratory tests;
    b. Pulmonary function test (FEV_1);
    c. Concomitant medications.
3. **On Days 21 and 77 visits only**: All patients will return prior treatment boxes, for accountability purposes and will receive re-supply of study treatment for their next treatment cycle(s);
4. **On Days 21 and 161 visits only**: Blood sample for the determination of fenretinide plasma through (C_{min}) concentrations, plasma lipidomic, ceramides and metabolipidomic tests, markers of inflammation and oxidative stress;
5. **On Day 77 visit only**: Ophthalmological evaluation: Complete ophthalmological examination (includes dark adaptation tests). If clinically indicated by the ophthalmologist, an electroretinogram will also be performed;
6. **On Days 77 and 161 visits only**, the following assessments will be done:
    a. Sputum will be induced using hypertonic saline for the assessment of the CFU for *PsA;*
    b. CFQ-R questionnaire to be filled out;
    c. At select sites participating in the investigation of the Matouk Disease Score, the Score will be determined.
7. **On Day 161 visit only:** systemic bone formation/resorption markers.

9.2.6 End-Of-Study Follow-up Visit (Visit 8, around Day 189)

The Investigator will review the totality of the safety information including the most recent test results and take appropriate actions as necessary. Adverse events still ongoing at the time of the visit will be assessed and evaluated for further follow-up as necessary. The following will also be performed:

1. Return prior treatment boxes, for accountability purposes;
2. Vital signs and 12-lead ECG;
3. Complete physical examination;
4. Ophthalmological evaluation: The NVQ will be self-administered and a complete ophthalmological examination (includes dark adaptation tests). If clinically indicated by the ophthalmologist, an electroretinogram will also be performed;
5. Pulmonary function tests (FEV_1);
6. Blood sampling and urine collection for safety laboratory tests, pregnancy test if applicable, lipidomic tests, plasma retinol and retinol-binding protein;

7. Body weight, with BMI calculated;
8. For patients participating in the bone density exploratory assessment, they will undergo their post-study assessment;
9. Final CFQ-R questionnaire will be filled out;
10. At select sites participating in the investigation of the Matouk Disease Score, the final Score will be determined.

9.3 Pulmonary Exacerbation Reporting and Evaluation

Below is the protocol definition of a pulmonary exacerbation, based on patient reporting it to the Study Staff at the time of scheduled visits of the study or patient reporting it by communicating with the Study Staff between scheduled study visits. Patients will be provided with contact information cards to facilitate the reporting, which is deemed to be standard of care at the site. Since there are several PEx-related efficacy endpoints, all potential PEx-related signs and symptoms will be carefully documented in the specific e-CRF screening tool, in order to determine during the analysis phase if they met the protocol definition of PEx. A sample of this PEx screening tool can be found in the Sample Case Report Form.

If indeed these result in a suspicion of a PEx requiring IV antibiotic treatment or any need to attend the clinical site, the patient will be invited to the clinical site to complete the assessment and for the Study Investigator to determine if an intervention is required and in particular if a new antibiotic (oral or IV) is required to treat the signs and symptoms. This visit at the clinical site should be scheduled at the earliest convenience to avoid any worsening of the patient's condition, e.g. not waiting for the next scheduled study visit if it is not happening soon enough to ensure the proper standard of care. If the PEx happens when the clinical site cannot attend to it, then the patient may miss the pre-PEx visit and procedures without impacting study participation.

At the time of the visit, the site staff including the Study Investigator will confirm the previously reported signs and symptoms and any changes affecting them, as well as perform PEx assessments such as pulmonary function tests, oxygen saturation and measure weight. It will also be an occasion to review concomitant medications and update adverse events. The Study Investigator will determine the proper treatment, if any, for the signs and symptoms, all to be reported as adverse events, and if the Study Investigator determines that they constitute a PEx, they will also be reported as such in the e-CRF and the patient's records, along with the prescribed treatment. If an IV antibiotic course is required and the patient can attend the clinical study site prior to starting the IV antibiotics, then this will trigger the actions listed below in Section 9.3.2, prior to starting the first dose of IV antibiotics. The IV antibiotics may be administered at clinics more convenient for the patient, but under some oversight by the Study Investigator.

During the IV antibiotic treatment days, the patients should receive the proper Standard of Care. They should be informed that another study visit will happen immediately or shortly after completing the IV antibiotic course. That visit can coincide with the visit to the clinical site to receive the last dose of the IV antibiotic if indeed the antibiotic was administered at the clinical site.

9.3.1 Protocol Definition of a Pulmonary Exacerbation

The presence of a protocol-defined pulmonary exacerbation is defined by the following, the criteria of Fuch et al. [94]: "Exacerbation of respiratory symptoms": a patient treated with parenteral antibiotics for any 4 of the following 12 signs or symptoms:

Change in sputum	New or increased hemoptysis	Increased cough
Increased dyspnea	Malaise, fatigue or lethargy	Temperature above 38°C
Anorexia or weight loss	Sinus pain or tenderness	Change in sinus discharge

Change in physical examination of the chest	Decrease in pulmonary function by 10 percent or more from a previously recorded value	Radiographic changes indicative of pulmonary infection

This definition is not a guide to determine the need for a given type of treatment (for example: Oral versus IV antibiotics, corticosteroids or no). Each Investigator will determine to the best of its clinical judgement and for the benefit to the patient, the best treatment for the exacerbation episode. However, if indeed an IV antibiotic course is selected based on the severity of the PEx and other factors and the patient can attend the study site prior to starting the IV antibiotics, this will trigger the performance of PEx-specific assessments prior to initiating the IV antibiotic course and at the end of the course. This is outlined below:

9.3.2 Specific additional testing for patients undergoing a pulmonary exacerbation treated with an IV antibiotic treatment cycle.

Prior to starting and after completing the IV antibiotic treatment:

1. Blood sampling for selective biomarkers such as:
    a. Plasma lipidomics: AA, DHA, calculated AA/DHA ratio and EPA;
    b. Markers of inflammation in:
        - Whole blood: total white cell count and absolute neutrophil count;
        - Serum: high sensitivity C-Reactive Protein (hsCRP) and serum amyloid A (SAA);
        - Plasma: interleukins IL-1ra, IL-6, IL-8, IL-10, Granulocyte-Colony Stimulating Factor (G-CSF), calprotectin, ceruloplasmin, haptoglobin, and neutrophil elastase antiprotease complexes (NEAPC).
2. Clinical parameters such as FEV_1 percent predicted, weight, with BMI calculated.

9.3.3 Pulmonary Exacerbation Evaluation and Conventions

The duration of PEx antibiotic treatment, whether IV or Oral, should be set at the shortest possible duration achieving the desired eradication of infection and alleviation of the PEx symptoms. It should always be 7 days or more in duration. Corticosteroids, albeit authorized for the treatment of PEx episodes, should be reserved for the more severe cases and their usage (dose and duration) set to the minimum required.

For reporting purposes, a PEx episode is considered an adverse event and its severity should be commensurate with the type of treatment required. The following is the information required at a minimum when reporting a PEx episode; please refer to the PEx case report form for more details.

1. Precise dates of initial reporting by the patient to the study staff;
2. Date of appointment set to evaluate the PEx;
3. Complete list of items from the Fuch scale listed in Section 9.3.1 observed for the patient;
4. Investigator judgment on the Severity of the PEx episode;
5. Prescribed antibiotic treatment (name of medication, route of administration and planned duration of treatment) and other support medications (e.g. corticosteroids);
6. Record of PEx-related specific blood sampling for pre-antibiotic treatment biomarkers, body weight and BMI, if the prescribed antibiotic treatment is intravenous;
7. Record of PEx-related specific pre-antibiotic treatment FEV_1 value, if the prescribed antibiotic treatment is intravenous;
8. Precise record of initiation of intravenous antibiotic treatment, if applicable;
9. Record of PEx-related specific blood sampling for post-antibiotic treatment biomarkers, body weight and BMI, if the prescribed antibiotic treatment is intravenous;

10. Record of PEx-related specific post-antibiotic treatment FEV_1 value, if the prescribed antibiotic treatment is intravenous;
11. Actual time of completion of intravenous antibiotic treatment, if applicable;
12. Any other information required in the case report form.

9.4 Study Drug Treatment

The total duration of study treatment is 6 cycles of 21 consecutive days of either LAU-7b or placebo treatment each followed by a 7-days treatment-free period. This represents approximately 24 weeks in total.

During the study, eligible CF patients aged 18 years and older will be randomized, after stratification according to 1- FEV_1 severity categories, 2- Number of PEx episodes in prior year, and 3- Co-administration or not of Kalydeco®, Orkambi®, Symdeko® or another commercially available CFTR modulator product, using a 1:1 allocation to LAU-7b 300 mg per day or matching placebo, in a double blind fashion. Treatment assignments will be determined through the IWRS system.

In all cases, the study treatment will be administered on top of current Standard of Care therapies.

9.4.1 LAU-7b (fenretinide)

It consists of an orange opaque, size 00, hard gelatin capsule containing 100 mg of fenretinide. The formulation contains the following non-medicinal ingredients: [redacted]

The capsules are dispensed in dedicated Patient Kit (Drug Packs, 21 sachets /Drug Pack). Study medication and their outer boxes must be stored in a -20°C freezer until dispensing to the patient who will then store in the refrigerator (2 to 8°C).

