Clinical Research Protocol

GALLIUM-IP-13

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Version Date:	28 August 2014
Investigational Product:	Gallium nitrate ($Ga(NO_3)_3$)
IND Number:	IND 104,363
Development Phase:	Phase 2
Sponsor:	Christopher H. Goss, MD MSc (University of Washington, Seattle Children's Hospital)
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Approval:

MD

PI or Sponsor Signature (Name and Title)

02 SEP 2014

Date

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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 Sponsor 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: GALLIUM-IP-13

Protocol Title: A Phase 2, Multi-Center, Randomized, Placebo-Controlled Study of IV Gallium Nitrate in Patients with Cystic Fibrosis (The IGNITE Study)

Protocol Date: 28 August 2014

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Investigator Signature *Date*

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Print Name and Title

Site # _____

Site Name _____

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Contents

LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	Area under the curve
BUN	blood urea nitrogen
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFTR	cystic fibrosis transmembrane conductance regulator
CFRSD-CRISS	Cystic Fibrosis Respiratory Symptom Diary- Chronic Respiratory Infection Symptom Score
CF	cystic fibrosis
CFFT	Cystic Fibrosis Foundation Therapeutics
CFU	colony forming units
CI	Confidence interval
CLSI	Clinical and Laboratory Standard Institute
CRF	case report form
C_{SS}	Steady state concentration
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data Safety Monitoring Board
EDC	Electronic Data Capture
EC	Ethics Committee
FDA	Food and Drug Administration
FEV_1	forced expiratory volume over one second
FVC	forced vital capacity
Ga	gallium
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act of 1996
hsCRP	highly sensitive C-Reactive Protein
IL-1ra	Interleukin-1ra
IL-6	Interleukin-6
IL-8	Interleukin-8
ICF	informed consent form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board

IV	Intravenous
ITT	intent-to-treat
LD50	Lethal Dose, 50%
LDH	lactate dehydrogenase
L	liter
LSMeans	least squares means
mEq	Milliequivalent
mL	Milliliter
MHB	Mueller Hinton broth
MCH	Major histocompatibility complex
mM	Millimole
MMRM	mixed model repeated measures
NIH	National Institutes of Health
NPD	nasal potential difference
P. aeruginosa	*Pseudomonas aeruginosa*
PI	Principal Investigator
PICC	peripherally inserted central catheter
PK	Pharmacokinetic
PMNeL	Neutrophil elastase antiprotease complex
QPIT	quantitative pilocarpine iontophoresis test
SAA	serum amyloid A
SAE	serious adverse experience
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase
SOC	System Organ Class
TNF-α	Tumor Necrosis Factor- α
TGF-β1	Transforming Growth Factor-β1
$t_{1/2\beta}$	elimination half-life
$\mathbf{t_{1/2}}$	Half life
WBC	White blood cells

PROTOCOL SYNOPSIS

TITLE	A Phase 2, Multi-Center, Randomized, Placebo-Controlled Study of IV Gallium Nitrate in Patients with Cystic Fibrosis
FUNDING ORGANIZATION	National Heart, Lung, and Blood Institute (NHLBI)
NUMBER OF SITES	Approximately twenty
RATIONALE	Much attention has been focused on the need for new antibiotics. One reason for this is that heavy antibiotic use has greatly increased resistance due to genetic mutations. But new agents are also needed because conventional antibiotics work poorly in chronic infections, even when the organisms are sensitive when tested *ex vivo*. These chronic infections resist treatment in large part because the organisms live in biofilms. Biofilms are communities of bacteria associated with surfaces and encased in a polymeric matrix making the bacteria far more resistant to killing than they are in the free-living (planktonic) state. Examples of biofilm infections include cystic fibrosis (CF) lung infections, endocarditis, osteomyelitis, wound, sinus, and device infections. We are pursuing a novel approach that uses the metal gallium (Ga) to disrupt intracellular bacterial iron metabolism. Gallium has a nearly identical ionic radius as iron, and many biologic systems are unable to distinguish gallium from iron (1;2). Gallium disrupts iron dependent processes because Ga^{3+} cannot be reduced and redox cycling is critical for iron's biological functions (1;2). Importantly, gallium is already approved by the Food and Drug Administration (FDA) for intravenous (IV) use. Our data shows that gallium kills *Pseudomonas aeruginosa* (*P. aeruginosa;* including antibiotic resistant strains), is active against biofilms, and treats three different animal models *P. aeruginosa* infections. In our phase 1b study, we demonstrated that IV gallium was both safe and had favorable pharmacokinetics (PK) in CF patients. We also found suggestions of preliminary efficacy. We will study the preliminary efficacy as assessed by both lung function and sputum microbiology of IV gallium in CF adults. We will also continue to assess PK and safety of IV gallium in CF patients.
STUDY DESIGN	This is a phase 2, multi-center, randomized, placebo-controlled trial in adults with CF chronically infected with *P. aeruginosa*. The study will evaluate the safety and clinical efficacy of a five-day infusion of IV gallium nitrate (IV gallium).

PRIMARY OBJECTIVE	To assess the efficacy of IV gallium to improve pulmonary function as measured by a 5% or greater relative improvement in forced expiratory volume in one second (FEV_1) from baseline to Day 28.
SECONDARY OBJECTIVES	• To assess the safety and tolerability of IV gallium • To assess the efficacy of IV gallium in improving measures of lung function including relative change in FEV_1, absolute change in FEV_1, and forced vital capacity (FVC) • To assess the efficacy of IV gallium in reducing *P. aeruginosa* in the lungs of CF patients based on quantitative cultures of sputum • To assess the efficacy of IV gallium in improving respiratory symptoms as measured by the CF Respiratory Symptoms Diary-Chronic Respiratory Infection Symptom Severity Score (CFRSD-CRISS) • To assess the sputum and blood PK profile of IV gallium • To assess the rate of acquired resistance of *P. aeruginosa* to gallium • To explore the anti-inflammatory properties of IV gallium • To explore the efficacy of IV gallium in reducing the use of antibiotics for an acute indication
NUMBER OF SUBJECTS	Approximately120, approximately 60 per treatment arm
SUBJECT SELECTION CRITERIA	**Inclusion Criteria:** 1. Greater than or equal to 18 years of age at Screening 2. Documented chronic colonization with *P. aeruginosa* defined as identification in two sputum or oropharyngeal cultures within the year prior to Day 1 3. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype **and** one or more of the following criteria: a. sweat chloride ≥ 60 mEq/liter by quantitative pilocarpine iontophoresis test (QPIT) b. two well-characterized mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene c. Abnormal nasal potential difference (NPD; change in NPD in response to a low chloride solution and

	isoproteronol of less than -5 mV) 4. $FEV_1 \geq 25$ % of predicted value at Screening 5. Able to expectorate sputum 6. Serum liver function tests ≤ 2.5 x upper limit of normal at Screening 7. Serum urea nitrogen (BUN) ≤ 1.5 x upper limit of normal at Screening 8. Serum creatinine ≤ 2.0 mg/dl and ≤ 1.5 x upper limit of normal at Screening 9. Hemoglobin ≥ 9 g/dl, platelets ≥ 100,000/mm^3, and white blood cells (WBC) ≥ 4,500/mm^3 at Screening 10. Ionized calcium ≥ lower limit of normal at Screening 11. Written informed consent obtained from subject or subject's legal representative, able to communicate with the Investigator and comply with the requirements of the protocol 12. If female and of childbearing potential, must have a negative pregnancy test on Day 1 prior to receiving study drug 13. If female and of childbearing potential, is willing to use adequate contraception for the duration of the study through Visit 5, as determined by the investigator 14. If male and able to father a child, is willing to use adequate contraception for the duration of the study through Visit 5, as determined by the investigator 15. Clinically stable with no significant changes in health status within 14 days prior to Day 1 **<u>Exclusion Criteria</u>:** 1. Use of inhaled antibiotics within seven days prior to Day 1 2. Unable or unwilling to withhold use of chronic inhaled antibiotics through Day 28 3. Use of intravenous, inhaled, or oral antibiotics for an acute indication within 14 days prior to Day 1 4. Use of bisphosphonates within seven days prior to Day 1 5. History of osteoporosis (defined as the most recent dexa scan with a T-score ≤ -2.5 with the dexa scan performed within the five years prior to Screening) 6. Lactating female 7. Known sensitivity to gallium

