Clinical Research Protocol
A DOUBLE BLIND, PLACEBO CONTROLLED, DOSE ESCALATION TRIAL OF GLYCEROL PHENYLBUTYRATE CORRECTOR THERAPY FOR CYSTIC FIBROSIS

Protocol Number:	GPBAc-CF-01
Version Date:	18 Feb 2021
Investigational Product:	Glycerol Phenylbutyrate (Ravicti®)
IND Number:	125024
Development Phase:	I/II
Sponsor:	Food and Drug Administration (FDA) 10903 New Hampshire Ave. Silver Spring, MD 20993
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Approval:

Pamela L. Zeitlin, M.D., Ph.D.

Date

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the Sponsors with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: GPBAc-CF-01

IND Number: 125024

Study Drug Name: Glycerol Phenylbutyrate (Ravicti®)

Protocol Title: A Double Blind, Placebo Controlled, Dose Escalation Trial of Glycerol Phenylbutyrate Corrector Therapy for Cystic Fibrosis

Protocol Date: 18 Feb 2021

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Investigator Signature *Date*

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Printed Name

Site # _____

Site Name _____

Address _____

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LIST OF ABBREVIATIONS

AE	adverse experience
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BAL	bronchoalveolar lavage
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CFTR	cystic fibrosis transmembrane conductance regulator
CF	cystic fibrosis
CRF	case report form
CRP	C-reactive protein
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ESR	erythrocyte sedimentation rate
FDA	Food and Drug Administration
$FEF_{25\%-75\%}$	forced expiratory flow
FEV_1	forced expiratory volume over one second
FVC	forced vital capacity
GPB	Glycerol Phenybutyrate
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IL-8	Interleukin-8
IRB	Institutional Review Board
IV	intravenous
LABA	Long-acting beta-agonist
mEq	milliequivalent
PI	Principal Investigator
PAA	phenylacetate
PAGN	phenacetylglutamine
PBA	phenylbutyrate
PK	pharmacokinetic
SABA	Short acting beta-agonist
SAE	serious adverse experience

SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase
SOP	Standard operating procedure
TDN-CC	Therapeutics Development Network- Coordinating Center

PROTOCOL SYNOPSIS

TITLE	**DOUBLE BLIND, PLACEBO CONTROLLED, DOSE ESCALATION TRIAL OF GLYCEROL PHENYLBUTYRATE CORRECTOR THERAPY FOR CYSTIC FIBROSIS**
SPONSOR	Food and Drug Administration (FDA)
NUMBER OF SITES	4 (up to 3 enrolling, 1 NPD Central Core Laboratory)
RATIONALE	The most common disease-causing mutation in CFTR is the F508del that promotes ubiquitination and premature degradation from the endoplasmic reticulum, as well as reduces the channel's open time for chloride transport. This double defect presents a challenge to therapeutic restoration of the cAMP-regulated chloride transporter. We were the first to test 4-phenylbutyrate (Buphenyl) as a systemic corrector of these defects in F508del under an IND held by P. Zeitlin. In a series of Phase 1 and 2 trials we established the maximum tolerated dose as 20 gm daily divided t.i.d. and the maximum induction of cAMP-mediated nasal epithelial chloride transport with 30 gm daily as a median of -10 mV on days 4 and 7 of treatment.(1, 2) Under those conditions there was no significant decrease in sweat chloride values or in amiloride-inhibited nasal potential difference (NPD). We interpreted these results as a proof of concept of corrector therapy, but corrector therapy alone was likely an insufficient therapy for this mutation in CF, and therefore closed the IND for 4-phenylbutyrate. 4-Phenylbutyrate tablets are formulated for oral delivery, and we showed that the pharmacokinetics were similar in CF to that in patients with urea cycle disorders. However the large number of tablets that had to be ingested at each meal was somewhat daunting at the 30 gm daily dose. A new pro-drug of 4-phenylbutyrate, glycerol phenylbutyrate or Ravicti® (owned by Horizon) was approved in February 2013 by the US FDA. This new formulation is a significant advance for patients with urea cycle disorders because it is an oral, odorless, tasteless liquid, that contains 3 molecules of 4-phenylbutyrate for every molecule of the triglyceride. Pancreatic lipase enzymes are required to break the covalent bonds and release the active drug in the intestines. Because most CF patients homozygous for F508del are pancreatic-insufficient and already on enzyme therapy, we propose to test the effectiveness of the combination of CF pancreatic enzyme replacement therapy (PERT) on absorption of Ravicti®. Alternatively, those CF patients with documented pancreatic sufficiency will have endogenous pancreatic enzymes to break the covalent bonds and release active drug in the intestines. We also propose to test the subsequent restoration of nasal epithelial CFTR-mediated chloride transport during the nasal potential difference (NPD) test in response to treatment with Ravicti®.
STUDY DESIGN	This is a randomized, double-blind, placebo-controlled, dose-escalation design in which 36 subjects will be randomized 2:1 study drug with placebo, resulting in 12 subjects at the low dose, 12 at the high dose, and 12 placebo. A screening visit (Visit 0) to establish eligibility will occur anywhere from 4 weeks to 1 week before visit 1 which is the first dosing day. There will be 5 study visits: Screening (-28 day to -7 days), Visit 1 on Day 1, Visit 2 on Day 4, Visit 3 on Day 7 and Visit 4 on Day 14. Study drug will be administered

	at visit 1, Day 1 and taken three times a day for the next 7 days, and there will be a 7 day washout period with a final visit on Day 14. Total participation will be 6 weeks at the maximum and 3 weeks at the minimum depending on length of time between screening and visit 1.
PRIMARY OBJECTIVE	*The primary objective* is to quantify the change in nasal epithelial CFTR-mediated chloride transport between pre-dose measurement and 4 and 7 days exposure to Ravicti® or placebo.
SECONDARY OBJECTIVES	*Secondary Objective 1* is to quantify the change in other NPD measures from baseline and Days 4,7, and 14 and the change in sweat chloride between pre-dose on Day 1 and Day 7 *Secondary Objective 2* is to measure safety and tolerability of Ravicti® *Secondary Objective 3* is to measure the pharmacokinetics of Ravicti® to determine the efficacy of PERT on absorption and metabolism of Ravicti® *Secondary Objective 4* is to be able to select a dose of Ravicti® for future combination with other modulators.
NUMBER OF SUBJECTS	36 evaluable subjects
SUBJECT SELECTION CRITERIA	Inclusion Criteria: 1. Male or female ≥ 18 years of age. 2. Confirmed diagnosis of CF based on the following criteria: any CFTR genotype combination EXCEPT two stop codons, and one or more clinical features consistent with the CF phenotype. 3. Taking pancreatic enzyme replacement therapy (PERT), or have documented pancreatic sufficiency. 4. Ability to perform acceptable spirometry. 5. Ability to understand and sign a written informed consent and comply with the requirements of the study. 6. FEV1 ≥30% of predicted normal for age, gender, and height (Hankinson standards): pre or post-bronchodilator at Screening. 7. Oxygen saturation by pulse oximetry ≥90% breathing either ambient air or regular oxygen regimen at screening and Day 1. 8. Hematology and clinical chemistry of blood and urine results with no clinically significant abnormalities that would interfere with the study assessments (as

	judged by the principal investigator) at screening. If electrolyte abnormality at screening, values must be corrected prior to dosing. 9. Subjects on chronic inhaled antibiotic therapy are eligible if they can continue their usual antibiotic regimen, or remain on their off-cycle period, for the duration of study drug exposure 10. Negative pregnancy test for women of child-bearing potential. 11. If of childbearing potential, agree to use one highly effective method of contraception from the time of consent through the Visit 4 study visit, per section 9.1.13 of the protocol. <u>Exclusion Criteria</u>: 1. Administration of any investigational drug or device within 30 days of Screening or within 6 half-lives of the investigational drug (whichever is longer). 2. History of any illness or condition that in the opinion of the investigator could confound the results of the study or pose additional risk in administering study drug to subjects. 3. Any change in chronic therapies for CF lung disease (e.g., Ibuprofen, Pulmozyme®, hypertonic saline, Azithromycin, TOBI®, Cayston®) within 4 weeks of Study Day 1. 4. Pregnant, planned pregnancy or breast feeding at Screening. 5. Clinically significant cardiac, liver or kidney disease. 6. Seizure disorder. 7. Acute upper respiratory infection within 2 weeks or acute pulmonary exacerbation requiring intravenous antibiotics within 4 weeks of Screening Visit. 8. Sinus surgery within 6 weeks of Screening Visit. 9. Abnormal renal function. 10. Abnormal liver function, defined as ≥3x upper limit of normal (ULN), of serum aspartate transaminase (AST) or serum alanine transaminase (ALT), or known cirrhosis. 11. Screening laboratory results which in the judgment of the investigator would interfere with completion of the study. 12. History of or listed for solid organ or hematological transplantation.

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION	Subjects usual pancreatic enzyme dosing will be taken about 15 min before ingestion of study drug, as required. Study drug should be taken with food. The dosing schedule is every 8 hours ± 2 hours. The low dose of Ravicti® (Oral liquid: 1.1 gm/m) will be administered as first daily dose 6 ml (6.6 gm) by mouth or gastrostomy tube (GT), and second and third daily dose of 5.5 ml (6.05 gm). The high dose of Ravicti® will be administered as 9.0 ml (9.9 gm) for the first dose, and, 8.25 ml (9.08 gm) for second and third daily dose. Placebo will be administered in the same volumes. Total exposure will be 7 ± 2 days followed by a 7 ± 2 day washout period.
CONTROL PRODUCT, DOSE AND MODE OF ADMINISTRATION	Days 1-7 1. Cohort 1; Low dose Ravicti® oral liquid or placebo every 8 hours ± 2 hour (for example, 6ml at 8 am, 5.5 ml at 4pm and midnight). 2. Cohort 2: High dose Ravicti® oral liquid, or placebo every 8 hours ± 2 hour (for example 9ml at 8 am and 8.25ml at 4pm and midnight). 3. Placebo – color and taste matched liquid taken every 8 hours ± 2 hour (for example, 8am, 4pm and midnight). Volume equivalent to either low (6ml/5.5ml) or high dose (9ml/8.25ml) depending on randomization. Product will be administered approximately every 8 hours for 7 days. Administration of study drug is oral taken as a liquid.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study up to 42 days depending on time between screening visit and dosing day visit 1. ***Screening:*** 7 to 28 days ***Treatment:*** 7 days ***Follow-up:*** 7 days The total duration of the study is expected to be 2 years. 8 months for subject recruitment and 16 months for final subject follow-up.
CONCOMMITANT MEDICATIONS	**Allowed:** All routine stable CF medications including one or two cycling inhaled antibiotics, daily nasal sinus irrigation, daily nasal steroid, mucolytics, bronchodilators, azithromycin and other suppressive oral antibiotics if unchanged or on usual alternating monthly schedule. **Prohibited:** Systemic corticosteroids, haloperidol, valproic acid and probenecid.
EFFICACY EVALUATIONS	Nasal Potential Difference (NPD) measurements sweat chloride, safety labs, PK of absorption, release, and excretion of Ravicti®.
PRIMARY ENDPOINT	The primary biological endpoint will be the change in average measurement of nasal potential difference between day 7 and baseline.
SECONDARY ENDPOINTS	Change in other NPD measures from baseline and Days 4, 7, and 14 to include baseline PD, change in amiloride, low chloride, and low chloride plus isoproterenol. Change in average sweat chloride measurement between pre-dose on Day 1 and Day 7. Safety and tolerability.

	Efficacy of PERT on absorption of Ravicti®.
OTHER EVALUATIONS	PK plasma will be sampled for PBA, PAA, and PAGN in this study on Visits 1 and 2 at time 0 (+/- 5 min) (delivery of first dose), 1 hour (+/-5 min), 2 hours (+/-10 min), 4 hours (+/-10 min) and 8 hours (+/- 10 min) post dose. The PK blood sampling done on Visits 3, Visit 4 and Early Withdrawal will include single post dose trough plasma levels. A single urine PK sample will be obtained at Visits 2, 4 and Early Withdrawal. Urine will be collected over 24 hours on Visit 1 and Visit 3.
SAFETY EVALUATIONS	Assessment of safety will include clinical observations, laboratory evaluations, and monitoring of subjects. Safety labs consisting of standard hematology, CMP, CRP, ESR and uric acid, sputum microbiology, and spirometry will be performed as outlined in Appendix 1. Schedule of Study Visits. AEs will be recorded from the Screening Visit through Day 14 Visit or Early Withdrawal Visit. Subjects will be examined by the Investigator or his/her designee for evidence of AEs. The presence or absence of specific AEs will not be solicited from subjects.
SAFETY MONITORING	Site investigators will be reviewing safety labs and notify the PI of any significant values. A Data Monitoring Committee (DMC) will be established for oversight of safety in this clinical trial. The Cystic Fibrosis Foundation Therapeutics Data Safety Monitoring Board will provide a DMC to serve in this capacity. The DMC will function to protect the safety and welfare of participants and to ensure the integrity of the clinical trial. Among the responsibilities are: Examining accumulated safety data, pharmacokinetic, compliance data, making recommendations concerning continuation, termination, or modification of the trial based on the safety of the interventions under study, reviewing the general progress of the study such as accrual, protocol violations, and study conduct. The study biostatistician will develop the randomization code, provide it to each of the site pharmacists, maintain the randomization code and provide oversight of the pharmacists. All other study personnel will be blinded to study drug assignment. If any subject develops signs or symptoms of butyrate toxicity such as headache, fatigue, peripheral neuropathy, seizures, tremor and/or dizziness, sleepiness, somnolence or confusion, the PI will be notified and will in turn notify the DMC. The study subject will be asked to stop drug immediately. The PI will determine if the patient should be withdrawn from the study. An interim review will commence at 50% enrollment or 9 months after the first patient is enrolled/or sooner if the DMC has any safety concerns. The DMC will advise as to whether the study can escalate to the higher dose and enroll subjects into Cohort 2. Study visits and enrollment may continue during the DMC review. The interim safety review will commence after 12 subjects on the lower dose of study drug have completed Day 7. The study biostatistician will provide the DMC with a summary of the safety labs and AEs by treatment group. Trial modification rules or formal stopping rules will be established. Since this is an early phase trial, stopping cannot be based on predefined statistical analyses. Determinations of safety require medical judgments of the DMC that cannot be

	exhaustively described prospectively. The reasons for stopping, interrupting, modifying the trial or changing the DMC safety monitoring plan include but are not limited to: Death of a patient for reasons that may possibly be drug-related, SAEs consistent with known effects of the drug, a clear temporal association with drug, and lack of alternative reasonable explanation, occurrence of a drug-related Class 3 or 4 AE or laboratory abnormality in $\geq$ 33% of subjects at a dose level, unexpected increases in the incidence or severity of disease-related symptoms that appears to be drug related and lack alternative explanations.
STATISTICS **Primary Analysis Plan**	The analyses will be conducted on all available data. Treatment groups will be described and compared with respect to baseline demographic and clinical characteristics including age, gender, race, sweat chloride, nasal potential difference measures, microorganisms in sputum, height, weight, and pulmonary function. Differences between the three groups on categorical variables in this descriptive analyses will be assessed using the chi-squared statistic. Difference between the three groups on continuous variables will be assessed using Analysis of Variance or the nonparametric Kruskall- Wallis test. The primary biological outcome will be the change in average measurement of nasal potential difference between day 7 and baseline. A two-sample t-test will be performed to compare the change in the combined treatment groups to that in the placebo group. An intention to treat analysis will be performed. However, treatment withdrawal or additional administration of PERT for malabsorption will be recorded and described. The analyses will be conducted on all available data