9.4.2 Placebo

A matching orange opaque, size 00 hard gelatin capsule without fenretinide will be used as a placebo for blinding purposes and will contain the same non-medicinal ingredients.

The capsules are dispensed in dedicated Patient Kit (Drug Packs, 21 sachets /Drug Pack). Study medication and their outer boxes must be stored in a -20°C freezer until dispensing to the patient who will then store in the refrigerator (2 to 8°C).

9.4.3 Packaging, Labeling, and Shipping

Study medication will be provided in patient kits containing enough medication for one treatment cycle. Study drug will be specifically identified and bear instructions to ensure proper order of intake at all times.

Each Patient Kit box will bear a label that conforms to the appropriate local regulations. The details will be described in the Pharmacy Manual.

Shipments will be done using a Sponsor-defined courier, under frozen temperature (target -20°C), a temperature data logger may accompany the shipment to ensure an adequate chain of custody.

9.4.4 Storage, Dispensing and Compliance Verification of Study Drug

Upon receipt of the study drug shipper container, the investigator should record the receipt, date, time, and temperature of the product based on inspection or on the temperature logger reading. Any study drug that arrives in improper storage conditions or is damaged in any way must be reported to the Laurent designee,

as instructed in the Pharmacy Manual, as soon as possible and shall not be administered until instructed otherwise.

At the clinical sites, the study drug kits will be stored in a suitable -20ºC (-15°C to -25°C) freezer in a secure location with limited access. Most preferably, the storage freezer will be continually monitored and alarmed in order to document any excursion outside the desired temperature range. Alternatively, the use of a calibrated min-max thermometer in the storage freezer is acceptable as long as the thermometer readings are taken daily during weekdays, at a minimum.

Upon instructions from the IWRS, the pharmacy/site staff will, at the time of dispensing, formally identify and remove the assigned Patient Kit box(es) from the -20ºC freezer and will check the expiry date on the labels on the Patient Kit box(es), at a minimum. Where applicable, the necessary Patient Kits will be sent (in an isolated container with ice packs) to the site staff actually dispensing the study drug to the patients.

The study drug may only be used as directed in this APPLAUD protocol. It is against regulations to use investigational products for other purposes.

Prior to some of the end-of-cycle visits (Days 21, 77 and 161), the patients will be reminded to bring their used box(es) of supplies including the capsule containers, for drug accountability. The number of capsules consumed per cycle will be recorded for the 21 days of study drug treatment. Patients will be encouraged toward full compliance with instructions.

9.4.5 Administration of Study Drug Treatment

At specific patient visits, the patient will receive a number of Patient Kit boxes sufficient until the next dispensing visit. To insure compliance (given the 7-days drug-free interval) SMS or phone reminders will be put in place at the beginning of each cycle.

Study drug will be taken orally, along with the first meal of the day, morning if possible, along pancreatic enzyme supplements, where applicable.

9.4.6 Study Drug Reconciliation and Destruction

Investigators must maintain accurate records regarding the receipt, dispensing, and return or destruction of study drug for each patient in the study. Any used study drug containers, as well as any unused containers or unused portions of containers, must be maintained until accounted for by the monitor. After accountability by the monitor and approval from the sponsor, the study drug and containers should be destroyed per the site's SOP for destruction of biological waste or returned to the Sponsor or Sponsor designee for disposition.

9.5 Treatment and Protocol Compliance

Patients are expected to receive oral daily doses based on the protocol schedule and have all procedures done within the allowable time windows (detailed in the schedule of events, page 10).

Regardless of allowable visit windows, study drug treatment cycles should always be 28 days, each consisting of 21 consecutive days of either LAU-7b or placebo treatment, followed by a 7-days treatment-free period. In the event a patient misses to start a cycle on time, he/she should start the cycle as soon as possible and inform the study site staff so that future visit schedule be adjusted accordingly.

In the event a patient misses a scheduled assessment visit, this should be rescheduled for the earliest possible date. Patients who are persistently noncompliant may be withdrawn from the study at the investigator's or the sponsor's discretion.

9.6 Allowed and Disallowed Concomitant Medications

The standard of care for patients with CF includes several medications, nutritional recommendations and procedures destined to improve their quality of life, treat symptoms and prevent pulmonary infections. This section outlines the general study specific requirements in terms of concomitant medications, vitamin supplements and potential drug interactions. Details and lists of specific allowed/disallowed medications/interactants will be provided as part of the Study Reference Manual.

9.6.1 Allowed Concomitant Treatments:

1. While it is understood that vitamin supplementation is essential for CF patients, in order to standardize and keep constant their intake, patients will discuss with study personnel their vitamin supplementation in daily standard dose oral multivitamin supplement (e.g.: AquADEK ™), so the amount of vitamins intake can be agreed upon and monitored carefully. Particular attention will be placed on Vitamin A, for which existing supplementation must be assessed in detail such as identifying their type (provitamin A carotenoids, preformed Vitamin A) and quantities, so as to derive their Retinol Activity Equivalents (RAE). Patients should report any deviation to their agreed upon Vitamin A, D, C, E or K intake, including over-the-counter or fitness-related supplements. Plasma retinol and retinol-binding protein levels will be monitored regularly during the study. [redacted];
2. Medications to treat concomitant health conditions are allowed as long as their intake has not changed in the in the 5 weeks prior to randomization, of which 2 weeks minimum are prior to screening and is kept constant throughout the entire duration of the study participation. Included in this category are pancreatic enzyme supplements, which should remain generally constant during the study. However, patient self-instructed acute dose increases justified by dietary fluctuations are acceptable, and should be noted. Patients will report immediately any unexpected change/addition to their concomitant medications, including medications added to treat a non-CF-related acute condition. Some classes of medication may be restricted; please see below the list of disallowed medications;
3. Inhaled corticosteroids for asthma/rhinitis control are allowed. Systemic corticosteroid for asthma control should be minimized and should be restricted to control asthma exacerbations or pulmonary infections associated with bronchospasm. A maximum dose of 5 mg/day of prednisone or equivalent (or a higher loading dose tapered down in subsequent days) will be acceptable on study for the above purpose but not chronic dosing;
4. Steroids (but not corticosteroids) for routine metabolic deficiency states are allowed, as long as their dose can be kept constant during the trial;
5. Antibiotics treatments recommended by the Cystic Fibrosis Foundation guidelines which includes chronic suppressive aerosol antimicrobial therapy (tobramycin, preservative free tobramycin (aerosol or powder), colistin or aztreonam) or azithromycin as an anti-inflammatory antibiotic. No other antibiotics than those recommended in the CCF guidelines are permitted. In all cases, changes or additions of oral, inhaled or intravenous antibiotics will be recorded on the Case Report Forms.

9.6.2 Disallowed medications:

After consulting the Study Reference Manual, in case of doubt about a patient's concomitant medication, study personnel should consult with the Sponsor's representative before enrolling/pursue participation of a patient on study.

1. Any drugs that can lower plasma retinol levels, such as cholesterol-lowering medications (cholestyramine, colestipol and colesevelam), neomycin, certain weight loss medications (orlistat, and olestra) and laxatives containing mineral oil;
2. Drugs known to cause night blindness such as chloroquine and phenothiazines;

3. Intake of omega-3 fatty acid is completely prohibited from the first date of screening and for the entire duration of participation to the study. The cessation of consuming any such supplements must be at least 2 weeks prior to randomization (Baseline);
4. Intake of medications that may potentially act as modulators of intracellular ceramide levels or ceramide cytotoxicity, sphingolipids transport, or p-glycoprotein "MDR1" or "MRP1" drug/lipid transporters, such as: cyclosporine A or analogue; verapamil; tamoxifen or analogue; ketoconazole, chlorpromazine and thioridazine; RU486 (mifepristone); indomethacin; or sulfinpyrazone;
5. Intake of CFTR potentiators/correctors. Only the intake of Kalydeco® (ivacaftor), Orkambi® (ivacaftor/lumacaftor), Symdeko® (ivacaftor/tezacaftor) or another commercially available CFTR modulator product, is allowed, as long as administered over a minimum of 3 months prior to screening if naïve to CFTR modulators and 1 month if switched from another CFTR modulator product deemed to be well tolerated by the treating physician;
6. Any other investigational drug is prohibited unless discontinued at least 30 days, meaning from the last study drug administration of the prior study (or five elimination half-lives, whichever is longer) prior to screening.

10 EFFICACY ASSESSMENTS

10.1 Spirometry

All sites will be provided with spirometers to be used for all study assessments. Spirometry data will be transmitted to a centralized spirometry service for quality review.

Spirometry will be performed according to the American Thoracic Society Guidelines at time points noted in the Schedule of Events, page 10, and according to the additional instructions that follow. Pre-bronchodilator spirometry is defined as spirometry testing performed for patients who have

- Withheld their short-acting bronchodilators (e.g., albuterol) or anticholinergic (e.g., ipratropium) for more than 4 hours before the spirometry assessment;
- Withheld their long-acting bronchodilator (e.g., salmeterol) for more than 12 hours before the spirometry assessment; and
- Withheld their once-daily, long-acting bronchodilator (e.g., tiotropium bromide) for more than 24 hours before the spirometry assessment.