	8. Use of any investigational drug and/or participated in any interventional clinical trial within 28 days prior to Screening 9. Presence of a condition or abnormality (e.g., pre-existing heart disease) that in the opinion of the site investigator would compromise the safety of the subject or the quality of the data
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION	Subjects will receive a five-day continuous infusion of 200 mg/m^2/day of IV gallium nitrate or volume-matched placebo (0.9% sodium chloride). Subjects will be permitted to remain in the clinical research center for the full five days of continuous infusion at the discretion of the investigator or can receive the infusion as an outpatient.
DURATION OF SUBJECT PARTICIPATION	Subjects will be on study for up to 63 days: **Screening:** 0-7 days (can be combined with Baseline/Day 1) **Treatment:** 5 days **Primary Efficacy Evaluation:** Day 28 **Final safety evaluation:** Day 56 **Total Follow-up time after first dose of study drug:** 56 days
CONCOMITANT MEDICATIONS	Allowed: Standard therapy for CF is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below. Ongoing chronic treatment (>30 days prior to Day 1) with oral antibiotics, including azithromycin, is allowed. Treatment for pulmonary exacerbations and as required for acute care is allowed, including acute oral, inhaled and IV antibiotics. Physicians are encouraged to prescribe acute antibiotic therapy only in the presence of symptoms. Prohibited: The following medications are prohibited within seven days prior to Day 1 through Day 28: • inhaled chronic antibiotics • bisphosphonates The use of nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin is prohibited from Day 1 through 24 hours after the study drug infusion is stopped (e.g., Day 7 for subjects that complete the entire course of study drug).
STUDY EVALUATIONS	
PRIMARY ENDPOINT	Difference between treatment groups in the proportion of subjects with a 5% or greater relative change in FEV_1 (liters) from baseline to Day 28

SECONDARY ENDPOINTS	• Difference between treatment groups in safety parameters including the incidence of adverse events (AEs), changes in clinical safety labs, and hospitalizations through the end of follow-up • Difference between treatment groups in the change in pulmonary function parameters from baseline through end of follow-up including relative change in FEV_1 (liters), absolute change in FEV_1 (liters) and in FEV_1 % predicted, and change in FVC (liters and % predicted) • Difference between treatment groups in the change in sputum *P. aeruginosa* density based on quantitative cultures from baseline through end of follow-up • Difference between treatment groups in the emergence of other CF pathogens, including gallium-resistant *P. aeruginosa*, from baseline through end of follow-up • Difference between treatment groups in the change in the CFRSD-CRISS from baseline through end of follow-up • Determination of the steady state concentration (Css) and elimination half life ($t_{1/2\beta}$) in those subjects receiving IV gallium • Difference between treatment groups in the change in blood inflammatory markers from baseline through end of follow-up • Difference between treatment groups in the rate of antibiotic usage including IV, oral, and inhaled for an acute indication from baseline through end of follow-up • Longitudinal trends in clinical features and CF pathogens following treatment through the CF Foundation National Patient Registry
SAFETY MONITORING & INTERIM ANLYSES	An independent data safety monitoring board, the CF Foundation Data Safety Monitoring Board (DSMB), will review safety data for this study. Summary reports tabulating SAEs by treatment group will be provided on a quarterly basis to the DSMB. Comprehensive safety interim reports will be provided twice yearly to the DSMB and will include detailed summaries of all SAEs, AEs, drug discontinuations, laboratory parameters and withdrawals. The DSMB will recommend continuation or discontinuation of the study based on these interim reviews.
STATISTICS **Primary Analysis Plan**	The primary analysis will be conducted on the intent-to-treat (ITT) population including all randomized subjects who had the study drug infusion started. The primary endpoint is the difference between treatment groups in the proportion of subjects with a 5% or greater improvement in the relative change in FEV_1 (liters) from baseline to Day 28. To test the null hypothesis that no difference exists between treatment groups, a two-sided 0.05 level of significance test of proportions will be performed

	adjusted for baseline lung function. The primary analysis will also be run on a per protocol population consisting of a subset of ITT population who had no protocol violations, did not use inhaled chronic antibiotics and bisphosphonates from seven days prior to Day 1 through Day 28 and received gallium infusion for five sequential days. Additional analyses of the relative change in FEV_1 through end of follow-up will include the development of a mixed-effect model for repeated measures (MMRM).
Rationale for Number of Subjects	With a sample size of 60 per group and assuming a responder rate of 30% in the placebo group, the study has 90% power to detect a 28.3% or greater improvement in the responder rate. Accounting for an attrition rate of 10% and interim analyses, the study will still have 90% power to detect a treatment difference of 30.0% or greater between groups.

1 BACKGROUND

Most adult patients with cystic fibrosis (CF) develop chronic bronchiectasis caused by life-long airway infections that is punctuated by recurrent acute exacerbations. This process progressively destroys the lungs, leading to premature death. The current median survival for CF patients is approximately 36 years (3). *Pseudomonas aeruginosa* (*P. aeruginosa)* accounts for approximately 60% of the chronic lung infections in CF (4) and ~ 90% of deaths are attributed to chronic airway damage caused by this organism (4). Despite intensive research, the airway colonization in CF patients cannot be eradicated by any known therapy and intensive antibiotic treatment is only effective at suppressing these persistent infections. Moreover, the frequent use of suppressive antibiotics has made antibiotic resistance a vexing problem in CF patients.

At present, new antimicrobial development by the pharmaceutical industry is at a historic low and the prospects for new classes of antimicrobial agents effective against *P. aeruginosa* (and other Gram-negative bacteria) are poor. Responding to this problem, ten federal agencies [chaired by the National Institutes of Health (NIH), Centers for Disease Control and Prevention (CDC), and Food and Drug Administration (FDA)] generated an intra-agency action plan that emphasized the importance of developing novel antimicrobial agents (5).

This negative outlook is compounded when one considers two additional points. First, *P. aeruginosa* is well known for its intrinsic and acquired antibiotic resistance, and this makes management of *P. aeruginosa* infections in all types of patients challenging (6). Second, in CF and other chronic infections, *P. aeruginosa* live in biofilms (7;8). Growth in biofilms makes organisms much more resistant to killing by antibiotics and biocides. Thus, new therapies directed against *P. aeruginosa* would optimally show activity against bacteria in the free-living (planktonic) state and in biofilms. Furthermore, these treatments would optimally work by novel mechanisms so that resistance to conventional antibiotic does not limit their use. Our studies suggest that gallium nitrate may fulfill these requirements.

1.1 Overview of Non-Clinical Studies

Biofilm growth gives bacteria many advantages. Biofilms are groups of bacteria, encased in a polymeric matrix (9). Compared to free-living (planktonic) cells, biofilm bacteria exhibit important differences in physiology (10). Most notorious is their resistance to killing by antibiotics and the immune system. Biofilms can be 1,000-10,000 times more resistant than planktonic bacteria (9). Resistance results from a combination of decreased antimicrobial penetration, metabolic inactivity of some biofilm cells, and other poorly defined changes (9). Biofilms accounts for the persistence of many chronic infections including endocarditis, medical device infections, and osteomyelitis (11). A prototypical biofilm disease is the chronic *P. aeruginosa* airway infections that afflict CF patients (11).