Rationale for Number of Subjects

Sample size calculations led us to plan for a sample size of 12 subjects in each of the three groups: placebo, low dose, and high dose Ravicti®. As we showed in ref 2, a group of 6 subjects on either study drug or placebo provides statistical power of 0.80, assuming a two- sided significance level of 0.05, to detect a mean change in PD of 5 mV between drug and placebo, assuming a standard deviation of the change of 1.1 mV in the placebo group and 2.9 mV in the drug group. More recent data (Steve Rowe) suggests a standard deviation of 3.0 mV for the 3 centers participating in the proposal. Using these estimates that are based on our two previously published studies of the low chloride/isoproterenol maneuver in NPD at the JHU site, and duplicated at UAB, Table 1 displays the sample size needed in the placebo group and in the combined treatment groups (using a 2:1 ratio of treatment:placebo) in order to detect differences in mean change in NPD under different assumptions of statistical power. A total sample size of 36 (12 in placebo, 12 in low-dose Ravicti® and 12 in high dose Ravicti®) will provide 80% power to detect a difference in mean change in PD of 3 or more mV between placebo and the combined treatment groups. Within subject reproducibility of sweat chloride is more than sufficient to detect physiologically relevant changes with this sample size. Randomization will be done by the study biostatistician and provided to the JHU and other site investigational pharmacists. The study pharmacist will dispense drug to the site coordinator.

Table 1. Sample Size Needed in the Placebo and Combined Treatment Groups (P:R) to Detect a Difference in Mean Change in PD (mV) under Varying Assumptions of Statistical Power and Standard Deviation (SD) of Mean Change (significance level (alpha) of 0.05).

	80% Power	85% Power	80% Power	85% Power
Mean Change	SD_1=3; SD_2=3	SD1=3; SD2=3	SD1=4; SD2=4	SD1=4; SD2=4
2.0 mV	27:54	31:62	48:96	54:108
3.0 mV	12:24	14:28	21:42	24:48
3.5 mV	9:18	10:20	16:32	18:36
4.0 mV	7:14	8:16	12:24	14:28

Table 2. Sample Size Needed in the Placebo and Combined Treatment Groups (P:R) to Detect a Difference in Mean Change in PD (mV) under Varying Assumptions of Statistical Power and Standard Deviation (SD) of Mean Change (significance level of 00.05).

	80% Power	85% Power	80% Power	85% Power
Mean Change	SD_1=1.1 SD_2=3	SD1=1.1 SD2=3	SD1=1.1 SD2=4	SD1=1.1 SD2=4
2.0 mV	12:24	13:26	19:38	21:42
3.0 mV	5:10	6:12	9:18	10:20
3.5 mV	4:8	5:10	6:12	7:14

1. BACKGROUND

Ravicti® is US FDA-approved/indicated for use as a nitrogen-binding agent in the chronic management of adult and pediatric patients > 2 years of age with urea cycle disorders (UCDs) that cannot be managed by dietary protein restriction and/or amino acid supplementation alone. It is specifically not indicated in the treatment of acute hyperammonemia in UCD, in patients less than 2 months of age, or in treatment of N-acetylglutamate synthase deficiency. The maximum daily dose in UCD is set at 17.5 ml divided three times a day rounded to the nearest 0.5 ml. The labeling says it must be given with protein restriction in UCD and with food at each dose (not on an empty stomach). The drug is provided as an oral liquid of 1.1 gm/ml glycerol phenylbutyrate (Ravicti®).

1.1 Overview of Non-Clinical Studies

4-phenylbutyrate (4-PBA) is the foundation of Ravicti®. We were the first to develop and test an in vivo corrector of F508del CFTR (1-9). Our approach was based on the failure of F508del at 37ºC to efficiently fold during translation in the endoplasmic reticulum. The end result in vitro in a number of cell lines and primary cells was the absence of F508del CFTR at the apical membrane surface of epithelial cells. At the time, butyrates were under development for a number of disease states and being recognized as histone deacetylase inhibitors (10-15). Sodium or arginine butyrate stimulated the over-expression of molecules such as fetal hemoglobin and CFTR, but had a short half-life and had to be administered intravenously. The oral 4-phenylbutyrate or 4PBA (Buphenyl) similarly increased the expression of CFTR(5). We published a series of investigations which demonstrated a dose-dependent up-regulation of Band C F508del CFTR, functional restoration of CFTR-mediated chloride current in vitro and in vivo, in nasal epithelia, and not in human sweat glands (1, 2).

We recognized that merely restoring more F508del to the trafficking pathway in vitro and in vivo was unlikely to fully compensate for the multiple ion transport defects observed in CF epithelia. In particular, CFTR down-regulates the epithelial sodium channel ENaC and facilitates the outwardly rectifying chloride channel (ORCC) chloride conductance. The Rubenstein group has studied the combination of genistein, a potentiator of CFTR(16) with 4-phenylbutyrate in Xenopus oocytes. They found that the combination of corrector and potentiator restored regulation of ENaC by F508del. The pilot clinical trial of 4PBA/Genistein run by Rubenstein had equivocal results (presented in abstract at NACFC in Denver, 2006), however the FDA limited delivery of genistein to short nasal perfusions in this trial. Subsequent studies of the related flavonoid quercetin by the Rowe group also revealed limitations with topical delivery (NACFC 2010). VX-770 is delivered in an oral formulation and has been well tolerated by CF pediatric and adult patients and represents a significant advance over the flavonoids. In addition, F508del may not be the only mutation to respond to 4PBA. Rowe et al demonstrated additive effects with sodium butyrate and stop codon suppressors for W1282X(17).

4-Phenylbutyrate (4PBA) is well tolerated at doses that lead to mMolar concentrations in the blood. It is rapidly metabolized to phenylacetate (PAA) which is also

an active F508del corrector in vitro and in vivo and then PAA is conjugated with glutamine to form phenacetylglutamine (PAGN) and excreted in the urine.

Figure 1 contains a Western blot of F508del expression in IB3-1 CF bronchial epithelial cells exposed to a range of concentrations of 4-phenylbutyrate and 27ºC as a positive control for F508del rescue (Panel A). In Panel B we have reproduced a similar experiment performed by Vertex and colleagues for comparison.

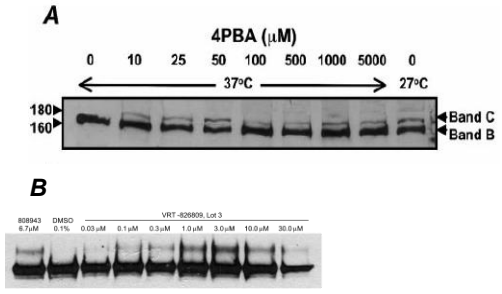


Figure 1: Comparison of corrector rescue by 4-PBA (A) in IB3-1 and VX-809 (B)

These data suggest that 4-phenylbutyrate was approximately as effective at 50 micromolar as VX-809 at 3 micromolar, but the cell lines are different and the expression levels of F508del in the two lines are non-identical as well. Van Goor has also published data showing peak maturation at 1 micromolar in HBE.(18)

Others have shown that F508del maintains a shorter half-life on the plasma membrane than wt. CFTR. Each of the different correctors in development—corr 4a, 4-phenylbutyrate, VX-809, VX-661 appear to have different mechanisms and are synergistic in combination in vitro.

Glycerol phenylbutyrate (Ravicti®)

The new pro-drug of 4-phenylbutyrate, glycerol phenylbutyrate or Ravicti® (Horizon) was approved in February 2013 by the US FDA(19). This new formulation (Figure 2) is a significant advance for patients with urea cycle disorders (UCDs) because it is an oral, odorless, tasteless liquid, that contains 3 moles of 4-phenylbutyrate for every mole of the triglyceride(20). The UCDs are inherited genetic deficiencies of enzymes or transporters required in the synthesis of urea from ammonia. Ammonia is produced in the body by catabolism of amino acids, amines, nucleic acids, glutamine and glutamate in peripheral tissues, primarily skeletal muscle. Gastrointestinal organisms also produce ammonia. Normally, this ammonia is absorbed through the portal circulation into the liver where the urea cycle enzymes convert it into urea which is then excreted via the kidney. Ravicti® is indicated for use as a nitrogen-binding agent in the chronic management of adult and pediatric patients 2 years of age and older with urea cycle disorders who cannot be managed by dietary protein restriction and/or amino acid supplementation alone(21, 22). It is not indicated for the treatment of acute hyperammonemic coma, since it would not act rapidly enough(23) although it is being considered in chronic hepatic encephalopathy due to liver disease. Ravicti® is contraindicated in patients less than two

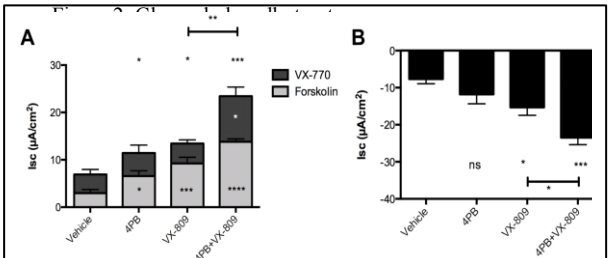


Figure 3. Effect of 4-Phenylbutyrate on CFTR-dependent ion transport. Well-differentiated primary HBE cells derived from a F508del homozygous donor were incubated for 24 hrs with 4-phenylbutyrate (4PB, 1 mM), lumacaftor (VX-809, 3 μM), or the combination, then mounted in Ussing chambers under short-circuit current (Isc) conditions.[2-5] A) Monolayers were sequentially stimulated with forskolin (20 μM, light grey) and ivacaftor (VX-770, 10 μM, dark grey) in the setting of amiloride (100 μM) and a chloride secretory gradient. B) Subsequently CFTR was inhibited in the same monolayers with $CFTR_{Inh}$-172 (10 μM) for specificity. N=4 monolayers/condition obtained in two separate experiments. *P<0.05,

months of age because of immature pancreatic exocrine function. In UCDs, the dose is adjusted based on fasting plasma ammonia and/or on urinary phenylacetylglutamine (24). In CF, we have the capability of titrating also on plasma phenylbutyrate levels and on NPD measures of CFTR function. Since the formulation is a liquid, it is easily titrated and it can be swallowed or given through a gastrostomy tube.

The published work on 4PBA has been described above. However, we have since begun experiments with 4PBA alone and in combination with VX-809 and VX-770. Ravicti® is a triglyceride that is inactive until hydrolyzed with PERT. Airway epithelial cell lines do not express PERT and thus the parent compound cannot be added alone to airway cultures. Instead, we model the effects of the pro-drug through the metabolite of PERT, e.g. 4PBA in vitro. The CF Corrector field is moving towards the idea that correctors working through independent mechanisms are more effective in combinations than alone in the rescue of F508del. We too, are asking not only whether 4PBA plus potentiator VX-770 will be effective, but whether 4PBA plus corrector VX-809 is more effective than either alone, and whether potentiator plus two correctors is the most robust.

We present two different but complementary investigations to address this hypothesis. The first is to measure CFTR mediated chloride transport using short circuit current (Isc) measurements from well-differentiated primary human CF bronchial epithelial cells homozygous for F508del CFTR. These cells were obtained by Dr. Steve Rowe through the UAB Clinical and Translational Core Laboratory. After 6 weeks in culture, transwell filters with polarized cell monolayers were treated for 24 hours with correctors added to the basolateral media. Then the filters were mounted in Ussing Chambers and studied under short-circuit current conditions, as described(25-27). A chloride gradient across the monolayer from basolateral bath to apical bath was imposed by substituting sodium gluconate for chloride in the apical bath. Sodium transport was inhibited using amiloride for all treatments; then the CFTR agonists forskolin and VX-770 (VX-770), followed by the CFTR inhibitor CFTRinh-172, were applied to the apical bath as described in the legend in Figure 2. In Figure 2A, the change in Isc was calculated after current stabilized in response to forskolin and VX-770, respectively. Figure 2B shows the subsequent inhibition of Isc by $CFTR_{inh}$-172. The data demonstrate that both 4PBA alone and VX-809 alone stimulate significant increases in CFTR-dependent Isc. Moreover, the two agents together are additive/synergistic; with addition of VX-770, the increase in Isc is over 20 μA/cm^2 (which

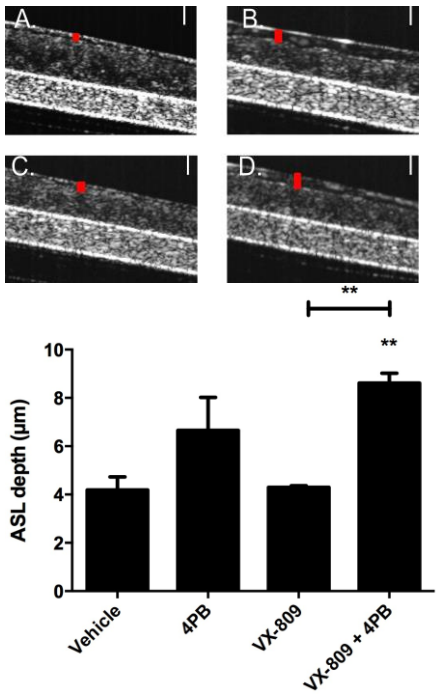


Figure 3. Effect of 4-Phenylbutyrate on airway surface liquid depth. Top: Well-differentiated primary HBE cells from a F508del homozygous donor were incubated for 24 hrs. with vehicle (A) 4-phenylbutyrate (4PB, 1 mM, B), VX-809 3 μM, C), or the combination (D), then ASL depth (red bar) was estimated using one micron resolution optical coherence tomography.[1] Scale bars (white) = 10 μm. **Bottom**: Summary data. Five regions of interest were obtained from two monolayers per condition. **P<0.01. Confirmatory studies including dose and time dependence are in progress.

is equivalent to ~44% of wild-type CFTR) a value sufficient to predict clinical success(28). The N for these experiments is currently being expanded with additional cultures.