During the Screening Period, spirometry assessments may be performed pre- or post-bronchodilators. At all other visits, all spirometry assessments should be performed "pre-bronchodilator." During the Treatment Period, spirometry assessments must be performed before dosing. In the event that a patient forgets to withhold bronchodilator(s), spirometry should be performed according to the following:

- If a patient's Day 1 spirometry is pre-bronchodilator but on a subsequent visit, the patient forgets to withhold bronchodilator use, a post-bronchodilator spirometry will be obtained for that visit only, and the visit will not be rescheduled.
- If, on Day 1, the patient forgets to withhold his/her dose of bronchodilator, spirometry should be performed post-bronchodilator, and all subsequent spirometry measurements (according to the Schedule of Events, page 10) should be performed post-bronchodilator.
- Each spirometry assessment will be recorded in the source documents as pre-bronchodilator or post-bronchodilator.

10.1.1 Parameters measured:

At a minimum, FEV_1 (percent predicted); this is the primary efficacy endpoint.
Other parameters: FEV_1 (L), Forced Vital Capacity (FVC, L), FEV_1/FVC and Forced Expiratory Flow ($FEF_{25-75\%}$ L/s).

The parameters listed below will be normalized using the Global Lung Initiative standards.

- FEV_1 (L)
- FVC (L)
- FEV_1/FVC (ratio)
- $FEF_{25\%-75\%}$ (L/s)

10.2 Lipidomic endpoints

Blood samples for the scheduled determination of lipidomic endpoints (AA, DHA and EPA) will be obtained at the time points listed in the Schedule of Events, page 10, specifically on Days 1, 21, 161 and 189 (Visits #2, 3, 7 and 8, respectively). Results will not be communicated to blinded staff, in order to maintain the study blind, unless required for emergency reasons. In addition, if the patient experiences a PEx episode during the study and this episode requires an IV course of antibiotic treatment, this will trigger pre- and post-antibiotic blood sampling for several markers including lipidomic; this PEx-related assessment is detailed in Section 9.3.2. Details of collection, storage and processing are provided in the Study Reference Manual and when instructed to do so, prepare all the samples in need of shipment to the designated central lab [redacted] for dispatch to the specialist laboratory, [redacted] Laval, Québec, Canada [redacted] and subsequently to [redacted], Laval, Quebec, Canada.

The lipids will be extracted from the sample and esterified by methods inspired by Folch [95] and Gellerman & Schlenk [96]. The esters will be quantified by chromatography and tandem mass spectrometry. The concentrations of the fatty acids AA, DHA and EPA, will be normalized by plasma phospholipids and free fatty acids content and will be expressed as nmol/mL. Extraction from and normalization by red blood cell phospholipids will also be explored using samples collected at specific clinical sites.

10.3 Markers of inflammation

Blood samples for the scheduled determination of systemic markers of inflammation will be obtained at the time points listed in the Schedule of Events, page 10, specifically on Days 1, 21 and161 (Visits #2, 3 and 7, respectively). In addition, if the patient experiences a PEx episode during the study and this episode requires an IV course of antibiotic treatment, this will trigger pre- and post-antibiotic blood sampling for several markers including inflammation; this PEx-related assessment is detailed in Section 9.3.2. Details of collection, storage and processing are provided in the Study Reference Manual and when instructed to do so, prepare all the samples in need of shipment to the designated central lab [redacted], for whole blood-based analyses and dispatch of plasma and serum to the specialist laboratory, [redacted], Aurora, CO.

For immunological, inflammation markers, an aliquot of whole blood, plasma or serum will be used according to the test, as following:

- Whole anti-coagulated blood will be used for the determination of: total white cell count and absolute neutrophil count;

- Plasma will be used for the determination of calprotectin, haptoglobin, ceruloplasmin, interleukins IL-1ra, IL-6, IL-8, and IL-10, granulocyte colony stimulating factor (G-CSF) and neutrophil elastase antiprotease complexes (NEAPC);
- Serum will be used for the determination of high sensitivity C-Reactive Protein (hsCRP) and serum amyloid A (SAA).

10.4 Body height, weight, with BMI calculated

Height (screening only) and weight will be measured with shoes off at time points listed in the Schedule of Events, page 10, specifically at screening and on Days 1, 21, 77, 161 and 189 (Visits #1, 2, 3, 5, 7 and 8, respectively). Weight will be measured before the morning dose of the study drug, where applicable. The body mass index (BMI) will be calculated using standard methods.

In addition, if the patient experiences a PEx episode during the study and this episode requires an IV course of antibiotic treatment, this will trigger pre- and post-antibiotic assessment of a few clinical variables including weight and BMI; this PEx-related assessment is detailed in Section 9.3.2.

10.5 Pseudomonas aeruginosa (PsA) density in sputum

Sputum will be obtained by hypertonic saline induction at time points listed in the Schedule of Events, Page 10, specifically at screening, Days 77 and 161 (Visits #1, 5 and 7, respectively) In order to minimize inter-site variability in the quality of the sputum samples, a protocol for inducing and obtaining sputum samples will be used across sites. It can be found in the Study Reference Manual. Training on the procedures will be provided to the clinical site staff.

The initial processing of the samples will be carried out at the clinical sites; the sputum induction will result in the patient producing a series of sputum samples. Upon obtaining the samples, their quality will be confirmed on the spot by macroscopic examination and weighing. Tubes containing suitable samples will be shipped overnight within 24 hours, in refrigerated state to the specialist laboratory [redacted], Denver, CO [redacted], USA.

10.6 Cystic Fibrosis Questionnaire – Revised (CFQ-R)

Patients will be asked to complete the CFQ-R in their native language, if validated translations are available. The CFQ-R will be completed before dosing at visits noted in the Schedule of Events, Page 10, specifically on Days 1, 77, 161 and 189 (Visits #2, 5, 7 and 8, respectively). The respiratory domain subscore will be extracted at time of data processing. The questionnaires provide information about demographics; general quality of life, school, work, or daily activities; and symptom difficulties (pertaining to CF). Copies of the CFQ-R used in this study will be provided in the Study Reference Manual. Validated translations of the CFQ-R, if available, will be provided for non-English-speaking patients/clinical sites. The CFQ-R must be completed before the start of any assessments scheduled at that visit as mentioned in the Timing of Assessments, Section 10.

10.7 Fenretinide Through Samples

In this study, patients will contribute PK samples for the determination of steady-state fenretinide through concentrations (C_{min}), 4-methoxy fenretinide (4-MPR), a significant but inactive metabolite as well as those

of 4-oxo-fenretinide, a minor active metabolite of interest. Blood samples for fenretinide and metabolites C_{min} will be obtained pre-dose or 24 hours post-previous dose, at the time of the safety lab sampling, on Days 21 and 161 time points, as listed in the Schedule of Events, Page 10. More specifically, since this is a double blind placebo controlled study, all patients whether or not on fenretinide, will undergo the sampling procedure. Results will not be communicated to blinded staff, in order to maintain the study blind, unless required for emergency reasons.

Patients will be informed at time of screening of the mandatory nature of the PK procedure and pre-dose requirements (arrive fasting at the clinical site on Days 21 and 161). All the blood collection and sample processing must be carried out in reduced light condition. Details of collection, storage and processing are provided in the Study Reference Manual and when instructed to do so, prepare all the samples in need of shipment to the designated central lab [redacted], for dispatch to the bioanalytical laboratory, [redacted] Laval, Quebec, Canada.

10.8 Other exploratory biomarkers: ceramides, metabolipidomics, systemic bone formation/resorption markers and oxidative stress markers

Blood samples for the determination of other biomarkers, such as ceramides, metabolipidomic, systemic bone formation/resorption markers and oxidative stress markers, will be obtained at the time points listed in the Schedule of Events, Page 10.

10.8.1 Ceramides

In this study, ceramides will be analyzed by, and according to a method developed by Dr. [redacted]). Details of collection, storage and processing are provided in the Study Reference Manual and when instructed to do so, prepare all the samples in need of shipment to the designated central lab [redacted], for dispatch to the specialist laboratory [redacted], Czech Republic.

10.8.2 Metabolipidomics

In this study, the metabolipidomic analysis will consist in quantifying eicosanoid metabolites (specific AA-derived leukotrienes, lipoxins, prostaglandins and thromboxane) and docosanoid metabolites (specific DHA-derived resolvins, protectins and maresins) in plasma phospholipids. Metabolite levels will be analyzed quantitatively by assessing the molecular decomposition using a LC/MS/MS method.

Details of collection, storage and processing are provided in the Study Reference Manual and when instructed to do so, prepare all the samples in need of shipment to the designated central lab [redacted], for dispatch to the specialist laboratory [redacted], The Netherlands.

10.8.3 Systemic bone formation/resorption markers

The selected markers are serum osteocalcin, serum C-telopeptide (CTx) and serum P1NP. Samples will be obtained at the time points listed in the Schedule of Events, page 10, specifically on Days 1 and 161 (Visits #2 and 7, respectively). Details of collection and processing are provided in the Study Reference Manual and when instructed to do so in the Study Reference Manual, prepare all the samples in need of shipment to the designated central laboratory [redacted], Tennessee [redacted], USA.