Iron limitation could be exploited in new antimicrobial and anti-biofilm treatments. Iron sequestration is an evolutionary ancient host defense strategy (12). The extremely low iron levels *in vivo* ($\sim 10^{-20}$ M) (13) prevent infection because all pathogens require iron for growth. Iron is also a key signal that promotes biofilm development, separate from its requirement for growth (14;15). Thus, disrupting bacterial iron metabolism could be effective against biofilm and planktonic bacteria. Unfortunately, exploiting this has been difficult. Treatment with iron chelators has been tried, but bacteria used the chelator-bound iron and secondary infection occurred (16). Another approach would develop drugs that interfere with iron uptake. However,

pathogens have many redundant acquisition systems; *P. aeruginosa* has over 30 genes encoding iron receptors. New approaches to interfere with iron-dependent processes are needed if the therapeutic potential of this approach is to be fully realized.

Gallium can substitute for iron and can disrupt iron-dependent processes. Gallium has many features similar to iron(III), including a nearly identical ionic radius, and biologic systems are often unable to distinguish gallium from iron(III). Unlike iron(III), gallium cannot be reduced to the divalent state. Thus placing gallium rather than iron in enzymes renders them non-functional (1;2). Our work suggests that *P. aeruginosa* may have a higher affinity for gallium than for iron. We have also found that gallium has bacteriostatic, bactericidal, and anti-biofilm actions *in vitro* and in three animal models (17).

1.2 Overview of Clinical Studies

All of the clinical studies noted in this section have been conducted using the FDA approved Gallium nitrate [$Ga(NO_3)_3$, trade name Ganite®]. This product is no longer available. The current study will use an identical formulation manufactured by the National Institutes of Health/National Heart, Lung, and Blood Institute (NHLBI) Science Moving towArds Research Translation and Therapy (SMARTT) Program.

Clinical uses of Gallium in humans. Gallium nitrate [$Ga(NO_3)_3$, trade name Ganite®] is an FDA-approved treatment for hypercalcemia of malignancy (18-20). The drug has also demonstrated activity against a variety of other skeletal conditions, including Paget's disease, metastatic bone disease, malignant hyperparathyroidism, and osteoporosis (presumably by inhibiting osteoclast activity (18)), as well as certain neoplastic diseases, including bladder cancer and lymphoma. Relative to doses used in skeletal diseases, substantially larger doses (~ 300-400 mg/m^2/day for seven consecutive days repeated every 21-28 days) have been broadly used for treatment of neoplastic diseases and have generally been well tolerated by debilitated patients. In addition, trace doses of Gallium-67 citrate have been used for nuclear scintiscanning to identify areas of neoplasia, inflammation and infection.

Pharmacokinetics of intravenous gallium nitrate in non-CF patients. As noted above, gallium nitrate is an FDA-approved agent to treat patients with cancer-related hypercalcemia that is resistant to hydration. In non-CF patients with disseminated cancer with normal renal function, gallium showed a biphasic plasma disappearance with an initial half-life ($t_{1/2}$) of 0.5 to 1.8 hours (mean 1.0) and a terminal $t_{1/2}$ of 10.5 to 50.4 hours (mean 25.1) (21). Gallium was distributed in total body water and localized in some body compartments as evidenced by a volume of distribution ranging from 0.25 to 2.53 L/kg (mean 1.19). The estimated steady state concentration (Css) of gallium at an infusion rate of 100 mg/m^2/day infusion is 1.0 to 4.3 μg/mL (21;22).

For more detail, refer to the Prescribing information.

Clinical experience in CF. We completed a Phase 1b non-randomized safety and pharmacokinetic (PK) trial in CF adults. The study involved two different dosing cohorts, 100 mg/m^2/day and 200 mg/m^2/day infused for a total of 5 days. The first dosing cohort included 9 CF subjects (4 males and 5 females) and the second included 11 CF subjects (6 males and 5 females). The mean baseline forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) were 2.25 liters (L) (range 1.06 to 4.59 L) and 3.60 L (range 1.84 to 6.11 L). The mean body mass index was 22.7 kg/m^2 (range 17.3 to 31.2 kg/m^2) thus representing a broad

spectrum of disease in CF adults. No serious adverse experiences (SAEs) were noted in the study. There were a total of 41 adverse events (AEs); 8 were deemed possibly related, one definitely related and 32 unrelated to study agent. Of the 9 AEs that were deemed related, 8 were categorized as mild and one was categorized as moderate severity. The AE categorized as definitely related to the study was bruising at the site of an intravenous line site (related to study procedure, not study drug). The other AEs included headache, dizziness, chest pain, gastroesophogeal reflux disease, increased sputum production, migraine headache, increased lethargy, gastrointestinal bloating and elevated blood glucose. Of the 41 AEs, 39 were deemed mild in severity with one moderate and one severe in severity. The severe AE was a headache. No AE led to a treatment discontinuation. The primary safety concern of IV gallium is renal toxicity. No evidence was found of renal injury during and after infusion using serum creatinine, blood urea nitrogen (BUN) and urine albumin/creatinine ratio. We found no evidence of perturbation of calcium homeostasis as noted by blood ionized calcium levels. Overall, the drug appeared safe and well tolerated in CF.

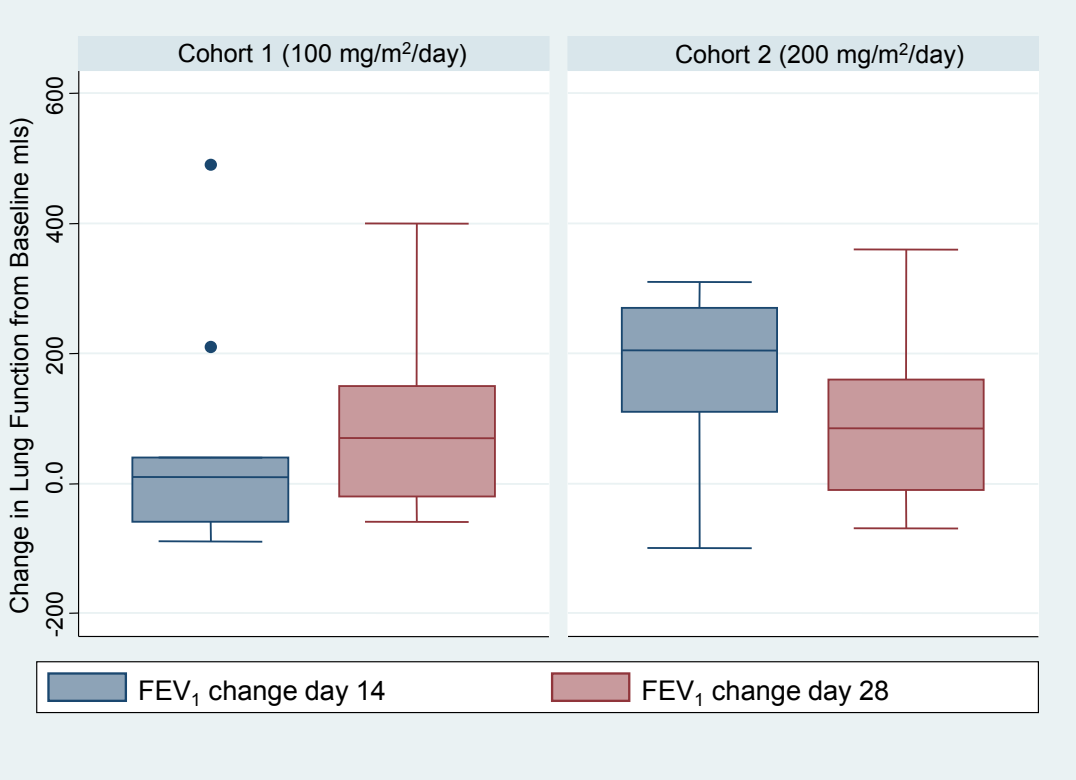
PK data has been determined in 9 patients receiving 100 mg/m^2/day and 10 patients receiving 200 mg/m^2/day in the Phase 1b study (the PK samples for one subject were lost). Steady state concentrations were achieved by 48 hours after initiation of the infusion. As shown in **Table 1**, there was no apparent increased in the gallium serum or sputum concentration area under the concentration time curve (AUC) with the increased dose. The clearance (Cl) was significantly increased with the higher dose due to increases in both renal and non-renal clearance. The lack of effect of dose on serum and sputum concentrations may reflect the uptake of gallium by white blood cells (WBC). The elimination half-life ($t_{1/2\beta}$) determined as the ratio of Cl and the elimination rate constant (K) reflects a prolonged elimination from a depot compartment.