The second investigation is based on quantifying the depth of airway surface liquid (ASL) on the cultures. This ASL is controlled by CFTR and the epithelial sodium channel ENaC (in turn also regulated by CFTR). Micro-Optical coherence microscopy (μOCT) is capable of estimating ASL depth to one micron resolution. Figure 3A-D contains representative images of ASL depth for vehicle, 4PBA, VX-809, and 4PBA plus VX-809 incubations for 24 hours. The graph is a composite of data for 5 regions of interest from two monolayers per condition. These data are consistent with Figure 2 and demonstrate significant ASL rehydration for 4 PBA alone and in combination with VX-809. These experiments are being run again to increase our N and evaluate the additive effect of VX-770 (which is required to maximize activity of VX-809 in this assay) (Rowe, S, Symposium NACFC 2012).

1.2 Overview of Clinical Studies

The most common disease-causing mutation in CFTR is the F508del that promotes ubiquitination and premature degradation from the endoplasmic reticulum, as well as reduces the channel's open time for chloride transport. This double defect presents a challenge to therapeutic restoration of the cAMP-regulated chloride transporter. We were the first to test 4-phenylbutyrate (Buphenyl) as a systemic corrector of these defects in F508del under an investigator-initiated IND held by P. Zeitlin. In a series of Phase 1 and 2 trials we established the maximum tolerated dose as 20 gm daily divided t.i.d. and the maximum induction of cAMP-mediated nasal epithelial chloride transport with 30 gm daily as a median of -10 mV on days 4 and 7 of treatment (1, 2). Under those conditions there was no significant decrease in sweat chloride values or in amiloride-inhibited nasal potential difference (NPD). We interpreted these results as a proof of concept of corrector therapy, but corrector therapy alone was likely an insufficient therapy for this mutation in CF, and therefore closed the IND for 4-phenylbutyrate. Now an improved version of 4-phenylbutyrate, Ravicti®, is available with more promising tolerability and pharmacokinetics.

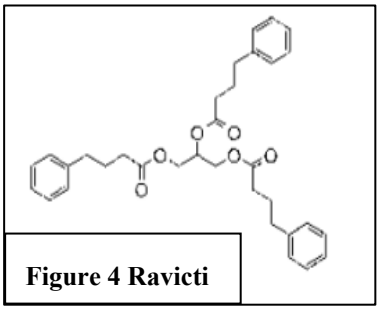


Figure 4 Ravicti

Ravicti® is a triglyceride of PBA (Figure 4) that is broken down by native pancreatic enzymes. Fortunately, in CF, exogenous enzymes are easily delivered. The pharmacokinetics for Ravicti® in UCD are favorable and superior to that of 4PBA. 4PBA is absorbed from the small intestine and converted by β-oxidation to phenylacetic acid (PAA) (Figure 5). PAA is conjugated with glutamine in the liver and kidney by N-acyl coenzyme A-L-glutamine N-acyltransferase to form phenylacetylglutamine (PAGN). Like urea, PAGN contains two waste nitrogens and is excreted in the urine.

Ravicti® has been tested in healthy adults and adults with cirrhosis. It contains no sodium (unlike 4PBA) and is palatable. Compared with 4PBA, peak plasma levels of metabolites occurred later and were lower, urinary phenacetylglutamine excretion was similar, but took longer, and steady state was achieved in 4 days. Importantly, safety in cirrhotic adults was demonstrated at levels equivalent to the highest approved dose for UCD (29). The most frequent adverse events were dizziness, headache, and nausea. For adults and children with UCDs, pharmacokinetic modeling has been based on Phase II/III clinical trials enrolling patients ages 2 months to 72 years. There is slower PBA absorption and greater presystemic conversion with Ravicti® than 4PBA. There is greater PAA exposure based on body surface area in younger patients (30, 31). None of these trials were conducted with concomitant PERT.

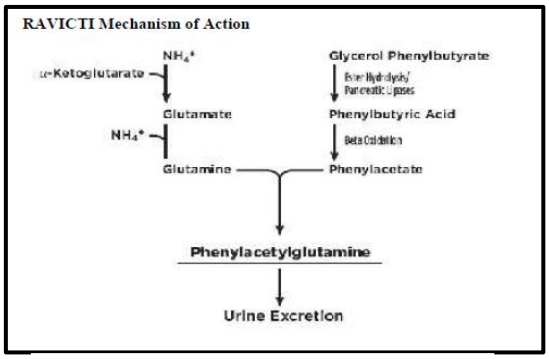


Figure 5: metabolism

We and others have investigated the mechanisms by which 4PBA increases functional F508del. When the CF bronchial epithelial cell line IB3-1 (F508del/W1282X) was grown in 2.5 mM 4 PBA for two days, single channel patch clamp recordings detected a 10.3 +/- 0.05 pS CFTR channel with open probability 0.201 +/-0.04.(5) in 9 of 15 patches as compared with 0 of 10 in IB3-1 at 37^{o}C without 4 PBA and in 54 of 67 patches in IB3-1 grown at 27^{o}C. Since Ravicti® is a triglyceride of 4PBA, the liberated molecule should be equally active. In the same publication we showed induction of band CFTR by 500 μM and 1 mM 4PBA, although, similar to patch data, the band C was slightly stronger in the low temperature control. Primary nasal epithelial cells harvested from a CF patient with F508del/unknown were treated in culture with 5 mM 4PBA and band C appeared at equivalent density on an immunoblot.

Rubenstein and Zeitlin conducted a double-blind, placebo-controlled, pilot clinical trial of 19 gm Buphenyl divided t.i.d. and administered as oral tablets in 18 adults homozygous for F508del for 7 days. Side effects were minimal and comparable in the two groups. At this maximum approved daily dose for UCDs, we saw small but significant inductions of chloride transport in the NPD, without change in the sweat chloride. We next performed a double-blind, placebo-controlled dose escalation study of Buphenyl in 19 adults homozygous for F508del, again for 7 days study drug exposure. Three dose levels: 20 gm, 30 gm, or 40 gm study drug or placebo divided t.i.d. were sequentially enrolled. Induction of CFTR-mediated chloride transport in the NPD was dose and time related, however side effects increased with dose level. The change in low chloride/isoproterenol compared with baseline day 0 data reached -11.9 (05% CI: -18.9, -5.0) on day 3 for the 20 gm cohort and -6.3 (95% Cci:-16.7, 4.1) on day 3 for the 30 gm group. Sweat chlorides did not change. The 40 gm cohort was suspended early because of unpleasant neurological symptoms (visual aurae and muscle cramping), although no SAEs were experienced. Urine and plasma samples were collected. Phenylbutyrate was efficiently converted to phenylacetate and excreted in the urine as phenacetylglutamine in the 20 gm cohort. Increasing the dose to 30

gm daily was associated with a doubling in the AUC24, and accumulation of phenylacetate in the plasma was observed in one individual in the 30 gm cohort. This suggests, that in this one subject, phenylbutyrate may have saturated the metabolic pathway to conversion to phenacetylglutamine.

The modest induction of CFTR function in nasal epithelia by Buphenyl led us to propose a combination of corrector and potentiator for treatment of F508del CF. At the time, the only clinically-eligible potentiator for CFTR was genistein, a tyrosine kinase inhibitor employed in some cancer trials. Rubenstein conducted a small pilot trial in which genistein was only allowed by the FDA to be perfused over minutes in the NPD. Risks of systemic delivery of genistein outweighed any potential benefits in this setting. Now that VX-770 is on the market, the potential to combine Ravicti® with VX-770 is in reach. However, the safety and efficacy of PERT in combination with Ravicti® must first be tested. It is also possible that a combination of correctors, such as Ravicti® with VX-809 would best overcome the barriers to trafficking F508del.

For more detail refer to the package insert (which serves as the Investigator's Brochure).

2. STUDY RATIONALE

We were the first to test 4-phenylbutyrate (Buphenyl) as a systemic corrector of these defects in F508del under an investigator-initiated IND held by P. Zeitlin. In a series of Phase 1 and 2 trials we established the maximum tolerated dose as 20 gm daily divided t.i.d. and the maximum induction of cAMP-mediated nasal epithelial chloride transport with 30 gm daily as a median of -10 mV on days 4 and 7 of treatment (1, 2). Under those conditions there was no significant decrease in sweat chloride values or in amiloride-inhibited nasal potential difference (NPD). We interpreted these results as a proof of concept of corrector therapy, but corrector therapy alone was likely an insufficient therapy for this mutation in CF, and therefore closed the IND for 4-phenylbutyrate.

In the ensuing years, Vertex Pharmaceuticals, Inc. has had success with the development of ivacaftor (32, 32, 33) (VX-770) as a potentiator of G551D CFTR and has studied the drug alone and in combination with their corrector lumacaftor (34) (VX-809) and VX-661. We at National Jewish Health (NJH), Johns Hopkins University (JHU), University of Alabama at Birmingham (UAB) and Children's' Hospital of Philadelphia/University of Pennsylvania (CHOP/Penn) have participated in many of the clinical trials and are pleased and encouraged by the success of VX-770. It is not yet certain that future combinations of corrector(s) and potentiator(s) will be safe and effective, and it is prudent to explore alternative correctors and potentiators. Furthermore, recent structural investigations in a number of laboratories support the idea that more than one corrector may be necessary to fully restore F508del to the trafficking pathway (35). Precedent for combination of 4PBA with other CFTR modulators has been established in vitro (4, 17). Therefore we anticipate that Ravicti® may be useful as an add-on corrector to a corrector/potentiator combination.

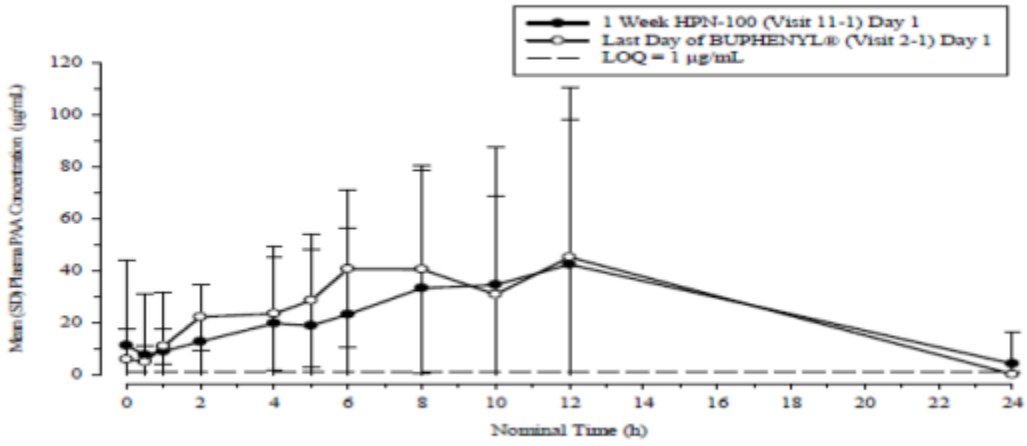
4-Phenylbutyrate tablets are formulated for oral delivery, and we showed that the pharmacokinetics were similar in CF to that in patients with urea cycle disorders. However

the large number of tablets that had to be ingested at each meal was somewhat daunting at the 30 gm daily dose. A new pro-drug of 4-phenylbutyrate, glycerol phenylbutyrate or Ravicti® (owned by Horizon Pharma) was approved in February 2013 by the US FDA. This new formulation is a significant advance for patients with urea cycle disorders because it is an oral, odorless, tasteless liquid, that contains 3 molecules of 4-phenylbutyrate for every molecule of the triglyceride. Simple arithmetic would suggest that one mole equivalent of the pro-drug provides three moles of active drug. However, pancreatic lipase enzymes are required to break the covalent bonds and release the active drug in the intestines. Because most CF patients homozygous for F508del are pancreatic-insufficient and already on enzyme therapy, we propose to test the effectiveness of the combination of CF pancreatic enzyme replacement therapy (PERT) on absorption of Ravicti®. Alternatively, those CF patients with documented pancreatic sufficiency will have endogenous pancreatic enzymes to break the covalent bonds and release active drug in the intestines. We also propose to test the subsequent restoration of nasal epithelial CFTR-mediated chloride transport during the nasal potential difference (NPD) test in response to treatment with Ravicti®.

Additional pharmacokinetic information on Ravicti®

In a PK and safety study UP1204-003, the systemic exposure of PBA following GPBA administration was 27% lower than NaPBA (540 vs 739 ug;h/mL). Exposure levels of the metabolites PAA and PAGN were similar between the two drugs, however GPBA showed less variability between peak and trough levels of PBA (desirable in CF) (see Fig 6 NDA

Figure 6 Mean Plasma PAA Concentration-Time Profile



[Ref: UP1204-003, Figure 7, p.68.]

PK parameters at steady state are compared below in the table for 4PBA and GPBA.

Table 2 PK Parameters (steady-state) NaPBA vs. HPN-100

PK Parameter	Arithmetic Mean (CV%)	
	NaPBA (N=10)	HPN-100 (N=10)
PBA in Plasma		
AUC (µg·h/mL)	739 (49)	540 (60)
Cmax (µg/mL)	141 (44.5)	70.1 (64.7)
Cmin (µg/mL)	0.588 (255)	2.87 (265)
PAA in Plasma		
AUC (µg·h/mL)	596 (124)	575 (169)
Cmax (µg/mL)	53 (94.7)	40.5 (148)
Cmin (µg/mL)	3.56 (194)	7.06 (311)
PAGN in Plasma		
AUC (µg·h/mL)	1133 (31)	1098 (44.2)
Cmax (µg/mL)	83.3 (25.8)	71.9 (56)
Cmin (µg/mL)	16.6 (86)	12.1 (134)

[Ref: UP1204-003, Table 18, p.67]

In Figure 6 shows that by hour twelve, both drugs behaved similarly with respect to PAA concentration.

PK parameters are compared for adult and pediatric UCD patients in the next table.