10.8.4 Markers of oxidative stress

Blood samples for the scheduled determination of systemic markers of oxidative stress will be obtained at the time points listed in the Schedule of Events, page 10, specifically on Days 1, 21 and 161 (Visits #2, 3 and 7, respectively).

Details of collection, storage and processing are provided in the Study Reference Manual and when instructed to do so, prepare all the samples in need of shipment to the designated central lab [redacted], for dispatch to the specialist laboratory, [redacted], Aurora, CO.

10.9 Bone density

The bone density will be determined at time points listed in the Schedule of Events, Page 10, specifically on Days 1 and 189 (Visits #2 and 8, respectively), at selected sites and in the first 24 patients who volunteer to participate in this additional investigation at the given study sites. The lumbar spine bone mineral density will be measured with a dual X-ray absorptiometry (DXA) instrument. Specifics to ensure consistency of DXA measurement such as the expression of results as a Z-score normalized according to age and gender, the need to use the same instrument and the need to follow the institutional procedure to ensure within-patient comparability of measurements will be described in the Study Reference Manual.

10.10 Matouk Disease Score

The Matouk disease score will be determined at time points listed in the Schedule of Events, Page 10, specifically on Days 1, 77, 161, 189, (Visits #2, 5, 7 and 8, respectively), at selected sites who will volunteer to participate in this exploratory investigation. The objective of this investigation is to further validate the Score in the framework of a well controlled therapeutic study. An example of the subsection questionnaires is presented in the Study Reference Manual. Given the lack of recognized biomarkers for CF inflammation clinical studies, and the long time required for FEV_1 to show changes, we believe that exploring additional alternatives will be welcomed by the CF community.

The Matouk Disease Score was previously published [97, 98], and reviewed by Hafen [99] and was used recently in two studies; 1- A non-interventional study on pulmonary exacerbations by Dr Matouk and 2- A Phase Ib study of fenretinide [3, 100]. Hafen recommends Matouk scoring as a potential tool to assess the clinical response to various treatment regimens on a short-term basis. The validation showed its value in prognostic evaluation of patients in end-stage disease, providing better discrimination for survival in the last 2 years of life than FEV_1 % predicted alone [98].

The Matouk Disease Score is a sum comprised of four subsections: clinical, pulmonary function tests (PFT), chest radiographic score (CXR) and complications score. The 3 key subsections with high impact on the total score internal consistency are the Clinical, the PFT and the Complication subsections. The CXR score is not essential for the Matouk score to perform well. If a new CXR is available, then its grading is used. Otherwise, the most recent CXR can be used instead. In the absence of a CXR, the radiographic score is omitted, and the total score internal consistency, as depicted by the Cronbach's coefficient alpha, is preserved. Consequently, the CXR score is optional.

The clinical subsection involves the evaluation of the patient's symptoms by the physician. The symptoms evaluated are weight, weight change, dyspnea, cough frequency, sputum colour, thickness and volume, physical examination (air entry, crackles, hyperinflation), respiratory rate/cardiac frequency, sputum culture, appetite and general condition.

The PFT subscore is based on the results of pulmonary function test.

The complication sub-score tabulates the serious events that are known to negatively impact CF disease: pulmonary exacerbation requiring therapy, pneumothorax, haemoptysis, respiratory failure, cardiac enlargement or congestive heart failure and pulmonary surgery.

The CXR score is the evaluation of the chest x-ray [optional in this study]

For the clinical subsection, the PFT and CXR subscores, the higher the better. For the complications score, the lower score is better. The total score is calculated by subtracting the complications score from the three subscores (total score = clinical + PFT + CXR (if available) – Complications).

The score uses information and measurements that should already be available at the clinical site. To facilitate the data entry/capture, the Sponsor will develop clear and simple templates to collect such data, and training will be provided. The sites will be instructed to send the completed forms to a centralized location for scoring and interpretation.

11 SAFETY ASSESSMENTS

Safety evaluations will include reporting of Adverse Events (AEs), clinical laboratory assessments, clinical evaluation of vital signs, ECGs, as well as ophthalmological questionnaires and examinations.

11.1 Adverse Events

All AEs will be assessed, documented, and reported in accordance with ICH GCP guidelines.

11.1.1 Documentation of Adverse Events

Adverse events will be collected from the time of informed consent until the last scheduled study follow-up, 4 weeks after the last dose of the study drug, or until the last unscheduled safety-related assessment whichever occurs latest. An AE is any untoward medical occurrence (which does not necessarily have to have a causal relationship with this treatment). An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the study drug. This includes any occurrence that was new in onset or aggravated in severity or frequency from the baseline condition. Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was screened in the study are **not** to be considered AEs unless the condition deteriorated in an unexpected manner during the study (e.g. surgery was performed earlier than planned).

Abnormal results of diagnostic procedures, including laboratory test abnormalities, are considered AEs if they result in any one of the following:

- Discontinuation of study treatment;
- Require treatment or any other therapeutic intervention;
- The necessity for further diagnostic evaluation (excluding a repetition of the same procedure to confirm the abnormality);
- Association with clinical signs or symptoms that may have a significant clinical impact, as determined by the Investigator.

Patients are encouraged to report AEs spontaneously or in response to general, non-directed questioning. All AEs are to be followed until resolution or until a stable clinical endpoint is reached. The investigator should question patients about AEs and changes in pre-existing illnesses since their last visit and must record the information in the patients' medical records. The onset and end dates, severity, relationship to study agent, action taken, and outcome must be recorded for each AE. All AEs are to be recorded on the appropriate e-CRF and in detail on the source documents.

Any AE that occurs within 4 weeks following the last dose of the study drug will be considered treatment emergent (TEAe) and must be recorded in the e-CRF and, if an SAE, reported immediately to the Sponsor. Adverse events that are ongoing at the end of the 4-week follow-up period should be marked as ongoing. However, it is the responsibility of the Investigator to follow up on these events until resolution, according to standard medical care.

Any AE or SAE that the investigator becomes aware of outside of the reporting period until the end of the study that has or is believed to have a causal relationship to the study drug should be reported to Laurent

Pharmaceuticals via telephone. Patients who experience AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the Investigator. All AEs and laboratory abnormalities encountered during the study should be followed until resolution or stabilization of the event(s). Any action taken and follow-up results must be recorded in the patient's medical record. Follow-up laboratory results should be filed with the patient's source documentation and e-CRF. For all AEs that require the patient to discontinue treatment, relevant clinical assessments and laboratory tests should be repeated on at least a monthly basis until final resolution or stabilization of the event(s). These assessments should be captured in the source data and SAE forms but will not be entered in the e-CRF.

All AEs for randomized patients will be recorded in the e-CRF and the patient's source documents. AEs for patients who are screened but not subsequently randomized in the study will be recorded only in the patient's source documents. The following data should be documented for each AE:

- Description of the event;
- Classification of "serious" or "not serious";
- Date of first occurrence and date of resolution (if applicable);
- Severity;
- Causal relationship to study drug(s);
- Action taken;
- Outcome;
- Concomitant medication or other treatment given

An independent DSMB will assess AEs that are reported from study sites. The DSMB will meet at regular intervals throughout the duration of the study or will meet as determined by the DSMB chair, and may recommend systematic treatment arm dose reduction or early termination of the study for safety reasons (see Sections 7.2.4 and 7.2.2).

11.1.1.1 Adverse Event Severity

The Investigator must determine and record the severity of all serious and non-serious AEs. The Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03, (Cancer Therapy Evaluation Program website; available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm ; accessed 01 December 2015) should be used for grading the severity of AEs. AEs of CTCAE Grades 4 and 5 should be documented as "life-threatening."

The severity of an AE that does not appear in the CTCAE scale should be determined according to the definitions below. Clinically significant laboratory tests should be recorded as AEs in the patient's source documents and e-CRF.

Severity will be assessed according to the following criteria:

Classification	Definition
Mild (Grade 1)	Mild level of discomfort and does not interfere with regular activities
Moderate (Grade 2)	Moderate level of discomfort and significantly interferes with regular activities

Severe (Grade 3)	Significant level of discomfort and prevents regular activities
Life-threatening (Grade 4)	Any adverse drug experience that places the patient, in the view of the investigator, at immediate risk of death

11.1.1.2 Adverse Event Causality

Causality will be assessed according to the following criteria:

Classification	Definition
Definitely Related	Reasonable temporal relationship to study drug administration Follows a known response pattern (i.e., drug is known to cause this AE) There is no alternative etiology
Probably Related	Reasonable temporal relationship Follows a suspected response pattern (i.e., based on similar drugs) Little or no evidence for a more likely alternative etiology
Possibly Related	Reasonable temporal relationship Some evidence for a more likely alternative etiology
Not Related	Does not have a temporal relationship. Or, Definitely due to alternative etiology

ICH guidelines (March, 1995) clarify "reasonable causal relationship" to mean "that there are facts [evidence] or arguments to suggest a causal relationship."

The causality assessment must be made by the Investigator based on information available at the time that the AE/SAE worksheet is completed. The initial causality assessment may be revised as new information becomes available.