Table 1: Pharmacokinetics of Galium (mean ± standard deviation) . C_{48hr}: steady state concentration at 48 hrs; C_{61hr}: steady state concentration at 61 hrs; $T_{1/2\beta}$: elimination half -life; Fe: Fractional excretion; Cl: clearance .

	100 mg/m ^{2}/day	200 mg/m ^{2}/day	P
SERUM	N = 9	N = 10	
C_{48hr} (μg/mL)	2.6 ± 0.6	2.3 ± 1.0	0.35
C_{61hr} (μg/mL)	2.1 ± 0. 6	2.2 ± 0. 6	0. 79
$T_{1/2\beta}$ (hr)	105 ± 14	129 ± 44	0.16
AUC (ug/mL ·hr)	503 ± 98	537 ± 153	0.59
Cl (L/hr)	3.8 ± 1.0	7.2 ± 2.1	0.0011*
(L/hr/m ^{2})	2.1 ± 0.4	4.0 ± 1. 1	0.007 *
Fe (%)	0.19 ± 0.07	0.20 ± 0.0 9	0. 67
Cl_{renal} (L/hr)	0.71 ± 0.29	1.35 ± 0. 52	0. 0077*
$Cl_{nonrenal}$ (L/hr)	3.0 ± 0.9	5.8 ± 2.2	0.0 052*
SPUTUM			
C_{max} (μg/mL)	0.92 ± 0.73	1.11 ± 0.80	0.61
AUC (ug/mL ·hr)	480 ± 397	567 ± 518	0. 69

Lung function increased in the pooled subjects (dosing cohorts 1 and 2). The mean change in FEV_1 and FVC by day 14 was 0.118 liters (95% confidence interval (CI): 0.041 to 0.196) and 0.124 liters (95% CI: 0.059 to 0.188) for 20 subjects. Both changes were statistically significant. The mean change in FEV_1 and FVC by day 28 was 0.095 liters (95% CI: 0.031 to 0.160) and 0.147 liters (95% CI: 0.063 to 0.231) for 20 subjects. Both changes were statistically significant. The effect on lung function appeared to be driven by cohort 2 (200 mg/m^2/day). In cohort 2, the mean change in FEV_1 and FVC by day 14 was 0.169 liters (95% CI: 0.08 to 0.260) and 0.204 liters (95% CI: 0.13 to 0.277) for 11 subjects. Both changes were statistically significant. The mean change in FEV_1 and FVC by day 28 was 0.085 liters (95% CI: -0.006 to 0.176) and 0.169 liters (95% CI: 0.04 to 0.293) for 11 subjects. Only the FVC change was significant. Figure 1 notes the change in FEV_1 in liters from baseline by study visit and cohort. These lung function changes are quite similar and comparable to other major treatments used in CF.

Figure 1. Box plots of change in lung function from baseline to day 14 and day 28 in mLs (Cohort 1: 9 subjects; Cohort 2: 11 subjects).



Interestingly, no significant change was noted in quantitative culture from the sputum of *P. aeruginosa* isolates. Sputum quantitative cultures noted wide variability with some subjects having a response akin to prior antimicrobial therapies in CF while others noted no clear treatment effect. The mean *P. aeruginosa* quantitative culture at baseline was 116.7 million colony forming units (CFUs)/gram of sputum (95% CI: 20.4 to 213.0 million CFU/gm). The mean change from baseline to day 14 was -5.5 million CFU/gm (95% CI: -110.5 to 99.5 million CFU/gm) and from baseline to day 28 was -28.7 million CFU/gm (95% CI: -111.0 to 53.7 million CFU/gm). Overall, the mean change was in the appropriate direction of a treatment response.

2 STUDY RATIONALE

2.1 Rationale for Gallium Treatment in CF Patients

Gallium nitrate is a promising therapeutic for CF airway infections for a number of reasons.

A large proportion of infecting organisms in CF airway infections are living in biofilms. Gallium nitrate could deliver "multiple hits" against infecting bacteria. At low concentrations, it blocks biofilm formation and inhibits bacterial growth; at higher concentrations, it kills planktonic and biofilm bacteria (17).

Gallium exhibits antimicrobial properties against laboratory *P. aeruginosa* strains and against mucoid and non-mucoid clinical isolates from CF patients. The vast majority of antibiotic resistant *P. aeruginosa* strains that we have tested so far are also susceptible to gallium (17).

Microbial resistance to gallium may be difficult for *P. aeruginosa* to acquire because gallium substitutes for iron in some metabolic processes and iron is an essential nutrient that is avidly taken up by bacteria. We have shown that mutations in three different iron uptake systems of *P. aeruginosa* did not change susceptibility to anti-biofilm or growth inhibitory actions of gallium (17). This suggests that multiple different iron uptake pathways can mediate the effects of gallium.

If resistance to gallium develops via mutation of gallium targets, it may not compromise the effectiveness of conventional anti-pseudomonal antibiotics currently in use. This is because gallium's antimicrobial mechanism differs from that of conventional antibiotics.

Laboratory data demonstrates that gallium's antimicrobial effect is enhanced in iron-limited conditions (17). Because bio-available iron is scarce in the lung (23), the antimicrobial action of gallium may be augmented *in vivo*. Thus, the concentrations of gallium required for efficacy in humans may be even lower than we have found *in vitro*.

Although *P. aeruginosa* is generally regarded as the most important pathogen in CF, patients also develop serious airway infections with other organisms including *Staphylococcus aureus* (methicillin sensitive and resistant strains), *Haemophilus influenzae*, and *Burkholderia cepacia* (24). Like *P. aeruginosa* infections, these organisms can be found in high concentrations in the sputum (3), develop antibiotic resistance (25;26), and can be difficult to treat with conventional antibiotics. Though evidence is incomplete, some of these organisms may live in biofilms in the CF sputum (27). Importantly, all of these organisms require iron for growth and thus they may also be sensitive to the antimicrobial action of gallium. Work by our group and others indicates that gallium nitrate has activity against *Stenotrophomonas maltophilia*, the *Burkholderia cepacia* complex, *Klebsiella pneumonia* (including ESB strains), *Escherichia coli*, *Acinetobacter baumannii*, *Bacillus anthracis, Francisella sp*. and *Salmonella typhi* (28).

Gallium may exhibit anti-inflammatory effects independent from the anti-microbial effect, which may be beneficial for CF lung infections. Extensive literature indicates that gallium has anti-inflammatory effects on various cells of the immune system and in intact animals. Gallium is a strong inhibitor of polyclonal and allo-antigen induced T-cell proliferation (29), and B-cell proliferation and antibody secretion (30). Gallium reduces Interleukin-6 (IL-6), Tumor Necrosis Factor- α (TNF-α), and nitric oxide release from lipopolysaccharide stimulated macrophage-like cells (31), and inhibits the expression of major histocompatibility complex and interferon-γ stimulated peritoneal macrophages (19).

In addition, we now have data from two non-blinded dosing cohorts in adults with CF noted in section 1.2 showing no evidence of toxicity but preliminary indications of clinical efficacy with improvement in both FEV_1 and FVC. We also found that although the PK did not correspond well to treatment response, the levels achieved in the sputum were similar to those used in *in vitro* experiments assessing the antimicrobial and anti-biofilm activity of gallium on *P. aeruginosa*. Here, we propose an initial proof of concept Phase 2 blinded efficacy study of gallium in CF patients using the maximum FDA-approved dose for patients with hypercalcemia (200 mg/m^2/day for a 5 day infusion).