Table 24 PK of HPN-100 and NaPBA in Adult and Pediatric UCD Patients

PK Variable	Adult UCD Patients (≥18 years)				Pediatric UCD Patients (29days – 17 years)			
	UP1204-003 (n=10)		HPN-100-006 (n=44)		HPN-100-005 (n=11) (6 years – 17 years)		HPN-100-012) n=15 (29 days – 6 years)	
	HPN-100	NaPBA	HPN-100	NaPBA	HPN-100	NaPBA	HPN-100	NaPBA
Mean (SD) Dose	12.3 (3.91)	12.6 (4.11)	12.50 (5.529)	12.33 (5.582)	11.04 (3.859)	10.94 (3.873)	5.16 (2.316)	5.27 (2.453)
Plasma PAA								
AUC_{0-24} (µg·h/mL)	574.6 (168.9)	595.6 (123.9)	447 (130.4)	599 (91.6)	964 (63.6)	773 (73.3)	1096 (214.0)	1458 (211.3)
Cmax (µg/mL)	40.5 (147.6)	53.0 (94.7)	38.5 (102.6)	52.2 (80.2)	90.5 (69.1)	75.1 (64.4)	84.7 (148.3)	98.0 (152.1)
C min (µg/mL)	7.06 (310.7)	3.56 (194.4)	2.11 (381.3)	0.903 (377.7)	2.99 (122.1)	0.674 (130.5)	26.1 (360.8)	49.2 (287.2)

[Ref: adapted from 120-day safety update, Table 2, p.19.]

2.1 Risk / Benefit Assessment

Known potential toxicity of the Ravicti®

Ravicti® is approved/indicated for use as a nitrogen-binding agent in the chronic management of adult and pediatric patients > 2 years of age with UCDs that cannot be managed by dietary protein restriction and/or amino acid supplementation alone. It is specifically not indicated in the treatment of acute hyperammonemia in UCD, in patients

less than 2 months of age, or in treatment of N-acetylglutamate synthase deficiency. The maximum daily dose in UCD is set at 17.5 ml divided three times a day rounded to the nearest 0.5 ml. The labeling says it must be given with protein restriction in UCD and with food at each dose (not on an empty stomach). The drug is provided as 1.1 gm/ml glycerolphenylbutyrate Ravicti®.

Specific warnings are given for neurotoxicity from the metabolite phenylacetate. If this occurs, the dose is recommended to be reduced. Pancreatic insufficiency is also a warning and in the case of UCD, ammonia is monitored as a biomarker of effective enzymatic release of the 4PBA. There is no specific experience with overdosing in a clinical trial, however accumulation of PAA could become toxic. Signs and symptoms of neurotoxicity include headache, fatigue, peripheral neuropathy, seizures, tremor and/or dizziness. No correlation between PAA levels and neurotoxicity symptoms were identified, but the levels were not always drawn during the symptom. Sleepiness, somnolence, or confusion should lead to consideration of reducing the dosage. As mentioned earlier, one CF subject in the 30 gm cohort of 4PBA accumulated PAA, but without signs or symptoms of neurotoxicity. Our experience with 40 gm daily Buphenyl in homozygous F508del CF patients (a higher equivalent dose than planned in this trial) was that patients experienced unpleasant neurological symptoms leading us to discontinue study drug and halt the third dose cohort and there were no serious AEs. We therefore have experience in early identification of the neurologic symptoms. Since we expect a slower absorption and metabolism of study drug, this is less likely to be a problem in the high dose cohort.

The most common AEs (in at least 10% of patients) are diarrhea, flatulence, and headache. Less commonly reported adverse reactions are: abdominal discomfort or pain, dyspepsia, nausea, vomiting, fatigue, reduced appetite, and dizziness. The drug should not be taken when pregnant or breast feeding. There are no adequate and well-controlled studies in pregnant women. It is not known whether the 4PBA or metabolites are excreted in human milk.

Drug interactions occur with corticosteroids, valproic acid, or haloperidol. In UCDs on these drugs, the ammonia level rises from ineffective Ravicti®. Corticosteroids may cause breakdown of body protein and increase the ammonia level in UCDs. We exclude systemic corticosteroid use, but allow inhaled and nasal corticosteroids since there is much lower systemic absorption and, unlike UCDs, patients with CF do not have underlying problems with protein breakdown. Haloperidol and valproic acid can induce hyperammonemia. Concomitant probenecid can affect renal excretion of phenylacetate and phenacetylglutamine.

Animal Studies

Oral administration of 350 mg/kg/day in pregnant rabbits led to maternal toxicity but no effects on embryonic/fetal development (2.7 times the human adult dosage). Oral administration of 300 mg/kg/day to rats also had no effects on fetal development. However,

in rats, doses of greater than 650 mg/kg/day led to maternal toxicity and reduced fetal growth. Juvenile rats at this high dose experienced reduced weight gain, but no effect on learning, memory, or motor activity.

Buphenyl (4PBA) was approved without carcinogenicity studies. In a 26 week study in transgenic (TG-rasH2), Ravicti® was not tumorigenic at doses up to 1 gm/kg/day. A 2 year carcinogenicity study of Ravicti® in male and female Sprague Dawley rats was positive at very high doses (0.65 gm/kg/day in males and 0.9 gm/kg/day in females) however a scientific advisory panel convened by the sponsor to review proliferative changes concluded that Ravicti® does not pose a carcinogenic risk to humans (NDA p18). The 0.65 gm/kg/day dose is 5 times the highest approved dosing of 12 ml. The FDA reviewer for the NDA application recommended including language to this effect in the product brochure and the drug was approved for lifetime use in UCD.

Genotoxicity studies were negative using the Ames bacterial reverse-mutation assay. The metabolites formed in humans are different that in rats. In humans the conversion to U-PAGN leads to lower glutamine and in rats the conversion ends at PAG and results in a depletion of glycine.

Ravicti® was studied in monkeys as the most relevant model of human metabolism. There were no unexpected toxicities with chronic oral administration at 4 fold higher dosing than for UCD patients. The PK studies suggested that the AUC of PAA at the NOAEL level (1.1 gm/kg/day) for male and female monkeys was 4 fold higher than steady state PAA exposure in adult UCD. Horizon feels this is because monkeys do not have a nitrogen retention state or elevated levels of glutamine that are present in UCD patients.

3. STUDY OBJECTIVES

3.1 Primary Objective

The primary objective **is to quantify the change in nasal epithelial CFTR-mediated chloride transport between pre-dose measurement and 4 and 7 days exposure to Ravicti® or placebo.**

3.2 Secondary Objectives

Secondary Objective 1 is to quantify the change in other NPD measures from baseline and Days 4,7, and 14 and the change in sweat chloride between pre-dose on Day 1 and Day 7

Secondary Objective 2 is to measure safety and tolerability of Ravicti®

Secondary Objective 3 is to measure the pharmacokinetics of Ravicti® to determine the efficacy of PERT on absorption and metabolism of Ravicti®

Secondary Objective 4 is to be able to select a dose of Ravicti® for future combination with other modulators.

4. STUDY DESIGN

4.1 Study Overview

This is a randomized, double-blind, placebo-controlled, dose-escalation design in which 36 subjects will be randomized 2:1 study drug with placebo, resulting in 12 subjects at the low dose (Cohort 1), 12 at the high dose (Cohort 2), and 12 on placebo distributed between the two cohorts.

A screening visit (Visit 0) to establish eligibility will occur anywhere from 4 weeks to 1 week before Visit 1 which is the first dosing day. There will be 5 study visits: Screening (-28 day to -7 days), Visit 1 on Day 1, Visit 2 on Day 4, Visit 3 on Day 7 and Visit 4 on Day 14. There will be an Early Withdraw Visit for anyone stopping study drug before the end of the 7 day dosing period. Study drug will be administered at Visit 1, Day 1 and taken three times a day for the next 7 days, and there will be a 7 day washout period with a final study visit on Day 14. Total participation will be 6 weeks at the maximum and 3 weeks at the minimum depending on length of time between screening and Visit 1.

Plasma concentrations of PBA, PAA, and PAGN will be measured at Visits 1- 4 and Early Withdrawal Urine concentrations of PBA, PAA and PAGN will be collected at study Visits 1-4 and Early Withdrawal with 24 hour urine collection at Visits 1 and 3 and single 1mL collections at all other visits. Traditional safety tests consisting of PFTs and baseline ECG will be performed. Labs including CMP, LFTs, CBC, ESR, CRP, uric acid, will be collected at Screening, Days 1, 7 and 14 or Early Withdrawal and sputum microbiology will be collected. Biologic markers of sweat chloride and NPD measures will be obtained.

Screening data will be reviewed to determine subject eligibility. Subjects who meet inclusion criteria and none of the exclusion criteria will be entered into the study.

The following treatment regimens will be used with a dosing window of ±2 hour:

Cohort 1: Low dose Ravicti® (Oral liquid: 1.1 gm/mL of Ravicti®) will be administered every 8 hours as 6 ml (6.6 gm) by mouth or gastrostomy tube (GT) at 8 AM, 5.5 (6.05 gm) ml at 4 PM, and 5.5 ml at midnight. Times are approximate, should be given every 8 hours ±2 hours.

Cohort 2: High dose Ravicti® (Oral liquid: 9 ml (9.9 gm) at 8AM, 8.25 ml (9.08 gm) at 4 PM and 8.25 ml at midnight). Times are approximate, should be given every 8 hours ±2 hours.

Placebo: A matching placebo will be used in each cohort with similar times and volumes as above, 6 subjects in Cohort 1 (low dose volume) and 6 in Cohort 2 (high dose volume).

The analyses will be conducted on all available data. Treatment groups will be described and compared with respect to baseline demographic and clinical characteristics including age, gender, race, sweat chloride, nasal potential difference measures, microorganisms in sputum, height, weight, and pulmonary function. For the descriptive analyses, Differences between the three groups on categorical variables will be assessed using the chi-squared statistic. Difference between the three groups on continuous variables will be assessed using Analysis of Variance or the nonparametric Kruskall-Wallis test.

The primary objective is to quantify the change in nasal epithelial CFTR-mediated chloride transport between pre-dose measurement and 4 and 7 days exposure to Ravicti® or placebo. A two-sample t-test will be performed to compare the change in the combined treatment group result to the change in the placebo group. An intention to treat analysis will be performed. However, treatment withdrawal or additional administration of PERT for malabsorption will be recorded and described.

Incidence of adverse events will be monitored and recorded during the trial.

5. CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

The primary endpoint is to quantify the change in nasal epithelial CFTR-mediated chloride transport between pre-dose measurement and 4 and 7 days exposure to Ravicti® or placebo.

5.2 Secondary Efficacy Endpoints

Secondary Objective 1 is to quantify the change in other NPD measures from baseline and Days 4, 7 and 14 and the change in sweat chloride between pre-dose on Day 1 and Day 7

Secondary Objective 2 is to measure safety and tolerability of Ravicti®

Secondary Objective 3 is to measure the pharmacokinetics of Ravicti® to determine the efficacy of PERT on absorption and metabolism of Ravicti®

Secondary Objective 4 is to be able to select a dose of Ravicti® for future combination with other modulators.

5.3 Safety Evaluations

Safety labs consisting of standard toxicity labs plus uric acid, sputum microbiology, and spirometry will be performed at visit as outlined in the Appendix 1.

Incidence of adverse events

5.4 Other Evaluations

PK plasma will be sampled for PBA, PAA, and PAGN in this study on Visits 1 and 2 at time 0 (+ 5 min) (delivery of first dose), 1 hour (+/-5 min), 2 hours (+/-10 min), 4 hours (+/-10 min) and 8 hours (+/- 10 min) post dose. A single PK blood sample will be drawn pre-dose on Visit 3 and a single PK blood sample obtained at Visit 4. Early Withdrawal will be

a single PK sample. Samples will be collected in a 4mL K2EDTA tube and placed immediately into an ice/water bath.

A 24 hour PK urine sample will be collected at Visit 1(Day1) and Visit 3 (Day 7) for PBA/PAA/PAGN in a urine collection container and held at room temperature, one ml from this urine will placed in a cryovial and stored at -80°C. Urine will be collected as single spot collection on Visits 2, 4 and Early withdraw. Blood and urine PK samples will be frozen at -80°C and sent to AIT Bioscience in Indianapolis, IN for analysis.

Pharmacokinetics will be evaluated with the association of symptoms of malabsorption if present.

At the National Jewish Health site only, a 4 ml K2EDTA tube of blood will be collected pre-dosing on day 1 and pre-dosing on day 7 for flow cytometry analysis of CFTR protein and cell surface markers of inflammation/activation on peripheral leukocytes.

6. SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of CF who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria:

1. Male or female ≥ 18 years of age.
2. Confirmed diagnosis of CF based on the following criteria:

   Any CFTR genotype combination EXCEPT two stop codons, and

   one or more clinical features consistent with the CF phenotype.
3. Taking pancreatic enzyme replacement therapy (PERT), or have documented pancreatic sufficiency.
4. Ability to perform acceptable spirometry.
5. Ability to understand and sign a written informed consent and comply with the requirements of the study.
6. FEV1 ≥30% of predicted normal for age, gender, and height (Hankinson standards): pre or post-bronchodilator at Screening.
7. Oxygen saturation by pulse oximetry ≥90% breathing ambient air or regular oxygen regimen at screening and Day 1.
8. Hematology and clinical chemistry of blood and urine results with no clinically significant abnormalities that would interfere with the study assessments (as judged by the principal investigator) at screening. If electrolyte abnormality at screening, values must be corrected prior to dosing.

9. Subjects on chronic inhaled antibiotic therapy are eligible if they can continue their usual antibiotic, or remain on their off-cycle period, for the duration of study drug exposure.
10. Negative pregnancy test for women of child-bearing potential.
11. If of childbearing potential, agree to use one highly effective method of contraception from the time of consent through the Visit 4 study visit, per section 9.1.13 of the protocol.

6.3 Exclusion Criteria

1. Administration of any investigational drug or device within 30 days of Screening or within 6 half-lives of the investigational drug (whichever is longer).
2. History of any illness or condition that in the opinion of the investigator could confound the results of the study or pose additional risk in administering study drug to subjects.
3. Any change in chronic therapies for CF lung disease (e.g., Ibuprophen, Pulmozyme®, hypertonic saline, azithromycin, TOBI®, Cayston®) within 4 weeks of Study Day 1.
4. Pregnant, planned pregnancy or breast feeding at Screening.
5. Clinically significant cardiac, liver or kidney disease.
6. Seizure disorder.
7. Acute upper respiratory infection within 2 weeks or acute pulmonary exacerbation requiring intravenous antibiotics within 4 weeks of Screening Visit.
8. Sinus surgery within 6 weeks of Screening Visit.
9. Abnormal renal function.
10. Abnormal liver function, defined as ≥3x upper limit of normal (ULN), of serum aspartate transaminase (AST) or serum alanine transaminase (ALT), or known cirrhosis.
11. Screening laboratory results which in the judgment of the investigator would interfere with completion of the study.
12. History of or listed for solid organ or hematological transplantation.

7. CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed (all routine stable CF medication, listed below)

- cycling inhaled antibiotics
- usual daily nasal sinus irrigation
- usual daily nasal steroid

- mucolytics
- bronchodilators
- azithromycin and other suppressive oral antibiotics if unchanged or on usual alternating monthly schedule

7.2 Prohibited

- systemic corticosteroids
- haloperidol
- valproic acid
- probenecid

8. STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Up to 36 subjects will be randomized into one of three groups (competitive enrollment at up to 3 study sites) Two of three subjects will be randomized to the Ravicti® arm for every one of three subjects allocated to placebo. Group/Cohort 1 will consist of 18 subjects taking either the low dose Ravicti or placebo and Group/Cohort 2 will consist of 18 subjects taking either the high dose Ravicti or placebo. Subjects will not be randomized into Group 2 (the high dose arm) until after the DSMB interim safety evaluation from Group 1. The overall study randomization assignments will be provided by the study biostatistician and managed by the Investigational Drug Service at the National Jewish Health under the direction of the pharmacist. Up to one week will be allotted to prepare the randomized product for dispensing. The pharmacist will dispense the randomized treatment allocated to the subject and give to the study coordinator.

8.2 Blinding

Due to the objectives of the study, the identity of test and control treatments will not be known to investigators, research staff, or patients. The following study procedures will be in place to ensure double-blind administration of study treatments.

The subject and the investigational team will be blinded to the treatment allocations. The study pharmacist will hold the unblinding codes. Activation of unblinding of the code should be done following consultation with the PI and only for a serious adverse event or emergency where the event or emergency is considered to be study drug related. All efforts should be made to contact the DMC before the randomization code is broken. The study pharmacist receiving the phenylbutyrate and metabolite levels and the study biostatistician are the only unblinded investigative staff.

The study blind will be broken on completion of the clinical study and after all adverse events have been evaluated for relationship to study drug, coded, and reviewed by the Sponsor.

During the study, the blind may be broken **only** in emergencies when knowledge of the patient's treatment group is necessary for further patient management. When possible, the Investigator should discuss the emergency with the DSMB Chairman prior to unblinding.

8.3 Test and Control Formulation

The study drug is Ravicti®, an oral liquid formulation of glycerol phenylbutyrate that is manufactured by Horizon Pharma. The placebo will be supplied by the site using pharmacy grade solutions. Placebo will be matched to study drug on color, taste and viscosity. It will be placed in an amberbottle(s) with quantity sufficient for entire dosing schedule and labeled appropriately. The bottle will have an AdaptaCap and oral syringes will be provided to draw up the correct dosage. Drug and placebo can be taken orally via dosing syringe or by medication cup.

The placebo is Thick-It® AquaCareH20 Beverage, Thickened Water, nectar consistency; The placebo is stable and sterile for 10 days at ambient temperature.

8.4 Dosage Forms and Strengths

Ravicti® is an oral liquid that is colorless to pale yellow as 1.1 g/mL of glycerol phenylbutyrate (delivers 1.02 g/mL of phenylbutyrate). This is packaged in an AdaptaCap bottle (trademark of Baxter International, Inc.) with Adapter making it easy to draw liquid from the bottle into an oral syringe. This can be taken by mouth, nasogastric tube or gastrostomy tube. If dispensing into NG or gastrostomy tube, flush with 30mL of water and allow the flush to drain. Flush a second time with an additional 30mL of water to clear tube.

8.4.1 Packaging and Labeling

Sample Label:

Date: __/__/___ Subject Name: _____ Subject ID #____

Directions: _____

Ravicti® ____ g/mL or Placebo

Qty: ___ syringes Exp: _____ RPh: ___

Dr. _____ Phone: _____

Ravicti® oral liquid 1.1gmL is supplied packaged in multi-use, 25mL glass bottles. The bottle will have an AdaptaCap and oral syringes will be provided to draw up the correct dosage. Drug and placebo can be taken orally via dosing syringe or by medication cup.

Labels will be provided by the pharmacy and will contain the dispensation date, subject name, study ID, directions for use, state "Ravicti® or Placebo", expiration date, study doctor's name and phone number, and pharmacist's name. Oral syringes will be dispensed with the number needed for dosing.

8.4.2 Handling/Dispensing

The PI or designee is responsible for maintaining accurate dosing records of the study drug throughout the clinical study. The designated research pharmacist is responsible for maintaining accurate accountability records of the study drug throughout the clinical study. The designated research pharmacist, who is not otherwise involved in the conduct of the study, will be unblinded. All other study staff including the Sponsor will remain blinded throughout the study until database lock.

The unblinded research pharmacist at the study site will prepare and dispense study drug for each subject according to the protocol, randomization list, and pharmacy manual. Study drug, glycerol phenylbutyrate and matched placebo, will be dispensed in identical bottles in identical volumes. The pharmacy will assure that no information related to randomized treatment is made available to personnel involved in the conduct of the study.
The drug accountability log includes information such as randomization number, amount dispensed, and amount returned to the pharmacy (if any). The Investigator or sub-Investigator will sign an order for the study medication and faxed to the site investigational pharmacy. Study drug will be picked up by the RC or delivered to the unit for dispensation to the subject. Enough study drug for use at home and clinic will be dispensed at Visit 1 to last until Visit 2, at Visit 2 to last until Visit 3, and at Visit 3 with enough drug for the two remaining afternoon and nighttime doses due. It is also permissible to dispense entire 7 days of study drug at Visit 1/Day 1. The first dose from each supply will be administered in the clinic at Visits 1, 2 and 3. Any unused study drug will be returned at Visits 2, 3, and 4 or entire amount returned at Visit 4 to the study team and subsequently, to the investigational pharmacy for accountability.

8.4.3 Dosage/Dosage Regimen

Study drug is to be taken by subjects with a meal or snack three times a day. Study drug will be administered after the start of a meal or snack. Prior to the first dose of study drug, subjects will be asked to fast from midnight (Visit 1/Day 1 only). For those pancreatic insufficient subjects, PERT will be administered 15 min (± 10 min) before study drug and the study drug will be given with a meal every 8 hours (dosing windows are ±2 hours). A typical dosing day may look something like this:

- **8am** (6am-10am) - PERT 15 min (±10 min) before dosing and ***study drug taken after the start of breakfast***. (If Day 1, fast from midnight).
- Noon (10am-2pm) lunch - PERT will be taken as usual before meal or snack
- **4pm** (2pm-6pm) - PERT 15 min (±10 min) before dosing and ***study drug taken after the start of a snack/meal.***

- 7 pm- (5pm-9pm) dinner – PERT will be taken as usual before meal or snack
- **Midnight** - (10pm-2am) PERT 15 min (±10 min) before dosing and ***study drug taken after the start of a snack.***

The low dose of Ravicti® (Oral liquid: 1.1 gm/mL) or placebo will be administered ~~to~~ every 8 hours ±2 hours. For Cohort 1, the first dose will be 6 ml (6.6 gm) by mouth or gastrostomy tube (GT), 5.5 (6.05 gm) ml, and 5.5 ml. For Cohort 2, the high dose of Ravicti® or placebo will be administered as 9 ml (9.9 gm), 8.25 ml (9.08 gm), and 8.25 ml.

Administration Instructions Study drug should be administered orally three times a day every 8 hours (for example, 8am, 4pm and midnight) with a ±2 hour dosing window for 7 ± 2 days. Study drug should be taken 8 ± 2hours apart and no less than 4 hours after the previous dose. PERT should be taken within 15 min (±10min) prior to each dose of study drug and also taken, as usual, before the other meals or snacks. Every dose of Study drug should be taken after the start of a meal or snack. Other medications can be taken as usual. Subjects will be dispensed study drug at Visits 1, 2, and 3 to complete all dosing requirements. All unused study drug should be returned at Visits 2, 3, and 4 or at an Early Withdrawal visit. Alternatively, subjects will be dispensed 7 days of study drug at Visit 1 and will return entire amount of unused drug at Visit 4.

8.4.4 Supply of Study Medication at the Site

The study drug will be housed at the Investigational Pharmacies for each site and will be sent to each site by the Sponsor or Sponsor's representative after the site has approval to enroll. Subjects will be given a study ID and after passing screening, will be randomized. If a subject withdraws from the study prior to treatment, they will be replaced.

8.4.5 Storage

RAVICTI® (glycerol phenylbutyrate) oral liquid 1.1 g/mL is supplied in multi-use, 25-mL glass bottles. The bottles are supplied in the following configurations:

NDC 76325-100-25: Single 25-mL bottle per carton
NDC 76325-100-04: Four 25-mL bottles per carton

Store at 20°-25°C (68°-77°F) with excursions permitted to 15°-30°C (59°-86°F).

8.5 Study Medication Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the site study staff. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record.

Measures of Treatment Compliance: Due to the brevity of the study and frequency of visits, we expect treatment compliance to be very good. Subjects will be asked to keep a patient diary noting the day, date and time they took their study medication, PERT prior to dose and if they had a meal or snack with the dose. They will also be asked to record any changes in medication, health or adverse events. They will be asked to bring the diary to

each study visit to review with the study coordinator. Any changes in health, medications or AEs will be recorded on the CRFs. Subjects will be asked to return any unused study drug at Visit 4 or Early Withdrawal to determine compliance and drug accountability. Missed doses will be documented on the diary or can be reported to the RC and then documented on the source documents.

9. STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, a local IRB- approved, written informed consent must be carefully reviewed with each subject then signed, dated, timed and witnessed after all questions and concerns are addressed. A copy of the consent form will be provided to the subject.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Screening (including the previous 28 days prior to screening) and verified at each visit. Any changes in medications/therapies from Screening will be documented on the Concomitant Medication/Therapy Forms. If subject has started any exclusionary medications between screening and Day 1, they will not be dosed. Name of drug, dose, route, unit frequency of administration, indication for administration as well as start and stop dates of each medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, gender, race, ethnicity) will be recorded at Screening.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Screening and reconfirmed on Day 1. Genotype will be documented from Port CF or medical records.

9.1.4 Full and Abbreviated Physical Examination

A complete physical examination will be performed at screening and will include an assessment of the following body systems: head/neck/thyroid, eyes/ears/nose/throat (EENT), chest, lungs, heart, lymph nodes, abdomen, skin, musculoskeletal, and neurological systems. The full physical examination will be performed by a physician at Screening and Early Withdraw Visits.

An abbreviated physical examination including evaluation of EENT, chest, heart, lungs, skin, and abdomen will be conducted at Visits 1-4. Qualified staff (MD, NP, RN, and PA) may complete the abbreviated physical exam.

If a clinically significant abnormality is found during a physical exam (after dosing, during the study, and/or at any of the follow-up visits) or if the investigator feels that there has been a clinically significant change from baseline, it should be recorded as an adverse event and the study subject will be followed until the findings have resolved or if ongoing, the findings are stable.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for ≥5 minutes at each study visit.

Height and weight will be conducted with shoes removed. Height will be obtained once at Screening. Weight will be collected at every study visit.

9.1.6 Pulse Oximetry

Pulse oximetry will be measured on either ambient air or regular oxygen regimen with the subject at rest for ≥ 5 minutes at every study visit (Screen, Visits 1-4 and Early Withdrawal).

9.1.7 Spirometry

Spirometry, meeting ATS criteria, will be performed at every study visit. The determination about whether or not to do pre-bronchodilator PFTs will be decided in a manner consistent with subject's usual bronchodilator regimen and maintained throughout the study. If a bronchodilator is routinely taken, then it will be given before each spirometry test as a post-bronchodilator PFT. This should be consistent at each study visit so if the subject used a bronchodilator prior to spirometry at Screening, it should be used at each study visit. If the subject does not use a bronchodilator prior to spirometry, the test will be done as a pre-bronchodilator PFT. This includes: the use of a SABA such as albuterol 4 hour before dosing, use of a LABA such as Advair® up to 12 hours before dosing or a daily bronchodilator (Spiriva®) up to 24 hours prior to spirometry. The spirometer used to assess lung function will be calibrated on study visit days and prior to the study visit.

Spirometry will be performed and in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests. Results will be reviewed and signed by the Investigator or sub-Investigator. Original copy will be maintained in the subject's binder. Any 20% decrease in subject's FEV1 from baseline (Day 1) will be recorded as an AE. Subjects who have an FEV1 of less than 30% predicted at Screening will not be eligible for study inclusion.

9.1.8 Questionnaires

The Cystic Fibrosis Questionnaire (CFQ-R)

The CFQ-R, version 2.0, is a validated, disease specific, developmentally appropriate questionnaire designed to measure the physical, emotional and social impact of CF on patients and their families. It should be administered in a quiet room prior to other study related procedures where possible. Since this study includes adults, the CFQ-R version for

ages 14 and over will be used and can be done in a self-report format. This will be done at the following study visits: Screening, Visit 1 (Day 1), Visit 3 (Day 7) and Visit 4 (Day 14). A sample of the questionnaire is provided in Appendix 2.

Epworth Sleepiness Scale (ESS)
Subjects will be asked to complete the Epworth Sleepiness Scale at the end of every visit. The Epworth Sleepiness Scale is widely used in the field of sleep medicine as a subjective measure of a patient's sleepiness. The test is a list of eight situations in which the subject rates their tendency to become sleepy on a scale of 0, no chance of dozing, to 3, high chance of dozing. When they finish the test, the staff can add up the values of their responses. The total score is based on a scale of 0 to 24. The scale estimates whether the subject is experiencing excessive sleepiness that possibly requires medical attention. A sample of the questionnaire is provided in Appendix 3.

9.1.9 Sputum collection:

A sputum sample will be collected for microbiology testing at Screening, and Visits 1 and 4. If the subject is unable to spontaneously expectorate, a throat culture may be performed. The sample will be collected in a sterile specimen cup or with a culturette and sent to the local lab for CF respiratory culture

9.1.10 12-lead Electrocardiogram

A standard 12 lead ECG will be obtained at Screening after the subject is resting supine for ≥5 min. The results will be interpreted and signed by the Investigator or sub-Investigator . The signed original ECG -will be maintained in the subject's binder.

9.1.11 Sweat Chloride Testing

Sweat Chloride testing will be performed pre-dose at Visit 1 and post-dose at Visit 3. The Macroduct® collection system (Wescor, Logan UT) will be used according to the CFFT TDN SOP 514 to collect sweat to evaluate sweat chloride. Collection of sweat samples will be performed using the approved collection device and stored frozen. Sweat samples will be sent to a central laboratory (TDN Center for Sweat Analysis, Denver) for testing, interpretation of results, and entry of the results into the database. Individual sweat test results will not be disclosed to the study sites except for screening results. Specific instructions for collection, handling, processing, and shipping of sweat chloride samples to the central laboratory will be provided separately in the Study Manual and/or Laboratory Manual. Ideally, the sweat chloride measurements will be collected prior to dosing on Visit 1 and within one hour after dosing on Visit 3.