11.1.1.3 Study Drug Action Taken

The investigator will classify the study drug action taken with regard to the AE. The action taken should be classified according to the categories shown below:

Classification	Definition
Dose Not Changed	Study drug dose not changed in response to an AE;
Dose Interrupted	Study drug administration interrupted in response to an AE;

Drug Withdrawn	Study drug administration permanently discontinued in response to an AE;
Not Applicable	Action taken regarding study drug administration does not apply. "Not applicable" should be used in circumstances such as when the investigational treatment had been completed before the AE began and no opportunity to decide whether to continue, interrupt, or withdraw treatment is possible.

11.1.1.4 Adverse Event Outcome

An AE should be followed until the investigator has determined and provided the final outcome. The outcome should be classified according to the categories shown below:

Classification	Definition
Recovered/Resolved	Resolution of an AE with no residual signs or symptoms;
Recovered/Resolved with Sequelae	Resolution of an AE with residual signs or symptoms;
Not Recovered/Resolved (Continuing)	Either incomplete improvement or no improvement of an AE, such that it remains ongoing;
Fatal	Outcome of an AE is death. "Fatal" should be used when death is at least possibly related to the AE;
Unknown	Outcome of an AE is not known (e.g.,a patient lost to follow-up).

11.1.1.5 Treatment Administered

The Investigator will ensure adequate medical care is provided to patients for any AEs, including clinically significant laboratory values related to study drug. In addition, the Investigator will describe whether any treatment was administered for the AE. "Yes" is used if any treatment was administered in response to an AE and may include treatments such as other medications, hospitalization, surgery, or physical therapy. "No" indicates the absence of any kind of treatment for an AE.

11.1.2 Clinically Significant Assessments

Study assessments including laboratory tests, ECGs, ophthalmological examinations and vital signs should be assessed and those deemed a clinically significant worsening from baseline documented as an AE. When possible, a clinical diagnosis for the study assessment should be provided rather than the abnormal test result alone (e.g. urinary tract infection, anemia). In the absence of a diagnosis, the abnormal study assessment itself should be listed as the AE (e.g. bacteria in urine or decreased hemoglobin).

An abnormal study assessment is considered clinically significant if the patient has one or more of the following:

- Concomitant signs or symptoms related to the abnormal study assessment;
- Further diagnostic testing or medical/surgical intervention;
- A change in the dose of study drug or discontinuation from study drug treatment.

Repeat testing to determine whether the result is abnormal, in the absence of any of the above criteria, does not necessarily meet clinically significant criteria. The determination of whether the study assessment results are clinically significant must be made by the Investigator. A laboratory abnormality judged to be Grade 4, in itself, may not constitute an SAE unless the clinical status of the patient indicates a life-threatening AE.

11.1.3 Serious Adverse Events

SAEs are generally any AEs that result in one or more of the following:

- Fatal (death, regardless of cause, that occurs during participation in the study or occurs after participation in the study but within 30 days post-study, and is suspected of being a delayed toxicity due to administration of the study drug);
- Is immediately life threatening (i.e., presents an immediate risk of death at the time of the AE, not an AE that hypothetically might have caused death if it were more severe);
- Requires or prolongs inpatient hospitalization*;
- Causes persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect;
- Other important medical events that may not be immediately life threatening or result in death or hospitalization but, based upon appropriate medical judgment, are thought to jeopardize the patient and/or require medical or surgical intervention to prevent one of the outcomes defining an SAE.

*An inpatient hospitalization is defined as an admission for any length of time. A hospitalization for administration of study drug, for routine or planned clinical procedures, or for "social" reasons (not the result of any adverse change in the patient's condition) should not be considered an AE and should not be reported as a SAE. If the patient experiences any adverse change in condition during hospitalization, the condition must be reported as an AE or SAE according to the above definitions.

For the APPLAUD study, the following AEs may not require expedited reporting based upon the judgment of the Investigator:

- AEs that occur due to Standard-of-Care CF therapies and are consistent with the package labels for such products;
- AEs due to Pulmonary Exacerbation and unrelated to the study drug.

The investigator should consult with the medical monitor if there is any doubt regarding classification of an SAE.

Definition of Life-Threatening Adverse Experience:
An adverse experience is life threatening if the patient was at immediate risk of death from the event as it occurred (i.e., it does not include a reaction that, had it occurred in a more serious form, might have caused death). For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening, even though drug-induced hepatitis can be fatal.

Definition of Disabling/Incapacitating Experience:

An adverse experience is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the patient's ability to carry out normal life functions.

Any SAE that occurs during this study, including death from any cause other than disease progression, must be reported to the designated contact for SAE reporting within 24 hours via the e-CRF (preferred), telephone, fax, or email, whether or not related to the study agents. If initially reported via telephone or email, this must be followed up by a e-CRF/written report and submitted within 24 hours of the occurrence of the SAE. Follow-up information after the initial report, including the causality assessment and a narrative, should be sent via e-mail using the paper SAE report form.

Medical Monitor:
Dr Homaira Moqadar, Pharmacovigilance Lead
JSS Medical Research
[redacted]
hmoqadar@jssresearch.com

Additional Medical Contacts may be provided separately for the Australian and European components of the study; they will work in collaboration with Dr. Moqadar of JSS.

11.1.3.1 Serious Adverse Event Reporting

It is imperative that the safety desk be informed within 24 hours of an SAE so that reporting to the health authority can be met within the required time frame.

Because of the need to report to health authorities all serious adverse reactions in a timely manner, it is vitally important that an Investigator report immediately any adverse experiences which would be considered serious, even if the Investigator does not consider the adverse experience to be clinically significant or drug related. Should the Investigator become aware of an SAE (regardless of relationship to study drug) that occurs within 4 weeks after the last dose of the study drug, the SAE must be reported in accordance with the procedures specified in this protocol.

All SAEs that are not resolved by the end of the study, or that were not resolved upon discontinuation of the patient's participation in the study, are to be followed until the AE resolves, the AE stabilizes, the AE returns to baseline values (if a baseline value is available), or it is shown that the AE is not attributable to the study drug or study conduct. If a patient or a female partner of a patient becomes pregnant during the study, the patient will be removed from the study without receiving further study medication. Follow-up regarding the outcome of the pregnancy and any postnatal sequelae in the infant is required. Pregnancies are considered immediately reportable AEs (within 1 working day) and are to be documented in the e-CRF.

11.1.3.2 Suspected Unexpected Serious Adverse Reactions (SUSAR)

SUSARs are SAEs that are possibly related or related to the study drug and are unexpected (i.e., not listed in the investigator brochure). SUSARs will be collected and reported expeditiously to competent authorities and independent ethics committees (IECs)/institutional review boards (IRBs) according to regulations. Medical and scientific judgment is to be exercised in deciding whether expedited reporting is appropriate in other situations, such as for important medical events that are not immediately life threatening or do not result in death or hospitalization, but jeopardize the patient or the patient population.

11.2 Clinical Laboratory Assessments

Blood and urine samples will be collected according to the Schedule of Events (page 10) and analyzed at the central laboratory [redacted]. Specific instructions for the collection, processing, and shipment of samples are presented in the Study Reference Manual. Laboratory test results that are abnormal and considered clinically significant must be reported as AEs (as per Section 11.1.1). Screening laboratory results must be available and reviewed by the Investigator before randomization. The safety laboratory test panels are shown in Table 1 below:

Table 1: Laboratory Tests Panels

Serum Chemistry	**Hematology**	**Urinalysis***
Albumin	Hematocrit	Nitrite
Creatinine	Hemoglobin	Urobilinogen
Total protein	Red blood cells	Protein
Potassium	Mean corpuscular hemoglobin	pH
Sodium	Mean corpuscular hemoglobin	Blood
Calcium	Mean corpuscular volume	Leucocyte esterase
Bicarbonate	Reticulocytes	Specific gravity
Phosphate	Platelet count	Ketones
Alkaline phosphatase	Leucocytes	Bilirubin
ALT	Differential (absolute and %)	Glucose
AST	Eosinophils	Appearance
GGT	Basophils	
Total bilirubin	Neutrophils	
Blood urea nitrogen	Lymphocytes	
Glucose	Monocytes	
Total cholesterol		
LDL cholesterol		
HDL cholesterol		
Triglycerides		
[redacted]		

* If urine is positive for leukocyte esterase, nitrite, urobilinogen, protein, or blood, microscopic examination of urine will be performed for leukocytes, erythrocytes, crystals, bacteria, and casts.

[redacted]

For the purpose of this study conduct, the laboratory tests performed in the central laboratory are the primary tests. Local laboratories may be used at the discretion of the local Investigator for management of emergent urgent medical conditions. If a local laboratory test result is found to be abnormal and clinically significant, it should be verified by the central laboratory as soon as possible after the Investigator is made aware of the abnormal test result. The Investigator may base the assessment of an AE on the local laboratory value if it is not possible to send a sample and obtain a timely report back from the central laboratory.

In addition, according to the Schedule of Events, the following tests are also performed:

- CF genotype (Screening Period only): If not already on file for the patient, CF genotyping will be performed locally.
- Pregnancy (β-human chorionic gonadotropin) tests for females of childbearing potential: Serum or urine samples will be obtained as specified in the Schedule of Events, page 10 and analyzed at the central laboratory (serum) or locally (urine). The urine pregnancy test on Day 1 must be negative before the first dose of study drug. A positive result during the study will result in immediate interruption of study drug.
- Follicle-stimulating hormone (FSH; Screening Period only): Blood sample for FSH will be measured for any suspected postmenopausal female with at least 12 months of continuous spontaneous amenorrhea. Serum FSH levels must be ≥40mIU/mL to be considered postmenopausal.
- Plasma retinol and retinol binding protein: while this is to support an exploratory correlation with ophthalmological assessments, measurements may be used to support decisions made in relation with ophthalmological TEAe. Abnormal values may result in ad-hoc follow-up determinations, when associated with ophthalmological TEAe. Post-screening results will not be communicated to blinded staff, in order to maintain the study blind, unless required for emergency reasons.