2.2 Risk / Benefit Assessment

No published studies exist that evaluate the safety of IV gallium nitrate in CF patients. However, there is extensive published information on the use of this compound over a wide range of doses both above and below the doses proposed in this trial in patients with both neoplastic as well as benign metabolic diseases. Moreover, this human experience extends over a period of more than 30 years, including a post-marketing experience of 17 years duration. Thus, the risks of the drug have been broadly described in thousands of patients and unique risks associated with the proposed dosing in CF patients would appear to be low. In this trial, we propose to limit our trial to stable CF subjects with normal renal function at a dose previously tested and shown to be safe in our Phase 1b CF study. Because of this, we anticipate potentially severe systemic adverse reactions to be unlikely.

A specific minimum human toxic dose has not been established. However, a "no effect" (placebo) dose of 0.05 mg/kg administered once per day subcutaneously was used in the randomized Phase 2 study in Paget's disease. A higher dose (0.5 mg/kg/d) demonstrated biological activity against markers of Paget's disease activity, but demonstrated no significant AEs. The lethal dose 50 (LD50) of gallium nitrate in mice is 4,360 mg/kg for oral doses and 600 mg/kg for subcutaneous doses (32). Renal toxicity is dose dependent and is usually the dose limiting adverse effect of parenteral gallium nitrate when administered by bolus injection or short (2 hours) IV infusion (2). Elevation of serum BUN and creatinine levels has been observed in approximately 12.5% of patients receiving recommended therapeutic doses of IV gallium nitrate (~ 400 mg/day for a typical adult). Toxicity depends on several factors: the total dose, the rate of administration, the underlying pathology, and use with other nephrotoxic drugs. Doses up to 1400 mg/m^2 by short IV infusion have been used in clinical trials. No significant toxicity has been observed at doses of 50 mg/m^2 (~ 100 mg for a typical adult) or less (33).

Nephrotoxicity occurs most commonly after large IV boluses are rapidly administered. While the exact mechanism of renal toxicity in humans is not known, studies in rats suggest that toxicity occurs when the influx of gallium into the circulation exceeds the ability of transferrin (or other binding proteins) to bind gallium (19;34). Unbound gallium forms gallate ($Ga(OH)_4^-$) which is rapidly excreted by the kidneys (35). Under conditions where gallate transiently reaches high levels, it is thought to precipitate within renal tubules and cause obstruction. Rats administered high levels of gallium nitrate developed flocculent complexes that were observed to be occluding the lumen of some renal tubules (34). X-ray energy spectrometry showed that gallium was found in the presence of calcium and phosphorus in the occluding precipitates (34).

This mechanism of renal toxicity is consistent with two additional observations. First, in rats and humans, osmotic diuresis significantly reduced the levels of gallium found in the urine and the incidence of renal toxicity when high doses were administered (34;35). Second, gallium

nephrotoxicity is markedly reduced by slow IV infusion or subcutaneous injection (19). These methods of delivery lower the rate at which gallium appears in the plasma, preventing transferrin saturation and reducing gallate delivery to the kidneys. Therapeutic doses of gallium have been administered to thousands of debilitated patients using slow infusions, without significant renal toxicity (36). If it occurs, renal toxicity is generally mild (transient proteinuria) at doses < 300 mg/m^2 (21;22).

Hematological toxicity has also been observed with IV gallium nitrate, although this is generally mild and interpretation of this adverse effect is complicated by the fact that patients receiving gallium nitrate had malignancies, were hospitalized, and were undergoing frequent phlebotomy (19). In one study, 38% of gallium-treated cancer patients developed a greater than 3 gm/100mL drop in the hemoglobin level; however, only 18% of patients required a blood transfusion (22). Thrombocytopenia (platelet count< $150,000/mm^3$) occurred in 15% of patients and was most frequent at doses > 450 mg/m^2/day (22). No bleeding was observed. Mild leukocytosis (mean increase of 0.9 $x10^3/mm^3$) has also been observed in a dose dependent manner (22). These toxicities were not observed in our Phase 1b CF clinical trial.

Gallium can also produce hypocalcemia; treatment of hypercalcemia of malignancy is the approved indication for gallium. In rats, a single injection produces a rapid increase in urinary excretion of calcium; however, this is transitory and was subsequently shown to be an effect of concomitant diuresis rather than the drug itself (19). Several studies suggest that gallium lowers serum calcium levels in large part by inhibiting bone resorption (19;35;37;38). In patients without elevated blood levels of calcium, the reduction in calcium levels is usually mild and asymptomatic and can usually be treated with oral calcium supplements (39). These toxicities were not observed in our Phase 1b CF clinical trial.

Other AEs associated with gallium include optic neuritis (observed at high doses), tinnitus and mild high frequency hearing loss (apparent on audiograms), anorexia, nausea, diarrhea, stomatitis, conjunctivitis, lethargy, mild respiratory alkalosis with hyperchloremia, and rash (19). A reversible pulmonary syndrome consisting of dyspnea, rales, rhonchi, pleural effusion, and pulmonary infiltrates has been reported in association with gallium nitrate infusion, but this is rare and has occurred in patients with serious underlying malignant conditions. Thus the relationship of these other AEs and the pulmonary syndrome to the gallium infusion is uncertain (Ganite® (gallium nitrate injection) package insert,(21;40)). A case of pulmonary toxicity occurred in a patient receiving 300 mg/m^2/day for seven (7) days every four (4) weeks (41). No pulmonary complaints were noted in our Phase 1b CF clinical trial; in fact, lung function improved with treatment.

2.3 Measures to Minimize Risk

All subjects will be instructed to ingest a minimum of two (2) liters of fluid per day during the five (5) day infusion of study drug (gallium nitrate or placebo) and for two (2) days thereafter to minimize the risks of nephrotoxicity. Fluid intake will be documented on a daily diary from Day 1 through Day 8. We will evaluate the respiratory status and general health of subjects before and after exposure to gallium. In the event of an allergic reaction during the six hour observation period on Day 1, adrenaline and anti-histamine will be immediately available. If needed, emergency services including an emergency room and medical ICU are immediately available in close proximity to the study site. Bronchospasm, if it occurs, will be treated immediately with nebulized albuterol. In addition, we will monitor the subject's physical exam, symptoms,

respiratory questionnaire results, spirometry, blood and urine clinical laboratory test results, and treat as indicated.

The effects of gallium on an unborn child (fetus) are unknown and may be harmful. Women cannot participate if they are pregnant, are breast feeding, or plan to become pregnant during the study. Men cannot participate if they plan to father a child during the study.

There is no proven benefit of IV gallium in CF. However, the risks of the infusion dose proposed in this trial appear to be both low and well-defined in non-CF populations. In the future, IV gallium might be proved to be useful in the acute and chronic management of CF patients infected with *P. aeruginosa*.

3 STUDY OBJECTIVES

3.1 Primary Objective

To assess the efficacy of IV gallium to improve pulmonary function as measured by a 5% or greater relative improvement in FEV_1 from baseline to Day 28

3.2 Secondary Objectives

- To assess the safety and tolerability of IV gallium
- To assess the efficacy of IV gallium in improving measures of lung function including relative change in FEV_1 absolute change in FEV_1, and FVC
- To assess the efficacy of IV gallium in reducing *P. aeruginosa* in the lungs of CF patients based on quantitative cultures of sputum
- To assess the efficacy of IV gallium in improving respiratory symptoms as measured by the CF Respiratory Symptoms Diary -Chronic Respiratory Infection Symptom Severity Score (CFRSD-CRISS)
- To assess the sputum and blood PK profile of IV gallium
- To assess the rate of acquired resistance of *P. aeruginosa* to gallium
- To explore the anti-inflammatory properties of IV gallium
- To explore the efficacy of IV gallium in reducing the use of antibiotics for an acute indication

4 STUDY DESIGN

4.1 Study Overview

This is a phase 2, multi-center, randomized, double-blind, placebo-controlled trial in adults with CF chronically infected with *P. aeruginosa*. The study will evaluate the safety and clinical efficacy of a five-day infusion of IV gallium nitrate. Approximately one hundred twenty eligible subjects will be randomized in equal allocation to receive either a single 5-day infusion of IV gallium or volume-matched placebo. Subjects will be randomized using an adaptive randomization scheme to prevent imbalances in treatment allocation within baseline FEV_1 strata.