9.1.12 Nasal Potential Difference (NPD) Test

The Nasal Potential Difference (NPD) Test will be performed at Screening and Visits 2, 3 and 4 by a certified operator at each site's local testing facility. The procedure will be done according to the CFFT TDN SOP 528.01. Specific instructions for the conduct of the NPD test, measurements to be collected, and submission of results to the central reading center)

will be provided in the Study Manual and/or Laboratory Manual. NPD results will be interpreted by a central reading center (UAB Center for CFTR Detection (CCD), and the results entered into the study database. If the Screening (baseline) NPD is deemed invalid by the Central Reader, the NPD may be repeated again at Visit 1.

9.1.13 Contraception/Pregnancy

The effects of glycerol phenylbutyrate on contraception, pregnancy, and lactation in humans are not known. For all subjects of child-bearing potential, all methods of contraception, including abstinence, must be in use from the time of consent through the Day 14 outpatient visit. Mothers will not be allowed to breast feed while in this study. Subjects who are not sexually active during the Screening period must agree to the contraceptive requirements if they become sexually active with a partner of the opposite sex during the study.

The contraceptive requirements for subjects are as follows:
Male subjects must agree to use one of the following highly effective methods of contraception:

- Condom with spermicide
- Abstinence
- Female partner who is not of child bearing potential (i.e., post-menopausal or post-surgical sterilization)
- Female partner of child bearing potential who uses hormonal contraception, has an IUD in place, or who uses either a cervical cap or diaphragm with spermicide.

Male subjects with documented infertility or surgical sterilization (performed at least 6 months before the first dose of study drug) are exempt from this requirement. Infertility may be documented through examination of a semen specimen or by demonstration of the absence of the vas deferens on ultrasound before the first dose of study drug (Day 1).

Female subjects who are considered of child-bearing potential must agree to use 1 of the following highly effective method of contraception:

- Hormonal contraceptives
- Occlusive cap (cervical cap or diaphragm) with spermicide
- Intrauterine device (IUD)
- Abstinence
- Condom with spermicide

Female subjects will be considered of child-bearing potential after the onset of their first menstrual period. Female subjects who are documented as being of non-child-bearing potential (Post-menopausal or surgical sterilization) are exempt from this requirement. Female subjects will be considered post-menopausal if they have had 12 months of consecutive spontaneous amenorrhea or less than 12 months of consecutive spontaneous amenorrhea and a serum FSH level > 40 mIU/mL. Female subjects will be considered surgically sterile if they are post-hysterectomy, 6 months post-surgical bilateral oophorectomy, or 6 months post-tubal ligation.

Subjects will be counseled to inform the investigator or study staff of any pregnancy that occurs from consent up to Visit 4. If a female subject or a male subject's female partner becomes pregnant, the study drug must be discontinued immediately and the pregnancy must be reported to the DSMB within 24 hours of the site's knowledge of the subject's (or partner's) pregnancy while confirmation is pending. Once the pregnancy is confirmed with a serum pregnancy test, study drug will be permanently discontinued. Pregnancy does not constitute an adverse event. Once a pregnancy is confirmed, subject treatment assignment will be unblinded; subjects on active study drug who become pregnant or impregnate their female partner will be followed until the end of pregnancy provided consent is obtained. The mother and infant exposed in utero will be followed for 1 year after the birth, provided consent is obtained.

9.1.14 Study Diary

A study diary will be initiated at Visit 1 and used to document the date and time each dose of study drug is taken, time of PERT prior to each dosing and whether the drug was taken with a meal or snack. The diary will also be used by the subject to collect any changes in their health or medications between visits and any adverse events during dosing and until the final visit. The diary will be reviewed by the study staff at Study Visits 2 and 3 and collected at Visit 4 or at the Early Withdrawal visit.

9.1.15 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

Below is a chart of labs collected at each study visit, amount, type of tube and lab where the sample is processed or shipped (local or outside). Up to 125mL of blood will be collected from Screening to Day 14.

Screening			
Collection	**Test**	**Local Lab**	**Outside Lab**
(1) 10mL SST	CMP	X	
	CRP	X	
	Uric Acid serum	X	
	HCG serum	X	
(1) 3mL Lavender	Heme-8	X	
	ESR	X	
(1) Sterile specimen cup with 10 cc urine	Urinalysis	X	
(1) Sterile specimen cup with sputum	CF Respiratory Culture	X	
Total blood = 13 mL			
Visit 1/ Day 1			
Collection	**Test**	**Local Lab**	**Outside Lab**
(1) .Sterile specimen cup with 10 cc urine	Urine HCG	X	
(1) 10mL SST	CMP	X	
	CRP	X	
	Uric Acid serum	X	
(1) 3mL Lavender	Heme-8	X	
	ESR	X	
(5-6) 4mL K2EDTA tube	PK blood sample: pre-dose, 1, 2, 4 & 8 hours post dose at all sites; biomarkers at NJH site only		X
(1) Sterile specimen cup with sputum	CF Respiratory Culture	X	
24 urine collection container	24 hr. urine PK		X

Total blood = 33-37mL			
Visit 2/Day 4 (±2 days)			
Collection	**Test**	**Local Lab**	**Outside Lab**
(5) 4mL K2EDTA tube	PK blood sample; pre-dose, 1, 2, 4 & 8 hours post dose		X
urine collection cup (1mL)	PK urine post dose		X
Total blood= 20mL			
Visit 3/Day 7 (±2 days)			
Collection	**Test**	**Local Lab**	**Outside Lab**
(1) 10mL SST	CMP	X	
	CRP	X	
	Uric Acid serum	X	
(1) 3mL Lavender	Heme-8	X	
	ESR	X	
(1) Sterile specimen cup with 10 cc urine	HCG Urine	X	
	Urinalysis	X	
(1-2) 4mL K2EDTA tube	Pre-dose PK blood sample (at all sites); biomarkers (at NJH site only)		X
24 urine collection container	24 hr. urine PK		X
Total blood= 17-21mL			
Visit 4/Day 14 (±2 days)			
Collection	**Test**	**Local Lab**	**Outside Lab**
(1) 10mL SST	CMP	X	
	CRP	X	
	Uric Acid serum	X	
(1) 3mL Lavender	Heme-8	X	
	ESR	X	
(1) Sterile specimen cup with 10 cc urine	HCG Urine	X	
	Urinalysis	X	

	Urine PK (1mL)		X
(1) Sterile specimen cup with __ mL sputum	CF Respiratory Culture	X	
(1) 4mL K2EDTA tube	PK blood sample only		X
Total blood = 17mL			
Early Withdrawal Visit- Only if needed			
Collection	**Test**	**Local Lab**	**Outside Lab**
(1) 10mL SST	CMP	X	
	CRP	X	
	Uric Acid serum	X	
(1) 3mL Lavender	Heme-8	X	
	ESR	X	
(1) Sterile specimen cup with 10 cc urine	HCG Urine	X	
	Urinalysis	X	
	Post dose urine PK (1mL)		X
(1) 4mL K2EDTA tube	PK blood sample only		X
Total blood= 17mL			

9.2.1 Hematology (Heme 8) and ESR:

Blood will be obtained and sent to the site's local clinical laboratory for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count) and erythrocyte sedimentation rate (ESR). This will be collected at Screening, Visit 1, 3 and 4 and Early Withdrawal.

9.2.2 Complete Metabolic Panel, CRP and uric acid:

Serum will be obtained and sent to the site's local clinical laboratory for determination of a blood chemistry profile consisting of sodium, potassium, chloride, bicarbonate/CO_2, glucose, BUN, total protein, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, albumin, C-reactive protein (CRP), and uric acid will be added. These samples will be collected at Screening, Visits 1, 3, 4 and Early Withdrawal.

9.2.3 Pregnancy Test:

β-human chorionic gonadotropin (β-HCG) using serum/urine samples for women of childbearing potential will be performed. Blood or urine will be obtained from all female subjects of child bearing potential and sent to the site's local clinical laboratory for a qualitative serum pregnancy testing. Blood will be collected for serum β-HCG at Screening and urine will be collected for β-HCG tests for Visits 1, 3, 4 and at Early Withdrawal.

9.2.4 Urinalysis:

Urine will be obtained for a urinalysis and sent to the site's local clinical laboratory for determination of color, specific gravity, pH, protein, glucose, ketones, and blood. This will be collected at Screening, Visits 3 and 4 and Early Withdrawal.

9.3 Pharmacokinetic Measurements

The PK blood samples will be collected in a 4mL K2EDTA tube and placed immediately into an ice/water bath for no longer than 30 minutes and spun and aliquoted within 60min of collection. Centrifuge under refrigerated conditions at 4°C between 1000 and 1300 RCF for 10 min.

Following centrifugation, divide plasma between two cryovial tubes (tube #1 and tube #2) for PAA, PBA, and PAGN analysis with at least 0.5 mL of plasma in the first tube. Place remaining plasma into a second cryovial (tube #2) for PAA, PBA, and PAGN. Samples should be frozen at -80°C. All samples will be labeled with the subject's ID. Blood for determination of plasma concentrations of Ravicti® will be collected pre-dose and at 1, 2, 4, and 8 hours after the start of dosing at Visit 1and Visit 2. At Visit 3 a pre-dose PK blood sample will be collected. At Visit 4, a PK blood sample will only be collected once. Early Withdrawal visits will consist of one PK blood sample.

Urine PK samples will be obtained at each visit after screening. 24 hour urine collection will be started in the clinic after dosing at Visits 1 and 3 and a single 1mL urine sample will be collected from the total volume of this urine. Urine will be collected as a single spot collection on Visits 2, 4 and Early Withdrawal. Blood and urine PK samples will be frozen at -80°C and sent to AIT Bioscience in Indianapolis, IN for analysis.

9.4 Research Laboratory Measurements

9.4.1 Plasma PBA, PAA and PAGN:

PK labs will be drawn at Study visits 1-4 and Early Withdrawal. Blood will be collected pre-dose and at 1, 2, 4 and 8 hours post-dose on Visits 1 and 2, and at Visit 3 pre-dose. On Visit 4, there will be only one PK blood sample collected.

9.4.2 Urine PBA, PAA and PAGN:

24 hour urine collection will begin immediately after dosing on Visit 1 and Visit 3. Subjects should fully void right before dosing. Subjects will be provided with a urine collection container to take home for the 24 hour urine collection starting immediately after dosing. Time of dosing and start of 24 hour urine collection are both time 0 so the urine will be

collected for 24 hours after dosing. The sample should be kept at room temperature at home during the collection time and then returned to the site at, or before, the next visit, within 50 hours of collection completion. The subject will be provided with a urine collection container and the whole sample should return in the container to the study site. Once returned to the site, 1mL will be removed as a representative sample by the study team and frozen at -80°C, the whole volume measured and the 1mL urine sample will be sent to the AIT Bioscience for PK analysis.

10 EVALUATIONS BY VISIT

10.1 Screening Visit, Day 0.

On Screening, Day 0 and prior to any procedures, the consent form will be signed, dated and timed by the participant and consent designee. Study staff will review the consent with the subjects and they will have the opportunity to ask any questions. A copy of the consent form will be provided to each subject.

The patient will complete the CFQ-R questionnaire before all other procedures then will undergo vital signs to include height, weight, pulse oximetry, heart rate, blood pressure, respiratory rate, and temperature. Phlebotomy, ECG and urine collection will be performed by study staff. Research staff will perform spirometry to consist of FVC, FEV1, and FEV1/FVC with flow volume loops, using ATS quality criteria. If participant routinely uses bronchodilator, it will be given prior to spirometry. An expectorated sputum sample will be collected. If the subject is unable to provide a spontaneous expectorated sputum sample, a throat culture may be obtained. A urine sample will be collected for urinalysis. A standard 12 lead ECG will be performed after the subject has rested supine for at least 5 minutes. A nasal PD according to the CFFT-TDN SOP 528.01 will be performed by a certified NPD operator at each site. The data will be over read at the UAB Center for CFTR Detection (CCD). The Epworth Sleepiness Scale will be administered at the end of the visit.

Visit 0 (Screening) Day -28 to Day -7 (4.25 hours)

1. Consent: Review the IRB-approved ICF with the subject and obtain written informed consent. Provide a copy to the subject.
2. Provide CFQ-R version 2.0 to subject to self-administer. This needs to be completed prior to all other procedures.
3. Assign the subject a unique study ID number.
4. Record demographics data.
5. Record medical history, including a history of CF and diagnosis date.
6. Record concomitant medications and concurrent therapies after confirming with subject.
7. Vital signs: Perform and record vital signs including temperature, pulse, respiratory rate and blood pressure.
8. Measure height and weight without shoes.
9. Perform oximetry.

10. Obtain an ECG after subject has been resting supine ≥5 minutes.
11. Perform spirometry using ATS criteria.
12. Collect expectorated sputum for culture. If unable to expectorate, use throat swab.
13. Collect urine sample for urinalysis.
14. Perform a complete physical examination.
15. Perform a Nasal Potential Difference (NPD) procedure.
16. Collect blood sample for clinical laboratory tests (CMP, hematology, pregnancy test, ESR, CRP, and uric acid).
17. Epworth Sleepiness Scale should be given to the subject to complete.
18. Schedule subject for Visit 1 within 7 to 28 days from Screening.
19. Document any adverse events.
20. Remind the subject to fast (NPO from midnight) the night before the first dose of study drug (Day 1)

10.2 Visit 1 (First Dosing, Day 1) (10 hours)

1. Record any Adverse Experiences.
2. Review of concomitant medications, lab results and reconfirmation of medical history to ensure subject meets all I/E criteria.
3. Confirm subject has fasted since midnight and until first dose of study drug. Ensure full PERT dose is consumed 15 min (±10min) prior to dosing (when applicable) and dosing is taken after the start of a meal/food.
4. Randomize subject (Day 1 or up to – 2 days before Day 1).
5. Provide CFQ-R version 2.0 to subject to self-administered.
6. Perform abbreviated physical examination.
7. Collect weight.
8. Perform and record vital signs after resting ≥5 minutes.
9. Perform and record oximetry.
10. Perform spirometry using ATS criteria.
11. Collect expectorated sputum sample. If subject is unable to expectorate, collect T/C.
12. Collect urine sample for pregnancy testing. Must confirm negative pregnancy test prior to dosing. A positive test is exclusionary.
13. Start IV or venipuncture to collect Pre-dose PK blood sample and clinical laboratory tests (CMP, hematology, ESR, CRP, and uric acid). PK blood sample must be placed immediately into an ice/water bath for no longer than 30 minutes and spun and aliquoted within 60min of collection.
14. Sweat chloride will be performed according to the CFFT TDN SOP 514 by study staff and evaluated at the CF-TDN Center for Sweat Analysis Laboratory in Denver.