Finally, clinical laboratory evaluations may be performed at other times if judged to be clinically appropriate by the Investigator.

11.3 Vital Signs and Physical Examinations

Vital signs, complete Physical Examinations (PE) and Focused PE will be performed according to the Schedule of Events, page 10.

A complete PE (review of all body systems) will be performed at screening and completion visits. At all other scheduled visits, a Focused (symptom-directed as well as mandatory pulmonary examination) PE will be performed as appropriate.

A complete PE includes a review of the following systems: head/neck/thyroid, eyes/ears/nose/throat, respiratory, cardiovascular, lymph nodes, abdomen, skin, musculoskeletal, and neurological. Breast, anorectal, and genital examinations will be performed only when medically indicated. After screening, any clinically significant abnormal findings in PE should be reported as AEs.

Vital signs include blood pressure (systolic and diastolic), temperature (oral or tympanic), pulse rate, oxygen saturation and respiratory rate. These will be assessed following a 5-minute rest (seated) and before any scheduled blood sample collection and Pulmonary Function Testing, but after ECG and questionnaires (see Section 9.1 for Timing of Assessments). At visits when study drug is taken at the site, vital sign assessments will be collected before dosing.

11.4 Electrocardiograms

Standard, digital 12-lead ECGs will be performed according to the Schedule of Events, page 10.

Sites will use their own ECG equipment. A window of ±15 minutes will be permitted around the nominal times for all post-dose ECG assessments. Additional 12-lead ECGs should be performed at any other time if clinically indicated and such instances should be recorded in the proper unscheduled event e-CRF.

Performance of all ECGs must adhere to the following guidelines in order to minimize variability and consistent assessments of each patient from time point to time point.

- ECGs will be performed after the patient has been supine for at least 5 minutes;
- The ECG will be performed before any other procedures that may affect heart rate (HR; e.g. blood draws, including PK, see Section 9.1 for Timing of Assessments);
- The ECG will be performed before the morning dose of study drug during the Treatment Period.

A hard copy of the ECG will be printed and signed by the Investigator at the site. To ensure the safety of the patients, the Investigator or designee at the clinical site will compare all QT segment-corrected (QTc) measured after predose on Day 1 to the average of the predose measurements of QTc on Day 1. If the QTc is increased by >45msec from the average of the predose measurements on Day 1 or the absolute QTc value is >500msec for any scheduled ECG, two (2) additional ECGs will be performed within 10 minutes, at least 1 minute apart, to confirm the original measurement. If either of the QTc values from these repeated ECGs remains above the threshold value (>45msec from the average of the 3 predose values on Day 1 or >500msec), a single ECG must be repeated at least hourly until QTc values from 2 successive ECGs fall below the threshold value that triggered the repeat measurement. If the QTc value remains above the threshold value (>45 msec from the average of the 3 predose values on Day 1 or >500 msec) on repeated measurement or is noted on >2 occasions with no identified alternative etiology for the increased QTc study drug, then discontinuation from study drug treatment may be required after discussion with the Medical Monitor. Patients in whom treatment is discontinued for increased QTc should have their QTc monitored closely until it normalizes or returns to baseline.

11.5 Ophthalmologic Examinations and Night Vision Questionnaire

A particular attention will be paid to ophthalmological safety, since fenretinide is known to cause a decrease in circulating vitamin A that may result in symptoms of nyctalopia, generally mild and rapidly reversible. In order to capture as much information as possible on ophthalmological TEAes, ophthalmological assessments are performed at planned timepoints, and include both specific tests and use of a patient questionnaire. At screening, a Night Vision Questionnaire (NVQ) will be self-administrated, along with a full ophthalmological examination, and any abnormality will be recorded in the e-CRF. Clinically significant abnormalities may result in exclusion from the study, in particular low light vision abnormalities or dark adaptation abnormalities.

Moreover, the NVQ will be used according to the Schedule of Events, Section 2, to ask three (3) specific questions probing possible ophthalmological AEs. These questions will cover the following elements: Question 1: Dark adaptation difficulties; Question 2: Vision in condition of poor luminosity; Question 3: Recovery after dazzling. Results will be compared to previous occurrence to detect changes. Furthermore, possible ophthalmological AEs will be sought using non-suggestive questions at intermediate assessments done via phone calls. Details and specific questionnaire sheets are presented in the Study Reference Manual.

Proposed escalating safety monitoring of dark adaptation in planned Phase 2

Step 1: Night Vision Questionnaire (NVQ)

> A specific questionnaire will be employed to assess the extent of reported symptoms and to trigger an escalation of objective assessments to determine if indeed the reported symptoms translate into a confirmed episode of nyctalopia. Also, a severity grading using CTCAE shall be used in order to qualify the confirmed episode of nyctalopia. The planned NVQ is presented below:

Question 1. Dark adaptation difficulties: In comparison with people around you, do you adapt easily to semi-obscurity when going from a bright to a dim environment, such as daylight to a movie theatre or tunnel.

Question 2. Vision in condition of poor luminosity: Are you able to perceive the outlines of objects in a domestic and semi-obscure environment, such in the indoor candlelight or poor streetlight, or to see the content of closets and kitchen cabinets?

Question 3. Recovery after dazzling: When you transition from a semi-obscure environment to a strongly lit one, do you remain dazzled?

Questions Q1 and Q2: Y (Yes) means normal. Questions Q3: N (No) means normal.

Assessment of the extent of reported symptoms: Level 0: no abnormal answer; Level 1: one abnormal answer; Level 2: two abnormal answers; and Level 3: three abnormal answers.

NVQ administration: Screening, Day 21, Day 77, Day 161, Day 189.

Level 1 NVQ will result in the given symptom to be considered a Grade 1 AE. However, it will not trigger an escalation to Step 2. Level 2 or 3 NVQ will automatically trigger a complete ophthalmological examination (Step 2) and its AE severity graded by the ophthalmologist.

Step 2: Complete ophthalmological examination, including specific dark adaptation tests

Administration: Screening, Day 77 and Day 189, and following any Level 2-3 NVQ report.
Suggested dark adaptation test to be included: a test able to evaluate dark-adaptation changes such as cone-rod break and the final rod threshold (see note below). Severity grading using CTCAE to be performed on confirmed nyctalopia episodes. If the reported symptoms do not get confirmed by the objective measures in Step 2, the default CTCAE severity Grade 1 will be used.

Step 3: Electroretinogram (ERG)

Administration: to be performed only if clinically indicated, in particular if the ophthalmological examination results are equivocal, or the type of signs/symptoms requires it.

Ophthalmologic examinations will be performed by (or under the oversight of) a licensed ophthalmologist at screening, Day 77 and Day 189 visits. It can also be triggered on demand if a patient reports signs of ophthalmologic nature upon questioning or at the time of the scheduled ophthalmologic questions posed on several occasions, according to the Schedule of Events, page 10.

The scheduled complete ophthalmologic examination will consist of the following: Examination of visual acuity, examination for amblyopia, examination of eyelids, conjunctiva, cornea, pupillary reactions to light and accommodation, low contrast visual function (Contrast Sensitivity), specific dark adaptation tests[iii], lens,

[iii] Specific Dark adaptation tests: May consist either of a dark adaptometry test performed with a dark adaptometer or equivalent **OR** a full field ERG test of the scotopic vision, and if possible the photopic vision, after dark adapting and light-adapting the patient, respectively. The order of testing may be done according to specific equipment in use.

vitreous humor, fundus examination of retina and retinal vessels, fundus visualization of optic nerve and macula.

The ad-hoc ophthalmologic examinations may differ since directed to potential medical condition based on the referral by the study Investigator. Such on-demand examinations should be recorded in detail, with results appended, in the proper unscheduled event e-CRF. The Medical Monitor should be notified of any additional ophthalmologic examinations.

The results of the post-study scheduled examination and any ad-hoc examination must be contrasted with the baseline ophthalmologic examination and any new adverse finding should be reported as an AE. Those that meet the definition of clinically significant assessment (see section 11.1.2 on clinically significant assessments) should be brought to the attention of the Medical Monitor, for forwarding to the DSMB.

The use of an ERG in lieu of the dark adaptometry test waives the need for further second line ERG testing, unless requested by the ophthalmologist. In extenuating circumstances such as the inability to locate the equipment to perform the above specific dark adaptation testing despite thorough feasibility assessments, such testing may be waived for a given clinical site. In all cases however, the site must have access, even distally, to an ERG in case a second line ERG examination is requested.

12 STATISTICAL ANALYSIS

A statistical analysis plan (SAP) will provide details of the methods of analysis to address all study objectives. The SAP may be amended during the course of the study, but will be finalized before the cutoff date for any analysis.