Five study visits will occur at Screening/Baseline (Day 1; Visit 1), Day 6 (Visit 2), Day 14 (Visit 3), Day 28 (Visit 4), and Day 56 (Visit 5). On Day 3, subject will be contacted by phone. Screening data will be reviewed to determine subject eligibility. Screening can occur on Day 1 prior to the start of study drug or within seven (7) days prior to Day 1. If Screening takes place prior to Day 1, then on Day 1, in order to reconfirm eligibility, an abbreviated physical exam, spirometry and a urine pregnancy test for females must be completed prior to dosing on Day 1. Subjects who meet all inclusion criteria and none of the exclusion criteria will be eligible for the study.

Subjects will be permitted to remain in the clinical research center for the full five (5) days of continuous infusion at the discretion of the investigator. During each day of the infusion and for two subsequent days (Day 1 to Day 8), the subject will be instructed to consume at least two (2) liters of fluid. Calcium, BUN and creatinine may be checked more frequently at the discretion of the investigator.

All subjects who had the study drug infusions started will be considered evaluable for safety and efficacy analyses. Incidence of AEs will be monitored during the trial.

Total duration of subject participation will be up to 63 days. Additional data regarding long-term health impact may be ascertained through the CF Foundation National Patient Registry with optional patient consent.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

Difference between treatment groups in the proportion of subjects with a 5% or greater relative change in FEV_1 (liters) from baseline to Day 28

5.2 Secondary Endpoints

- Difference between treatment groups in safety parameters including the incidence of AEs, changes in clinical safety labs, and hospitalizations through the end of follow-up
- Difference between treatment groups in the change in pulmonary function parameters from baseline through end of follow-up including relative change in FEV_1 (liters), absolute change in FEV_1 (liters) and in FEV_1 % predicted, and change in FVC (liters and % predicted)
- Difference between treatment groups in the change in sputum *P. aeruginosa* density based on quantitative cultures from baseline through end of follow-up
- Difference between treatment groups in the emergence of other CF pathogens, including gallium-resistant *P. aeruginosa*, from baseline through end of follow-up
- Difference between treatment groups in the change in the CFRSD-CRISS from baseline through end of follow-up
- Determination of the steady state concentration (Css) and elimination half life ($t_{1/2\beta}$) in those subjects receiving IV gallium
- Difference between treatment groups in the change in blood inflammatory markers from baseline through end of follow-up
- Difference between treatment groups in the rate of antibiotic usage including IV, oral, and inhaled for an acute indication from baseline through end of follow-up
- Longitudinal trends in clinical features and CF pathogens following treatment through the CF Foundation National Patient Registry

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of CF who meet the inclusion and do not meet the exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Greater than or equal to 18 years of age at Screening
2. Documented chronic colonization with *P. aeruginosa* defined as identification in two sputum or oropharyngeal cultures within the year prior to Day 1
3. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype **and** one or more of the following criteria:
    a. sweat chloride ≥ 60 mEq/liter by quantitative pilocarpine iontophoresis test (QPIT)
    b. two well-characterized mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene
    c. Abnormal nasal potential difference (NPD; change in NPD in response to a low chloride solution and isoproteronol of less than -5 mV)
4. $FEV_1 \geq 25$ % of predicted value at Screening
5. Able to expectorate sputum
6. Serum liver function tests ≤ 2.5 x upper limit of normal at Screening
7. Serum urea nitrogen (BUN) ≤ 1.5 x upper limit of normal at Screening
8. Serum creatinine ≤ 2.0 mg/dl and ≤ 1.5 x upper limit of normal at Screening
9. Hemoglobin ≥ 9 g/dl, platelets ≥ 100,000/mm^3, and white blood cells (WBC) ≥ 4,500/mm^3 at Screening
10. Ionized calcium ≥ lower limit of normal at Screening
11. Written informed consent obtained from subject or subject's legal representative, able to communicate with the Investigator and comply with the requirements of the protocol
12. If female and of childbearing potential, must have a negative pregnancy test on Day 1 prior to receiving study drug
13. If female and of childbearing potential, is willing to use adequate contraception for the duration of the study through Visit 5, as determined by the investigator
14. If male and able to father a child, is willing to use adequate contraception for the duration of the study through Visit 5, as determined by the investigator
15. Clinically stable with no significant changes in health status within 14 days prior to Day 1

6.3 Exclusion Criteria

1. Use of inhaled antibiotics within seven days prior to Day 1
2. Unable or unwilling to withhold use of chronic inhaled antibiotics through Day 28

3. Use of intravenous, inhaled, or oral antibiotics for an acute indication within 14 days prior to Day 1
4. Use of bisphosphonates within seven days prior to Day 1
5. History of osteoporosis (defined as the most recent dexa scan with a T-score ≤ -2.5 with the dexa scan performed within the five years prior to screening)
6. Lactating female
7. Known sensitivity to gallium
8. Use of any investigational drug and/or participated in any interventional clinical trial within 28 days prior to Screening
9. Presence of a condition or abnormality (e.g., pre-existing heart disease) that in the opinion of the site investigator would compromise the safety of the subject or the quality of the data

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

Standard therapy for CF is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below. Ongoing chronic treatment (>30 days prior to Day 1) with oral antibiotics, including azithromycin, is allowed. Treatment for pulmonary exacerbations and as required for acute care is allowed, including acute oral, inhaled and IV antibiotics. Physicians are encouraged to prescribe acute antibiotic therapy only in the presence of symptoms.

7.2 Prohibited

The following medications are prohibited within seven days prior to Day 1 through Day 28:

- inhaled chronic antibiotics
- bisphosphonates

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin is prohibited from Day 1 through 24 hours after the study drug infusion is stopped (e.g., Day 7 for subjects that complete the entire course of study drug).

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Approximately 120 eligible subjects will be randomly assigned to receive study drug (IV gallium or placebo) in equal allocation. An adaptive randomization will be employed with the goal of ensuring equal representation in each study arm as noted in the Statistical Analysis Plan (SAP). Subject randomization will be stratified by baseline FEV_1 group (FEV_1 < 50% of predicted, 50-70% of predicted and >70% of predicted). Randomization will occur centrally using a web based randomization system linked to the electronic data capture (EDC) system. Only authorized site personnel are given access to the randomization module. Authorized site

personnel will enter subject eligibility information and whether or not the subject signed informed consent. The system will verify that the subject is eligible and has provided signed informed consent, and then provide the appropriate authorized site personnel with a randomization assignment for that subject that matches a specific treatment.

8.2 Blinding

Study treatments will be administered in a double-blind fashion (i.e., subjects and site research staff will not know the treatment assigned, with the exception of the dispensing site pharmacist, central pharmacy, and designated TDNCC personnel. The following study procedures will be in place to ensure double-blind administration of test and control (IV gallium/placebo) study treatments:

- Randomization assignments will be stored in a secure database and appropriately protected and backed up.
- Access to the randomization code will be strictly controlled and limited to designated TDNCC study personnel. All other study personnel, investigators, and subjects will remain blinded to their study drug assignment throughout the study.
- A volume and appearance-matched placebo will be utilized.
- The site pharmacist will apply a blinded label to each one-liter bag of study drug.

The study blind will be broken on completion of the clinical study and after the study database has been locked and study results released. The site investigators will be provided with each subject's treatment assignment following completion of data analysis.