15. NPD may be repeated ***only if good results were not obtained at screening.***
16. PERT will be administered 15 min (±10 min) before study drug when applicable.
17. Ask subject to fully void just prior to dosing.
18. Study drug will be given after the start of a meal or snack. Remind subject to take study drug at every 8 hours (±2 hours) daily. PERT should be taken when applicable 15 min (±10 min) prior to each dose and the dose should be taken after the start of a meal or snack.
19. Post dose PK urine collection will be initiated immediately after dosing for 24 hours. Sample should be at room temperature during the collection period and the whole sample returned to clinic within 50 hours of collection completion. Subjects will be provided with a urine collection container and asked to return whole sample to the clinic.
20. Post dose PK phlebotomy will be obtained in a 4mL K2EDTA tube at 1 hour (±5 min), 2 hours (±10 min), 4 hours (±10 min) and 8 hours (+/- 10 min) after study drug administration.
21. Initiate diary to collect all dosing times, PERT times and AEs.
22. Provide subject with lunch (PERT to be taken as usual).
23. Epworth Sleepiness Scale should be given to the subject to complete.
24. Patient will be discharged with enough study drug to last through the next study visit (Visit 2 Day 4 ±2 days). Alternatively, subject may be dispensed with entire dose regimen. Subjects will be provided oral syringes to measure the correct dose of the drug. Drug can be taken from oral syringe or from medicine cup once measured.
25. Remind the subject to collect all urine for 24 hours from dosing time on Day 1, keep at room temperature and bring whole sample back to the clinic. Remind them to bring any unused study drug, syringes and completed study diary.

10.3 Visit 2 (Day 4 Dosing visit) ± 2 days (10 hours)

1. Review subject diary for adverse experiences and dosing compliance.
2. Collect used syringes and unused study drug, if applicable.
3. Record changes to concomitant medications.
4. Perform and record vital signs and weight (no shoes).
5. Perform and record oximetry.
6. Perform and record spirometry.
7. Perform abbreviated physical examination.
8. Start IV or venipuncture and collect pre-dose PK blood
9. PERT will be administered 15 min (±10 min) before study drug when applicable
10. Study drug will be taken just after the start of the meal with breakfast

11. Collect 1mL of urine from the post dose PK 24 hour urine collection.
12. Collect a 1mL freshly voided single spot urine sample post dosing.
13. Post dose PK phlebotomy will be obtained at 1 hour (±5 min), 2 hours (± 10 min), 4 hours, (±10 min), and 8 hours (+/- 10 min) after study drug administration. The PK blood samples will be placed immediately into an ice/water bath for no longer than 30 minutes, spun and aliquoted within 60min of collection.
14. Perform Nasal potential difference (NPD) test after dosing.
15. Epworth Sleepiness Scale should be given to the subject to complete.
16. If not previously dispensed, subject will be discharged with enough study drug to last through Visit 3 (Day 7). Remind subjects to return to clinic for Visit 3 on day 7±2 days.

10.4 Visit 3 (Day 7) (±2 days) (4 hours)

1. Provide the CFQ-R v2.0 questionnaire to subject to self-administer.
2. Review subject diary for adverse experiences and dosing compliance.
3. Collect used syringes and unused study drug, if applicable.
4. Record changes to concomitant medications.
5. Perform and record vital signs and weight (without shoes).
6. Perform and record oximetry.
7. Perform and record spirometry.
8. Perform abbreviated physical examination.
9. Collect blood sample for clinical laboratory tests: CMP, Hematology, CRP, ESR, and uric acid.
10. Collect urine for urinalysis and pregnancy test.
11. PERT will be administered 15 min (±10 min) before study drug when applicable.
12. Study drug will be taken just after the start of breakfast which may be provided by the study staff
13. Initiate 24 hour urine collection immediately after dosing. Subject should collect for 24 hours after dosing and keep at room temperature. Return whole urine sample to the clinic within 50 hours of collection completion.
14. Collect pre dose PK blood sample.
15. Perform a nasal potential difference test (NPD) after study drug dosing.
16. Perform a sweat chloride test after study drug dosing: ideally within 1 hour of dosing.
17. Epworth Sleepiness Scale should be given to the subject to complete.
18. If not previously dispensed, subject will be discharged with enough study drug to last through remaining doses.

10.5 Visit 4 (Follow-up Visit, Day 14) (3.25 hours)

1. Provide the CFQ-R v2.0 questionnaire to subject to self-administer.
2. Review subject diary for adverse experiences and dosing compliance.
3. Collect all syringes and unused study drug.
4. Record changes to concomitant medications.
5. Perform and record vital signs and weight (without shoes).
6. Perform and record oximetry.
7. Perform and record spirometry.
8. Collect expectorated sputum sample. If subject is unable to expectorate, collect T/C.
9. Perform abbreviated physical examination.
10. Collect urine for urinalysis and pregnancy test.
11. Collect PK blood sample and clinical laboratory tests: CMP, Hematology, CRP, ESR and uric acid.
12. Collect 1mL PK urine sample from the post dose PK 24 hour urine collection.
13. Collect a 1mL freshly voided single spot PK urine sample post dosing.
14. Perform a nasal potential difference test (NPD) after study drug dosing.
15. Epworth Sleepiness Scale should be given to the subject to complete.

10.6 Early Withdrawal Visit for subjects who discontinue after dosing between Visits 1 and 3 only. (1.5 hours)

1. Review subject diary for adverse experiences and dosing compliance.
2. Collect syringes and unused study drug, if applicable.
3. Record changes to concomitant medications.
4. Perform and record vital signs and weight (without shoes).
5. Perform and record oximetry.
6. Perform and record spirometry.
7. Perform complete physical examination.
8. Collect blood sample for PK blood and clinical laboratory tests: CMP, Hematology, CRP, ESR, and uric acid.
9. Collect urine for urinalysis, PK urine and pregnancy test.

10. Epworth Sleepiness Scale should be given to the subject to complete.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study medication, or if unrelated, the cause.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0, as modified for cystic fibrosis, should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.3 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) on an SAE Report Form. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

All SAEs will be reviewed by the site investigator and reported to the IRBs and CRUs within one business day of the site learning of the event. Sites will fax the SAE report to the Data Safety Monitoring Board.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

11.4 Medical Monitoring

Dr. Scott Sagel should be contacted directly at these numbers to report medical concerns or questions regarding safety.

Phone: (720) 777-5546

Email: scott.sagel@childrenscolorado.org

12. DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Withdrawal of Subjects and Drop-outs

A subject may be discontinued from the study or study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study or treatment discontinuation:

- Subject withdrawal of consent
- Subject is not compliant with study procedures
- Adverse event
- Lost to follow-up
- Sponsor request for early termination of study
- At the discretion of the DMC, if deemed appropriate, for any reason
- Development of any significant neurological effects (as determined by the Investigator)
- Significant increase in abdominal pain or other signs and symptoms of GI discomfort or hemorrhage.

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized. Subjects will be encouraged to complete all remaining scheduled and follow-up visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw from the study will be replaced.

13. PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol Violations for this study include, but are not limited to,

the following: failure to meet inclusion/exclusion criteria, non-compliance with study drug regimen, and use of a prohibited concomitant medication.

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor -Investigator. A copy of the form will be filed in the site's regulatory binder.

14. DATA SAFETY MONITORING

A Data Monitoring Committee (DMC) will be provided for oversight of safety in this clinical trial. The Cystic Fibrosis Foundation Therapeutics Data Safety Monitoring Board will provide a Data Monitoring Committee to serve in this capacity.

A biostatistician from the Johns Hopkins Bloomberg School of Public Health will act as the study statistician. She will develop the randomization code, provide it to the site pharmacist, maintain the randomization code and provide oversight of the pharmacist. All other study personnel will be blinded. Activation of unblinding the code should be done following consultation with PI and only for a serious adverse event or emergency, where the event or emergency is considered to be study drug related.

The unblinded study pharmacist will inform the PI when two subjects have received study drug and completed PK collections. The PK labs from the first 2 subjects on study drug along with the remaining subjects dosed with placebo will be batched and sent to AIT Biosciences for urgent analysis. Enrollment will continue without hold. The unblinded study pharmacist will review the early PK data and notify the PI if no PBA or PAA is detectable in the first two subjects that receive study drug OR if there are excessive levels. The PI will then halt the study to discuss modifications to the protocol with the medical monitor and DSMB. Otherwise, the study will continue.

If any subject develops severe headache, fatigue, peripheral neuropathy, seizures, tremor and/or dizziness, sleepiness, confusion or other significant clinical neurological or GI symptoms as assessed by the Investigator, the PI will be notified and in turn, will notify the DMC. The subject will be asked to stop the study medication immediately. The PI will determine if the patient should be withdrawn from the study.

The primary responsibility of the DMC is to protect the safety and welfare of patients participating in this clinical trial and to ensure the integrity of the clinical trial. Specifically, for this study, the DMC will be responsible for:

- Examining accumulated safety data, pharmacokinetic (PK), and compliance data in order to make recommendations concerning continuation, termination or modification of the trial based on the safety of the interventions under study.
- Review of the general progress of the studies regards accrual, protocol violations, and study conduct.

The DMC will review the interim data from the study once 50% enrollment or 9 months after the first patient is enrolled/and or sooner if the DMC has any safety concerns. The treatment phase (Day 7) and sufficient safety data are available for review. The DMC chair will convene a DMC conference call for the review of a safety report by all members of the DMC to determine one of three recommendations: 1) dose escalation to Cohort 2; 2) reduction of the dose for the remainder of the study subjects, or 3) extend the low dose cohort for all remaining subjects (no dose escalation). If the DMC deems the study safe, Cohort 2 enrollment can begin after 12 subjects complete Day 7 and randomization will be opened to the higher dose group. Screening visits for Cohort 1 may continue during the DMC meeting period. If the DMC finds the low dose safe but is concerned with moving forward with dose escalation, they may recommend randomizing the remaining subjects to only the low dose and placebo groups. In this case, randomization will be done in a 2:1 ratio, Ravicti® to placebo, respectively. Those who would have been randomized according to the randomization list to the high dose of Ravicti® will instead be given the low dose of Ravicti®.

A report will be prepared by the PI and will include a summary of adverse event rates by treatment group. Adverse events will be tabulated by body system, intensity, seriousness, duration, treatment given, and their relationship to the study drug and/or study procedure. In addition, the study biostatistician will prepare the DMC Safety report including FEV1, CBC, chemistry, ESR, CRP, and PBA levels. Abnormal changes in laboratory values will also be summarized. This report will be accompanied by listings of adverse events and other safety data by subject.

15. STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

15.1 Data Sets Analyzed

All eligible patients who are randomized into the study and receive at least one dose of the study drug (the Safety Population) will be included in the safety analysis.

15.2 Demographic and Baseline Characteristics

The analyses will be conducted on all available data. Treatment groups will be described and compared with respect to baseline demographic and clinical characteristics including age, gender, race, sweat chloride, nasal potential difference measures, microorganisms in sputum, height, weight, and pulmonary function. Differences between the three groups on categorical variables will be assessed using the chi-squared statistic. Difference between the three groups on continuous variables will be assessed using Analysis of Variance or the nonparametric Kruskall- Wallis test.

15.3 Analysis of Primary and Secondary Endpoint

The primary biological outcome will be the change in average measurement of nasal potential difference between day 7 and baseline. . A two-sample t-test will be performed to compare the change in the combined treatment groups to that in the placebo group. An intention to treat analysis will be performed. However, treatment withdrawal or additional administration of PERT for malabsorption will be recorded and described. The analyses will be conducted on all available data

Rationale for Sample Size

Sample size calculations led us to plan for a sample size of 12 subjects in each of the three groups: placebo, low dose, and high dose Ravicti®. As we showed in ref 2, a group of 6 subjects on either study drug or placebo provides statistical power of 0.80, assuming a two- sided significance level of 0.05, to detect a mean change in PD of 5 mV between drug and placebo, assuming a standard deviation of the change of 1.1 mV in the placebo group and 2.9 mV in the drug group. More recent data (Steve Rowe) suggests a standard deviation of 3.0 mV for the 4 centers participating in the proposal. Using these estimates that are based on our two previously published studies of the low chloride/isoproterenol maneuver in NPD at the JHU site, and duplicated at UAB, Table 1 displays the sample size needed in the placebo group and in the combined treatment groups (using a 2:1 ratio of treatment:placebo) in order to detect differences in mean change in NPD under different assumptions of statistical power. A total sample size of 36 (12 in placebo, 12 in low-dose Ravicti® and 12 in high dose Ravicti®) will provide 80% power to detect a difference in mean change in PD of 3 or more mV between placebo and the combined treatment groups. Within subject reproducibility of sweat chloride is more than sufficient to detect physiologically relevant changes with this sample size. Randomization will be done by the study biostatistician and provided to the JHU and other site investigational pharmacists. The study pharmacist will dispense drug to the site coordinator.

	80% Power	85% Power	80% Power	85% Power
Mean Change	SD_1=3; SD_2=3	SD1=3; SD2=3	SD1=4; SD2=4	SD1=4; SD2=4
2.0 mV	27:54	31:62	48:96	54:108
3.0 mV	12:24	14:28	21:42	24:48
3.5 mV	9:18	10:20	16:32	18:36
4.0 mV	7:14	8:16	12:24	14:28

Table 1. Sample Size Needed in the Placebo and Combined Treatment Groups (P:R) to Detect a Difference in Mean Change in PD (mV) under Varying Assumptions of Statistical Power and Standard Deviation (SD) of Mean Change (significance level (alpha) of 0.05).

Table 2. Sample Size Needed in the Placebo and Combined Treatment Groups (P:R) to Detect a Difference in Mean Change in PD (mV) under Varying Assumptions of Statistical Power and Standard Deviation (SD) of Mean Change (significance level of 00.05).

	80% Power	85% Power	80% Power	85% Power
Mean Change	SD_1=1.1 SD_2=3	SD1=1.1 SD2=3	SD1=1.1 SD2=4	SD1=1.1 SD2=4
2.0 mV	12:24	13:26	19:38	21:42
3.0 mV	5:10	6:12	9:18	10:20
3.5 mV	4:8	5:10	6:12	7:14

Safety and tolerability data will be summarized by treatment group.