Data summaries by treatment group will be presented. For continuous variables, data will be summarized with the number of patients, mean, standard deviation, median, and minimum and maximum values by treatment group. For categorical variables, data will be tabulated in frequency tables to display the number and proportion of patients for each category by treatment group. Baseline assessments for each outcome variable will be defined as the last measurement obtained before the first dose of study drug except the baseline FEV_1 which will be the average of the screening and baseline best values, to reduce variability. Statistical significance will be set at the 5% level.

Diligent efforts will be made to prevent missing data in the study. For example, patients who discontinue the study treatment early or initiate medication changes (including those prohibited by the protocol) should still be followed for all regularly scheduled visits for safety and relevant efficacy assessments, or for a minimum of a 1-month follow-up. A clear distinction should be made between treatment discontinuation and study withdrawal. Efficacy assessments occurring after treatment discontinuation should not be included in the per-protocol analysis.

12.1 Sample Size Determination

The primary measure of efficacy is the absolute change in percent predicted FEV_1 from baseline to Week 24, fenretinide 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 following is calculated based on comparing changes in the parameter (compared to baseline) between groups:

FEV_1 % predicted (absolute change): 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 6, 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 6: 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 FEV1 % 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.

12.2 Analysis Populations

- **The intent-to-treat (ITT) population** will include all patients randomized to a treatment group who complete the baseline evaluations. The ITT population will be used for the primary analyses of all study endpoints, taking into consideration the specific populations described below.
- **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. The PP population will serve as the basis for secondary analyses of all study endpoints, taking into consideration the specific populations described below.
- **The FEV_1 population** 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.

- **The PEx subset population** will include all patients who experience an IV antibiotic treated PEx episode during the study after completing at least one cycle of study drug treatment.
- **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 pharmacokinetic 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.

12.3 Baseline and Demographic Characteristics

Demographic and baseline data will be summarized by treatment group using descriptive statistics. Demographics will include, among others, date of birth, gender, 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. Major protocol deviations/ violations will be identified for analysis purposes.

The demographics and baseline characteristics summary will be presented for the ITT and the PP populations to allow review of the characteristics of those included in the efficacy analyses, which will be based on these analysis sets. The PEx subset will also be summarized separately.

12.3.1 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.

12.3.2 Study Drug Exposure

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 study group, using the safety population subset of the ITT population.

12.3.3 Study Drug 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 study group.

12.4 Efficacy Analysis

12.4.1 Analysis of Primary Endpoint

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.

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 for the primary efficacy endpoint, change from baseline in FEV_1 % predicted (absolute change), including all measurements up to Week 28 (both during and after treatment measurements, including after treatment discontinuation) will be analyzed based on a mixed-effect repeated-measure model. Even though no imputations will be used in the primary analysis, a sensitivity analysis will be conducted where missing data points will be imputed using appropriate interpolation methods; extrapolation methods will be used for truncated FEV_1 % predicted values.

The planned model will include the absolute change from baseline in FEV_1 % predicted as the dependent variable; treatment, visit, and treatment-by-visit interaction as fixed effects; and patient as a random effect with adjustments for a) FEV_1 % predicted determined at the Screening/Baseline Visits (average where possible), b) PEx frequency in the prior year, and c) Co-administration or not of Kalydeco® (ivacaftor), Orkambi® (ivacaftor/lumacaftor), Symdeko® (ivacaftor/tezacaftor) or another commercially available CFTR modulator product. Additional post-hoc analyses may be performed in an exploratory manner depending on the frequency of other phenotypes or concomitant drugs. 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. Alternative methods will be used if necessary due to data not meeting the standard requirements for the proposed analysis.

Full details will be specified in the SAP.

12.4.2 Analysis of Secondary Efficacy Endpoints

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

Pre-defined Clinical Parameters:

- Count data variables: The time to first antibiotic use, 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) and the number of protocol-defined PEx will be tabulated by treatment and compared using Count regression will be used for the group comparison of the number of antibiotic treatments and the number of days of antibiotic treatment. The number of protocol-defined PEx will take

into account in the model of the time spent on study and considering the same covariates as those described below for the time to event data.

- Time to event data variables: The time to first protocol-defined PEx, the time interval between IV antibiotic-treated PEx episodes will be tabulated by treatment and compared using a Cox proportional hazards regression analysis. The model will include treatment as main effect with covariates for age category at baseline, FEV_1 % predicted category (as per entry stratification), PEx number category in prior year (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 and to estimate cumulative survival rates by treatment.
- Relative change (in %) in FEV_1 % predicted from baseline to Week 24: Analysis of this variable will be similar to that of the primary analysis of the primary efficacy endpoint
- Body weight and BMI changes from baseline/screening will be summarized using descriptive statistics by treatment and compared between treatments using a linear mixed-effects model with treatment as fixed effect; visit and treatment-by-visit interaction as random effects; and adjustment for FEV_1 % predicted category (as per entry stratification), PEx number category in prior year (as per entry stratification) and weight or BMI at baseline as covariates.

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) will be calculated for the end-of-treatment cycle values, and the treatments will be compared using two-sided multivariate logistic regression with baseline AA/DHA ratio as a covariate. Alternative methods will be used if necessary.

Values for pharmacodynamic parameters 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} dataset.

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.

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) will be performed similarly to that of the primary efficacy endpoint with the addition of the CFQ-R respiratory domain score at baseline as a covariate.

12.4.3 Analysis of Exploratory Endpoints

- On the subset of patients that exacerbates and are treated with IV antibiotics, the changes of selective biomarkers in blood, serum and plasma 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 through 12 and 24 weeks of treatment, and the AUC of the CFU from baseline to 24 weeks 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 datasets meet standard pre-requisites for such analysis (normal or log-normal distribution and homogeneity of variance).
- 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 datasets meet standard pre-requisites for such analysis (normal or log-normal distribution and homogeneity of variance).
- Data from this trial may be collected and analyzed in various other retrospective analyses as detailed in the SAP.

12.5 Safety Analyses

The safety analyses will be performed at least thrice, 1- 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, 2- when at least half of the enrolled patients have completed a minimum of 3 dosing cycles and corresponding safety information is available, along with Day 77 plasma retinol and retinol binding protein concentration data, and 3- at the time of final analysis. Safety analyses will be performed on the safety population (those patients who receive at least one dose of study drug). Blinded safety data will be assessed by the DSMB at regular meetings. The DSMB may request unblinding of patients for safety concerns in addition to receiving the unblinded mid-study analysis results. The study will remain double blinded to the Investigators, patients and sponsor's staff until the end of the study, unless circumstances require unblinding.

Safety and tolerability will be assessed by the following:

- Adverse events described and categorized according to the MedDRA, version 16 or more recent.
- Clinically relevant changes from baseline in vital signs
- Clinically relevant changes from baseline in 12-lead ECG
- Clinically relevant changes from baseline in physical examinations
- Clinically relevant changes from baseline in safety laboratory assessments (hematology with differential count, biochemistry, and urinalysis)

- Plasma retinol and retinol-binding protein values, changes from screening/baseline, correlated with ophthalmological symptoms, more specifically nyctalopia
- Change in PEx incidence and antibiotic treatment requirement.

12.5.1 Adverse Events

Adverse events will be tabulated with reported incidences by treatment, number of patients that presented adverse events by treatment, serious adverse events by treatment, number of adverse events by body system and by treatment. Summaries will be presented by MedDRA system organ class and preferred term.

For the purpose of analyses and tabulations, AEs will be classified as pretreatment AEs, TEAEs, or post-treatment AEs. More specifically: Pretreatment AE are those that started before the first dose of study drug. TEAE are those that increased in severity or that was 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. 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.

AE summary tables will be presented for TEAE only and will include the following: All TEAEs, TEAEs by relationship, TEAEs by maximal severity, TEAEs leading to treatment discontinuation, Serious TEAEs and fatal TEAEs. All AEs, including pre-and post-treatment AEs, will be presented in individual patient data listings.

The number and percentage of patients with at least one AE, as classified by preferred term and system organ class, will be summarized for each treatment group. For these summaries, patients with multiple events will be counted only once per preferred term. AEs will also be summarized by severity and relationship to study drug. At each level of summarization, the event with the highest level of severity or strongest drug relationship will be presented.

All AEs must be listed. In addition, detailed listings will be provided for patients who die, experience a SAE, or discontinue the study because of an AE. These listings will include treatment, patient's age, duration of follow-up, amount of fenretinide received, and time since last intake. Patients who develop Grade 3-4 toxicities will also be listed separately.

A Fisher's Exact test will be used in an exploratory sense to evaluate differences between treatment groups in the incidence of treatment-emergent and clinically significant AEs, graded according to CTCAE outlined in Section 11.1.1.1.

12.5.2 Clinical Laboratory Assessments

Safety laboratory variables (e.g., 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.

Summary tables of laboratory results will be prepared to examine the worst toxicity grade on study. Cross-tabulations of patients by maximum post-baseline toxicity will be presented, as appropriate. Patients who develop Grade 3-4 laboratory toxicities will also be listed separately.

A Fisher's Exact test will be used in an exploratory sense to evaluate differences between treatment groups in the incidence of laboratory toxicities graded according to CTCAE grading outlined in section 11.1.1.1.