During the study, the blind may be broken **only** in emergencies when knowledge of the subject's treatment group is necessary for further patient management.

8.3 Test and Control Formulation

8.3.1 Test product

Gallium, a group IIIA transition metal, is the active component in gallium nitrate, $Ga(NO_3)_3$. Gallium nitrate crystallizes from the reaction of elemental gallium with nitric acid. Anhydrous $Ga(NO_3)_3$ has a molecular weight of 258.76. The hydrated form of gallium nitrate, $Ga(N0_3)_3 \bullet 9H_2O$ with molecular weight 417.87, is a white, crystalline powder. Gallium nitrate is fully soluble in water and in normal saline up to 1 mM. To achieve higher concentrations, gallium nitrate can be dissolved in chelators like sodium citrate.

Gallium nitrate is a member of the calcium regulator drug class and is used primarily in the treatment of hypercalcemia of malignancy (19;21;35). Gallium nitrate for injection is a clear, colorless, tasteless, odorless, sterile solution of gallium nitrate.

Each vial contains 100 mM gallium nitrate (on an anhydrous basis) and sodium citrate dihydrate. Each milliliter in the prepackaged 25 mL vials , contains 25 mg gallium nitrate (on an anhydrous basis) and 28.75 mg sodium citrate dihydrate. The osmolarity of this solution is 570 mOsm. The pH is adjusted to 6.0 to 7.0 using NaOH or HCl.

8.3.2 Placebo Control

Placebo vials will be provided for this study. Each placebo contains 25 mL of 0.9% sodium chloride

8.3.3 Packaging and Labeling

The study drug (gallium nitrate/placebo) is supplied in prepackaged 25 mL single-use vials. Each vial of study drug will be packaged and labeled according to current ICH GMP and GCP guidelines and FDA requirements.

The site investigational pharmacy will affix a blinded label to each one-liter bag of study drug. The label will comply with FDA requirements (e.g., the required FDA warning statement, the protocol number, the name of the sponsors, and directions for patient use and storage). Further details are provided in the Pharmacy Manual.

8.3.4 Preparation/Dispensing

To prepare a one-liter solution of study drug, the site investigational pharmacy will dilute the study drug vials with 1,000 mLs of 0.9% sodium chloride for a dose of 200 mg/m^2/day. The site investigational pharmacy will label the solutions as described in section 8.3.3.

The study drug will be dispensed by the site's investigational pharmacist. The pharmacist or appropriate designee will instruct the subject on the infusion of the study drug. On Day 1, the investigator and clinical research nurse will review the use of the continuous infusion with the subject and inspect the catheter used for infusion.

8.3.5 Dosage/Dosage Regimen

Study drug will be infused continuously over five (5) days at 200 mg/m^2/day.

8.3.6 Administration Instructions

The administration of study drug can occur via one of the following types of venous access. The type of access is determined by the site PI.

- An existing long term vascular access (e.g. subcutaneous venous Port)
- A midline catheter
- A peripherally inserted central catheter (PICC) line
- A peripheral IV

Depending on the type of venous access and according to institutional standard practice, additional procedures may need to be performed to confirm the proper placement of the venous access (e.g., chest x-ray or ultrasound for PICC line placement).

Study drug will be administered using an ambulatory infusion pump infused over 24 hours for five (5) sequential days. The specifics of administration (e.g., pump type, bag vs. syringe, inpatient vs. outpatient) can be determined by each site according to institutional policy.

Study drug will be started on Day1, in the presence of the clinical research nurse. The first six (6) hours of the infusion will be directly observed in the clinical research center (or equivalent) of the institution. Subsequent to leaving the clinical research center, the subject will receive

doses for Day 2 through Day 5 to be refrigerated upon their return home. The subject will be instructed on changing the infusion once the first 24-hour infusion is complete. A one-liter solution bag will be infused each day for five days. Subjects will be permitted to remain in the clinical research center for the full five (5) days of continuous infusion at the discretion of the investigator.

During each day of the infusion and for two (2) days following the infusion (Day 1 to Day 8), the subject will be instructed to consume at least two (2) liters of fluid. Examples will be provided to assist the subject in determining the adequate volume to consume.

8.4 Supply of Study Drug at the Site

Gallium nitrate will be donated by the CFFT.

Each site's investigational pharmacy will receive study drug in the form of vials containing either 100 mM gallium nitrate (on an anhydrous basis)/sodium citrate dihydrate or 0.9% sodium chloride (placebo).

For the dilution of the study drug, 0.9% sodium chloride will be provided by each investigational pharmacy.

8.4.1 Storage

Vials of study drug for injection and one-liter solutions of 0.9% sodium chloride will be stored at controlled room temperature (20 to 25 degrees Celsius; 68 to 77 degrees Fahrenheit) until the day prior to randomization. The one-liter solution of study drug will remain stable for 48 hours at room temperature and for 7 days if refrigerated (2 to 8 degrees Celsius) [per the Ganite® (gallium nitrate injection) package insert]. All study drug must be stored separately from normal hospital inventories, in a locked facility with access limited to the investigator and authorized personnel.

Once dispensed to the subject, the five one-liter solutions of study drug will be stored at 2 to 8 degrees Celsius until 60 minutes prior to the start of the infusion.

8.5 Study Drug 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 study site staff. The number of bags dispensed and returned by the subject and the volume remaining will be recorded on the Investigational Drug Accountability Record.

8.6 Measures of Treatment Compliance

Subjects will be asked to keep a subject diary noting the date, start and stop times of the infusion, and any AEs. They will be asked to bring their diary to each study visit along with all used and unused study drug bags.

8.7 Discontinuation of Study Drug

Indications for study drug discontinuation include symptoms of hypocalcemia confirmed on blood assay and development of an acute fall in urine output confirmed by a rise in creatinine. Additional indications for discontinuation include the development of a study drug related SAE

during infusion or any other study drug related adverse event that the investigator believes warrants discontinuation.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Study Visits 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, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Screening and at all Study Visits and at early termination, when applicable. In addition, the use of concomitant inhaled, oral, and IV antibiotics from 30 days prior to Screening through Visit 5 will be documented. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, sex, race, CF genotype) 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 as noted in the Schedule of Events.

9.1.4 Physical Examination

A complete or abbreviated physical examination will be performed by a licensed professional [MD (either the investigator or a sub-investigator), NP, RN, PA] as noted in the Schedule of Events. A complete physical includes examination of the following systems: head/neck/thyroid, EENT, respiratory, cardiovascular, lymph nodes, abdomen, skin, musculoskeletal, and neurologic. An abbreviated physical includes examination of the following systems: respiratory, cardiovascular and abdomen.

After initiation of Study Drug, new clinically significant abnormal physical exam findings must be documented as an AE.

9.1.5 Weight and Height

Weight and height will be measured on the same scale and recorded as noted in the Schedule of Events. Subjects may remain in clothes (without shoes). A standing height will be measured and recorded.

9.1.6 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes as noted in the Schedule of Events.

9.1.7 Oximetry

Oximetry will be measured on room air with the subject at rest as noted in the Schedule of Events.

9.1.8 Spirometry

Spirometry will be performed as noted in the Schedule of Events and in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests. Predicted equations for the computation of FEV_1 and FVC % predicted will utilize the reference equations by Hankinson (42).

Subjects who routinely use bronchodilators should use them prior to spirometry as noted below:

- Subjects who routinely use short acting inhaled bronchodilators should use them 15 minutes to 2 hours prior to PFTs during study visits.
- Subjects who routinely use long acting bronchodilator agents should use them 15 minutes to 6 hours prior to PFTs during study visits.

9.1.9 CFRSD-CRISS and Subject Diary

The subjects will be given a CF specific symptom diary called the CFRSD-CRISS. This diary includes 8 questions and takes less than 5 minutes to complete. The diary will be completed daily as noted in the schedule of Events.

Subjects also will be given a diary to document date, start and stop times they take their study drug and any AEs from Day 1 through Day 5. In addition, at least two (2) liters each day of fluid consumption from Day 1 through Day 8 will be documented.