Adverse event rates will be coded by body system and MedDra classification term. Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug. Fisher's exact test will be used to compare the various treatment groups with respect to incidence of the more commonly occurring AEs.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

Study personnel at each site will enter data on source documents and then into the protocol-specific web based data base when the information corresponding to that visit is available. Source documents will serve as CRFs for this study. Subjects will be identified by a site number, subject number and initials.

All clinical information requested in this protocol will be entered on the CRFs. If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator.

16.2 Data Management Procedures

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

16.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved query reports. All changes to the study database will be documented.

16.4 Archival of Data

At all times, appropriate backup copies of the database and related software files will be maintained and the information will be appropriately protected from illegitimate access. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

16.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of 2 years following marketing of the investigational product or for 2 years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

16.6 Monitoring

This study will be monitored by the Sponsor (or designee) in accordance with current Good Clinical Practice. By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16.7 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will be used to identify study subjects on CRFs and other documentation submitted

to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

17. ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain a list of IRB/IEC members or other assurance of compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonization and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study. A copy of the signed consent form will be given to the subject and the original will be maintained with the subject's records.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.

5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. SCHEDULE OF STUDY VISITS

	VISIT 0 (SCREEN) Day -28 to Day -7	VISIT 1 (Day1)	VISIT 2 (Day4)[a] ±2 days	VISIT 3 (Day7)[a] ±2 days	VISIT 4 (Day14)[a] ±2 days	EARLY WITHDRAWAL (IF DROP V1-3)
Informed Consent	X					
Medical History	X	X				
Complete Physical Exam	X					X
Abbreviated Physical Exam		X	X	X	X	
Height	X					
Weight	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X
Oximetry	X	X	X	X	X	X
Spirometry	X	X	X	X	X	X
Sputum collection (expectorated)	X	X			X	
ECG	X					
Pharmacokinetics – plasma PBA, PAA, PAGN		X[C] Serial draws	X[C] Serial draws	X[C]	X[C]	X[C]
Pharmacokinetics- urine PBA, PAA, PAGN		X[g] 24 hour	X[b]	X[g] 24 hour	X[b]	X[b]
CMP, CRP and uric acid	X	X		X	X	X
Pregnancy Test (urine or serum[f])	X[f]	X		X	X	X
Hematology and ESR	X	X		X	X	X
Urinalysis	X			X	X	X
Randomization		X (-2 days)				
Study drug administration [e]		X	X	X		
Study drug dispensation		X	X [h]	X [h]		
Subject Diary Initiate[d] and Review		X[d]	X	X	X	X

	VISIT 0 (SCREEN) Day -28 to Day -7	VISIT 1 (Day1)	VISIT 2 (Day4)[a] ±2 days	VISIT 3 (Day7)[a] ±2 days	VISIT 4 (Day14)[a] ±2 days	EARLY WITHDRAWAL (IF DROP V1-3)
Concomitant Medication Review	X	X	X	X	X	X
Adverse Experiences	X	X	X	X	X	X
CFQ-R	X	X		X	X	X
Epworth Sleepiness Scale	X	X	X	X	X	X
Nasal Potential Difference	X	X (repeat only if needed)	X	X	X	
Sweat chloride[i]		X		X		

[a] ±2 days

[b] Freshly-voided Urine- a single1mL sample will be collected post-dose. The sample should be frozen at -80° at the end of the visit.

[C] PK blood samples will be drawn in a 4mL K2EDTA tube as follows: Visit 1 pre-dose, 1 hour (±5 min), 2 hours (±10min), 4 hours (±10min) and 8 hours (+/-10 min) post dose. Visit 2 pre-dose, 1 hour (±5min) 2 hours (±10min) 4 hours (±10min), and 8 hours (+/- 10 min) post dose and on Visit 3 one pre-dose PK sample and on Visit 4 one PK blood sample. Early Withdrawal will be a single PK sample. Blood will be collected in a 4mL K2EDTA tube, placed immediately into an ice/water bath for no longer than 30 minutes. Spin and aliquot within 60min of collection. Centrifuge under refrigerated conditions at 4°Cbetween 1000 and 1300 RCF for 10 min. Divide the plasma with at least 0.5mL of plasma in the first tube. Place remaining plasma into a second cryovial for PAA, PBA and PAGN.

[d] Initiate Subject Diary on Day 1. Complete the time PERT and drug are taken on Day 1 with the subject and ask them to complete at each dosing and return it at each visit.

[e] PERT should be taken approximately 15 min (±10 min) prior to dosing, if applicable. Drug should be taken at the start of a meal or snack for each dose. Dosing is scheduled daily at every 8 hours (for example, 8am, 4pm and midnight). There is a ±2 hour window for each dose. On Visits 1, 2 and 3, the first dose will be given in the clinic, however, depending on study visit length the second dose may be given in clinic or taken at home. Please be sure to have adequate meals/snacks in clinic for up to two doses. Remind subject to bring in adequate PERT for each day.

[f] A serum pregnancy test will be collected at Screening. A urine pregnancy test will be done at Visits 1, 3, 4 and Early Withdrawal.

[g] On Day 1 and Day 7 a 24 hour urine collection will be obtained. To collect the 24 hour sample, the subject should void fully just prior to dosing. The urine collection time starts at the same time as dosing. Urine should be collected and kept at room temperature, then returned to the site at or before the next visit, within 50 hours of collection completion. The total urine volume should be returned to the site, mixed well and inverted. Then 1mL of the total volume should be removed as a representative sample by the study team and frozen at -80 degrees, the whole volume measured and the 1mL urine sample sent to AIT Bioscience for PK analysis.

[h] If applicable

[i] Begin sweat chloride measurements prior to dosing on Visit 1 and within one hour after dosing on Visit 3.

Appendix 2- CFQ-R Adult, Version 2.0

CFQ - R Adolescents and Adults (Patients 14 Years Old and Older)

CYSTIC FIBROSIS QUESTIONNAIRE - REVISED

Understanding the impact of your illness and treatments on your everyday life can help your healthcare team keep track of your health and adjust your treatments. For this reason, this questionnaire was specifically developed for people who have cystic fibrosis. Thank you for your willingness to complete this form.

Instructions: The following questions are about the current state of your health, as you perceive it. This information will allow us to better understand how you feel in your everyday life.

Please answer all the questions. There are **no** right or wrong answers! If you are not sure how to answer, choose the response that seems closest to your situation.

Section I. Demographics

Please fill-in the information or check the box indicating your answer.

A. What is your date of birth?
Date ☐☐ ☐☐ ☐☐☐☐
Mo Day Year

B. What is your gender?
☐ Male ☐ Female

C. During the past **two weeks**, have you been on vacation or out of school or work for reasons **NOT** related to your health?
☐ Yes ☐ No

D. What is your current marital status?
☐ Single/never married
☐ Married
☐ Widowed
☐ Divorced
☐ Separated
☐ Remarried
☐ With a partner

E. Which of the following best describes your racial background?
☐ Caucasian
☐ African American
☐ Hispanic
☐ Asian/Oriental or Pacific Islander
☐ Native American or Native Alaskan
☐ Other (please describe) _____
☐ Prefer not to answer this question

F. What is the highest grade of school you have completed?
☐ Some high school or less
☐ High school diploma/GED
☐ Vocational school
☐ Some college
☐ College degree
☐ Professional or graduate degree

G. Which of the following best describes your current work or school status?
☐ Attending school outside the home
☐ Taking educational courses at home
☐ Seeking work
☐ Working full or part time (either outside the home or at a home-based business)
☐ Full time homemaker
☐ Not attending school or working due to my health
☐ Not working for other reasons

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CFQ - R Adolescents and Adults (Patient: 14 Years Old and Older)

CYSTIC FIBROSIS QUESTIONNAIRE - REVISED

Section II. Quality of Life

Please check the box indicating your answer.

During the past two weeks, to what extent have you had difficulty:	A lot of difficulty	Some difficulty	A little difficulty	No difficulty
1. Performing vigorous activities such as running or playing sports	☐	☐	☐	☐
2. Walking as fast as others	☐	☐	☐	☐
3. Carrying or lifting heavy things such as books, groceries, or school bags	☐	☐	☐	☐
4. Climbing one flight of stairs	☐	☐	☐	☐
5. Climbing stairs as fast as others	☐	☐	☐	☐

During the past two weeks, indicate how often:	Always	Often	Sometimes	Never
6. You felt well	☐	☐	☐	☐
7. You felt worried	☐	☐	☐	☐
8. You felt useless	☐	☐	☐	☐
9. You felt tired	☐	☐	☐	☐
10. You felt energetic	☐	☐	☐	☐
11. You felt exhausted	☐	☐	☐	☐
12. You felt sad	☐	☐	☐	☐

Please circle the number indicating your answer. Please choose only one answer for each question.

Thinking about the state of your health over the last two weeks:

13. To what extent do you have difficulty walking?
    1. You can walk a long time without getting tired
    2. You can walk a long time but you get tired
    3. You cannot walk a long time because you get tired quickly
    4. You avoid walking whenever possible because it's too tiring for you
14. How do you feel about eating?
    1. Just thinking about food makes you feel sick
    2. You never enjoy eating
    3. You are sometimes able to enjoy eating
    4. You are always able to enjoy eating
15. To what extent do your treatments make your daily life more difficult?
    1. Not at all
    2. A little
    3. Moderately
    4. A lot

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CFQ - R Adolescents and Adults (Patients 14 Years Old and Older)

CYSTIC FIBROSIS QUESTIONNAIRE - REVISED

16. How much time do you currently spend each day on your treatments?
    1. A lot
    2. Some
    3. A little
    4. Not very much

17. How difficult is it for you to do your treatments (including medications) each day?
    1. Not at all
    2. A little
    3. Moderately
    4. Very

18. How do you think your health is now?
    1. Excellent
    2. Good
    3. Fair
    4. Poor

Please select a box indicating your answer.

Thinking about your health during the past two weeks, indicate the extent to which each sentence is true or false for you.	Very true	Somewhat true	Somewhat false	Very false
19. I have trouble recovering after physical effort	☐	☐	☐	☐
20. I have to limit vigorous activities such as running or playing sports	☐	☐	☐	☐
21. I have to force myself to eat	☐	☐	☐	☐
22. I have to stay at home more than I want to	☐	☐	☐	☐
23. I feel comfortable discussing my illness with others	☐	☐	☐	☐
24. I think I am too thin	☐	☐	☐	☐
25. I think I look different from others my age	☐	☐	☐	☐
26. I feel bad about my physical appearance	☐	☐	☐	☐
27. People are afraid that I may be contagious	☐	☐	☐	☐
28. I get together with my friends a lot	☐	☐	☐	☐
29. I think my coughing bothers others	☐	☐	☐	☐
30. I feel comfortable going out at night	☐	☐	☐	☐
31. I often feel lonely	☐	☐	☐	☐
32. I feel healthy	☐	☐	☐	☐
33. It is difficult to make plans for the future (for example, going to college, getting married, advancing in a job, etc.)	☐	☐	☐	☐
34. I lead a normal life	☐	☐	☐	☐

Cystic Fibrosis Foundation CFQ Teen/Adult, English Version 2.0 Page 3

CFQ - R Adolescents and Adults (Patient: 14 Years Old and Older)
CYSTIC FIBROSIS QUESTIONNAIRE - REVISED

Section III. School, Work, or Daily Activities

Questions 35 through 38 are about school, work, or other daily tasks.

35. To what extent did you have trouble keeping up with your schoolwork, professional work, or other daily activities during the past two weeks?
    1. You have had no trouble keeping up
    2. You have managed to keep up but it's been difficult
    3. You have been behind
    4. You have not been able to do these activities at all

36. How often were you absent from school, work, or unable to complete daily activities during the last two weeks because of your illness or treatments?
    ☐ Always ☐ Often ☐ Sometimes ☐ Never

37. How often does CF get in the way of meeting your school, work, or personal goals?
    ☐ Always ☐ Often ☐ Sometimes ☐ Never

38. How often does CF interfere with getting out of the house to run errands such as shopping or going to the bank?
    ☐ Always ☐ Often ☐ Sometimes ☐ Never

Section IV. Symptom Difficulties

Please select a box indicating your answer.

Indicate how you have been feeling during the past two weeks.	A great deal	Somewhat	A little	Not at all
39. Have you had trouble gaining weight?	☐	☐	☐	☐
40. Have you been congested?	☐	☐	☐	☐
41. Have you been coughing during the day?	☐	☐	☐	☐
42. Have you had to cough up mucus?	☐	☐	☐	☐ Go to Question 44

43. Has your mucus been mostly: ☐ Clear ☐ Clear to yellow ☐ Yellowish-green ☐ Green with traces of blood ☐ Don't know

How often during the past two weeks:	Always	Often	Sometimes	Never
44. Have you been wheezing?	☐	☐	☐	☐
45. Have you had trouble breathing?	☐	☐	☐	☐
46. Have you woken up during the night because you were coughing?	☐	☐	☐	☐
47. Have you had problems with gas?	☐	☐	☐	☐
48. Have you had diarrhea?	☐	☐	☐	☐
49. Have you had abdominal pain?	☐	☐	☐	☐
50. Have you had eating problems?	☐	☐	☐	☐

Please be sure you have answered all the questions.

THANK YOU FOR YOUR COOPERATION!

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Appendix 3 - Epworth Sleepiness Scale

EPWORTH SLEEPINESS SCALE (ESS)

The following questionnaire will help you measure your general level of daytime sleepiness. You are to rate the chance that you would *doze off or fall asleep* during different routine daytime situations. Answers to the questions are rated on a reliable scale called the Epworth Sleepiness Scale (ESS). Each item is rated from 0 to 3: with 0 meaning you would never *doze or fall asleep* in a given situation; and 3 meaning that there is a very high chance that you would *doze or fall asleep* in that situation.

How likely are you to *doze off or fall asleep* in the following situations, in contrast to just feeling tired? Even if you haven't done some of the activities recently, think about how they would have affected you.

Use this scale to choose the most appropriate number for each situation:

0 = would never doze
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing
It is important that you circle a number (0 to 3) for EACH situation.

SITUATION	CHANCE OF DOZING			
Sitting and Reading	0	1	2	3
Watching Television	0	1	2	3
Sitting inactive in a public place (theater/meeting)	0	1	2	3
As a passenger in a car for an hour without a break	0	1	2	3
Lying down to rest in the afternoon	0	1	2	3
Sitting and talking to someone	0	1	2	3
Sitting quietly after lunch (with no alcohol)	0	1	2	3
In a car, while stopped in traffic	0	1	2	3

TOTAL SCORE _____

Name: _____

Date: _____

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