12.5.3 Vital Signs

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 for with shift changes from baseline of the vital signs 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, Diastolic Blood Pressure ≤45 mmHg and decrease from baseline ≥10 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 (and reported as an AE).

No statistical analyses of vital signs are planned.

12.5.4 ECG

For ECG measurements, a summary of raw values and change from baseline 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 baseline (normal/missing, not clinically significant, and potentially 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 ≤500msec and >500msec, as well as maximum on-treatment change from baseline value of QT/QTc intervals, categorized as >30msec.

No statistical analyses of ECG data are planned.

12.5.5 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 post-study ophthalmologic examinations will be presented and summarized by treatment group. Ad-hoc examinations and findings will also be presented and contrasted with 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.

13 DATA HANDLING AND RECORD KEEPING

13.1 Case Report Forms

All patient data generated by the study will be recorded in each patient's e-CRFs. Data reported on the e-CRFs that are derived from source documents should be consistent with the source documents or the discrepancies should be explained. e-CRFs will be considered complete when all missing and/or incorrect data have been resolved and all safety data have been recorded.

The Investigator, or designated representative, should complete the e-CRF as soon as possible after information is collected. e-CRFs must be completed only by persons designated by the Investigator. The e-CRF system should enable an audit trail that will provide the user's identification information and the date and time of any entry/correction. The completed e-CRF will be reviewed by Laurent Pharmaceuticals or its agents on a routine basis.

The Investigator must approve formally all the information in the e-CRFs for the patients for whom he/she is responsible. The United States Food and Drug Administration (FDA) may inspect all records related to the study.

13.1.1 Source Documentation

Source documents are considered to be all information in original records and certified copies of original records of clinical findings, observations, data, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. The Investigator and designees agree to maintain accurate e-CRFs and source documentation as part of the case histories. Source documents are the originals of any documents used by the Investigator, sub-Investigator, or hospital/institution that will allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial. All data entered into the e-CRF also must be available in the source documents. The Investigator will allow designated representatives of Laurent Pharmaceuticals, IRB and regulatory bodies, including the FDA to have direct access to the source documents to verify the data reported in the e-CRFs. Personally identifiable source documentation shall not be copied or removed from the Investigator site, and to the extent permitted by law and/or regulations, will not be made publicly available. All representatives of Laurent Pharmaceuticals, IRB and regulatory bodies must respect confidentiality.

13.1.2 Record Retention

Study records and source documents need to be preserved for at least 15 years after the completion or discontinuation of/withdrawal from the study, or 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region, whichever is the longest time period.

14 MONITORING

In accordance with current applicable regulations, Good Clinical Practice (GCP), and Laurent Pharmaceuticals procedures, monitors will contact the site before the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and Laurent Pharmaceuticals requirements. When reviewing procedures for data collection, the discussion will include identification, agreement, and documentation of data items which will be recorded in each patient's e-CRF.

The study will be monitored to ensure the following:

- Data are authentic, accurate, and complete;
- The safety and rights of patients are being protected;
- The study is being conducted in accordance with the currently approved protocol, any other study agreements, GCP, and all applicable regulatory requirements.

The Investigator and the head of the medical institution (where applicable) agree to allow the monitor direct access to all relevant documents.

15 QUALITY CONTROL AND QUALITY ASSURANCE

The sponsor or its designee will perform the quality assurance and quality control activities of this study. However, responsibility for the accuracy, completeness, and reliability of the study data presented to Laurent Pharmaceuticals lies with the principal or qualified Investigator generating the data.

Laurent Pharmaceuticals or its designated representative will conduct a study site visit to verify the qualifications of the principal Investigator and sub-Investigators, inspect clinical site facilities as needed, and inform the Investigator of responsibilities and procedures for ensuring adequate and correct study documentation.

Study data for randomized patients will be entered into an e-CRF by clinical site staff using a secure, validated web-based electronic data capture (EDC) application. Select data for patients not randomized will also be collected in e-CRFs.

Instances of missing, discrepant, or uninterpretable data will be queried with the Investigator for resolution. Any changes to study data will be enacted in the e-CRF and documented in an audit trail, which will be maintained within the clinical database.

16 COMPLIANCE, PROTOCOL AMENDMENT AND DEVIATION

16.1 Compliance

It is very important that no modifications to the protocol should be made without the approval of Laurent Pharmaceuticals and Investigators. Changes that significantly affect the safety of the patients, the nature, the scope and the scientific integrity of the study will require IRB/IEC notification/approval before their implementation. Exceptions are cases where the modification is necessary to abrogate an apparent immediate risk to the patients. Laurent Pharmaceuticals or designee will submit all protocol modifications to IRB/IEC and the required regulatory authorities. When there is a need for immediate deviation from procedures enunciated in the protocol, the Investigator will contact Laurent Pharmaceuticals to discuss the course of action and possible alternatives, if at all possible, before any implementation of changes. Any deviation from protocol must be fully documented in the source documentation and in the study documentation on protocol deviations.

16.2 Protocol Amendment

Administrative amendments to the protocol will be classified as amendments of typographical errors, clarifications of confusing wording, name changes, and minor modifications that have no impact on the safety of the patients or the science of the study. Administrative amendments will be submitted to the IRB/IEC for information only. Laurent Pharmaceuticals will ensure that acknowledgement is received and filed. Any other amendment will be classified as a substantial amendment and will be submitted to the appropriate regulatory authorities and the IRBs/IECs for approval.

16.3 Protocol Deviation

Should a protocol deviation occur, Laurent Pharmaceuticals must be informed as soon as possible. Important protocol deviations and their reasons will be summarized in the clinical study report. In accordance with applicable regulatory authority mandates, the investigator is responsible for reporting protocol deviations to the IRB/IEC.

17 STUDY TERMINATION

At any time, Laurent Pharmaceuticals may terminate this study in its entirety or at specific clinical site. In addition, for reasonable cause, the IRB/IEC and/or the Investigator at a clinical site may terminate the study at their center. In such cases, Laurent Pharmaceuticals should be informed immediately and if at all possible, before implementation.
Conditions that may lead to reasonable cause and warrant termination include, but are not limited to:

- Investigator noncompliance and/or lack of adherence to protocol procedures;
- Unsatisfactory patient enrollment;
- Lack of evaluable and/or complete data;
- Potentially unacceptable risk to patients (see Section 7.2.3 for additional guidance);
- Changes in Laurent Pharmaceuticals drug development plans;
- Decision by Health Canada and/or the FDA.

The reason(s) for clinical study termination must be properly documented.

18 ETHICAL CONSIDERATIONS

The study will be conducted according to current GCP, including any future revisions, all relevant local laws and regulations, as well as the principles of the Declaration of Helsinki and its amendments. IRB/IEC committees will review and approve this protocol and informed consent. All patients must provide written informed consent where applicable, before participation in the study.

This study will be performed by qualified clinical investigators and in accordance with GCP. The study specifically incorporates all of the following features:

- Multicenter, randomized study design;
- Prospectively stated objectives and analytical plan;
- Accepted, pre-specified outcome measures for safety and efficacy;
- Investigator meeting prior to study start and a detailed protocol to promote consistency across sites;
- Compliance with current GCP, with assessment via regular monitoring;

- Quality assurance procedures performed at study sites and during data management to ensure that safety and efficacy data are adequate and well documented.

The study will only be conducted at sites where IRB/IEC approval has been obtained. The protocol, Investigator's Brochure, sample ICF, advertisements (if applicable), written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC by the investigator or Laurent Pharmaceuticals, as allowable by local applicable laws and regulations.

19 FINANCING AND INSURANCE

Financial aspects of the study are addressed in a separate clinical study agreement.

The Investigator/institution is required to have adequate current insurance to cover claims for negligence and/or malpractice according to national regulations. Laurent Pharmaceuticals will provide insurance coverage for the clinical study as required by national regulations.

20 PUBLICATION POLICY AND CLINICAL STUDY REPORT

20.1 Confidentiality and Publication Policy

Both the use of data and the publication policy are detailed within the clinical study agreement.

Any and all scientific, commercial, and technical information disclosed by Laurent Pharmaceuticals in this protocol or any other documents and communications should be considered the confidential and proprietary property of Laurent Pharmaceuticals. The Investigator shall hold such information in confidence and shall not disclose the information to any third party except to the Investigator's staff on a “need to know” basis, as long as the said staff has been made aware that the information is confidential and who are bound to treat it as such.

The Investigator shall not use any and all information for any purpose other than determining interest in performing the study and, if the parties decide to proceed with the study, for the purpose of conducting the study. The Investigator understands that the information developed from this clinical study will be used by Laurent Pharmaceuticals for the development of the study drug and therefore may be disclosed as required to other clinical Investigators, potential and current business partners and associates, Health Canada, the FDA, and possibly other agencies, without bearing any personally identifiable information. The Investigator also understands that, to allow for the use of the information derived from the clinical study, he/she has the obligation to provide Laurent Pharmaceuticals with complete results and accompanying data developed in the study.

No publication or disclosure of study results will be permitted except under the terms and conditions of a separate written agreement between Laurent Pharmaceuticals and the Investigator and/or the Investigator's institution. In all instances, personally and individually identifiable information shall not be published.

20.2 Clinical Study Report

A clinical study report, written in accordance with the ICH E3 Guideline, will be submitted in accordance with local regulations.

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