9.1.10 Adverse Events

Information regarding occurrence of AEs will be captured throughout the study. Duration (start and stop dates), severity, outcome, treatment and relation to study drug will be recorded on the case report form (CRF). Subjects will be specifically instructed to report peri-oral tingling, paresthesia (pins and needles sensation) of the extremities, or muscle spasms. If these symptoms are reported, calcium, BUN, and creatinine may be checked more frequently at the discretion of the investigator.

If a subject has study drug discontinued because of an AE, the subject will be followed and treated by the site investigator until the abnormal parameter or symptom has resolved or stabilized. Subjects who discontinue study drug early will be encouraged to return for an Early Discontinuation of Study Drug Visit (Refer to section 10.6) and to complete all remaining visits and procedures.

9.2 Clinical Laboratory Measurements

After initiation of Study Drug, new clinically significant abnormal laboratory findings must be documented as an AE.

The collection of blood samples may occur via the study drug administration access point if study drug is not being administered. If study drug is being administered, blood collection must occur at a different access point.

9.2.1 Hematology

Blood will be obtained as noted in the Schedule of Events and sent to each site's clinical hematology lab for a complete blood count with differential (i.e., hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count).

9.2.2 Serum Chemistry Profile

Blood will be obtained as noted in the Schedule of Events and sent to each site's clinical chemistry lab for determination of serum sodium, potassium, chloride, bicarbonate, random glucose, BUN, creatinine, calcium, ionized calcium, phosphate, magnesium, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, total bilirubin, direct bilirubin, gamma-glutamyl transferase (GGT), albumin and lactate dehydrogenase (LDH).

Calcium, BUN, and creatinine may be checked more frequently at the discretion of the investigator.

9.2.3 Pregnancy Test

A serum or urine pregnancy test will be obtained from female subjects who are of childbearing potential as noted in the Schedule of Events.

9.2.4 Urinalysis

Urine will be obtained as noted in the Schedule of Events and sent to each site's clinical laboratory for determination of color, specific gravity, pH, protein, glucose, ketones, and blood.

9.3 Pharmacokinetic Measurements

Because of limited correlation between clinical efficacy and PK measurements done in the previous Phase 1b study in CF, only limited PK assessments will be made. Expectorated sputum and blood (plasma) for determination of concentrations of gallium and iron will be collected pre-dose and post-dose as detailed in the Table below:

PK Collection Day	Blood	Sputum
Day 1	• Pre-dose • 1hr (+/- 10 minutes) post infusion start	1 hr (+/- 10 minutes) post infusion start

PK Collection Day	Blood	Sputum
Day 6	Prior to infusion stop (+/- 10 minutes)	Prior to infusion stop (+/-10 minutes)
Day 14	At visit	At visit
Day 28	At visit	At visit
Day 56	At visit	At visit

The blood will be processed for plasma as detailed in the Study Laboratory Manual. All specimens will be labeled and placed immediately into a -70°C freezer. All frozen specimens will be shipped at the specified time frames, as detailed in the Study Laboratory Manual, throughout the study to a central laboratory.

Gallium and iron concentrations will be measured using inductively coupled plasma mass spectrometry (ICP-MS) done at a central laboratory.

9.4 Research Laboratory Measurements

9.4.1 Sputum Culture

Expectorated sputum will be collected for culture as noted in the Schedule of Events. All sputum specimens should be collected in a sterile specimen cup, labeled, and shipped on ice overnight to a central laboratory for culture. Specimens will be processed within two (2) days of collection. Quantitative culture for typical *P. aeruginosa* and qualitative culture for other CF pathogens will be performed. Detailed instructions for specimen collection, packaging, and shipping will be provided in the Study Laboratory Manual.

9.4.1.1 Gallium Susceptibility Testing

The CF pathogens (e.g., *Haemophilus influenzae*, *Staphylococcus aureus*, *P. aeruginosa*, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans*) isolated from the subject's sputum will be tested for susceptibility using Clinical and Laboratory Standard Institute (CLSI) broth microdilution methods. Susceptibility will be performed using standard low iron BM-2 broth (for the Gram-negative organisms) or 1:8 diluted MHB (for the Gram-positive organisms).

9.4.2 Plasma Collection for Assessment of Blood Cytokines and Chemokines

Blood will be collected and processed for plasma as specified in the Schedule of Events. Detailed instructions will be provided in the Study Laboratory Manual. The plasma aliquots will be placed immediately into a –70°C freezer for storage until shipment. All frozen specimens will be shipped to a central laboratory at the specified time frames detailed in the Study Laboratory Manual throughout the study.

A panel of cytokines and chemokines assays will be performed on plasma to evaluate inflammatory biomarkers in CF (e.g.,(43;44)(45) Granulocyte Colony Stimulating Factor (GCSF), Interleukin-1ra (IL1ra), IL6, Interleukin-8 (IL8), TNF-α, Transforming Growth Factor-β1 (TGFβ1), Neutrophil elastase antiprotease complex (PMNeL), highly sensitive C-Reactive Protein (hsCRP), Calprotectin, Arginase-1, and Serum Amyloid A (SAA)).

These assays will be performed at a central laboratory as specified in the Study Laboratory Manual.

10 EVALUATIONS BY VISIT

10.1 Screening/Baseline/Visit 1 (Day 1)

Screening may occur on the morning of Day 1 prior to start of study drug. In this case, Screening, Baseline and Start of Treatment (Day 1) occur on the same day. Alternatively, Screening and Baseline/Day 1 may be separated into two visits as long as Screening takes place within 7 days of Baseline/Day 1.

10.1.1 Visit 1 (Day 1) – Combined Screening / Baseline

Screening/Pre-Dose Baseline (Day 1)

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization. Ensure subject has opted in or out of CFF Registry ID collection.
2. Assign the subject a unique subject number.
3. Record demographics data.
4. Record medical history, including a history of CF, diagnosis date, and prior CF treatments.
5. Record concomitant medications.
6. Administer CFRSD-CRISS.
7. Record height and weight.
8. Perform a complete physical examination.
9. Perform and record vital signs.
10. Perform and record oximetry.
11. Collect blood for clinical laboratory tests (hematology and serum chemistry), pre-dose gallium and iron concentrations, cytokine and chemokine testing.
12. Collect urine for urinalysis.
13. Collect blood or urine for pregnancy test (if female of child-bearing potential).
14. Perform and record spirometry.
15. Collect baseline expectorated sputum for culture.
16. Confirm all eligibility criteria when lab results received.
17. Randomize subject.
18. If existing long-term vascular access (e.g., a subcutaneous venous Port) is not available, place a midline catheter, PICC or peripheral IV.

Post-Dose (Day 1)

1. Initiate infusion and dispense study drug.
2. Observe first six (6) hours of infusion.
3. Administer and dispense subject diary. Dispense CFRSD-CRISS for Days 2-6.

4. Collect blood for gallium and iron levels one (1) hour (± 10 minutes) after initiation of study drug infusion.
5. Collect expectorated sputum for gallium and iron levels one (1) hour (± 10 minutes) after initiation of study drug infusion.
6. Record any AEs.
7. Instruct the subject to drink two liters of fluid per day during the infusion.
8. Schedule subject for Visit 2 and remind them to return all study drug bags, the subject diary, and the CFRSD-CRISS at the next visit.

10.1.2 Visit 1 (Day 1) – Separate Screening / Baseline

Screening (-1 to -6 days)

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization. Ensure subject has opted in or out of CFF Registry ID collection.
2. Assign the subject a unique subject number.
3. Record demographics data.
4. Record medical history, including a history of CF, diagnosis date, and prior CF treatments.
5. Record concomitant medications.
6. Record height and weight.
7. Perform a complete physical examination.
8. Perform and record vital signs.
9. Perform and record oximetry.
10. Collect blood for clinical laboratory tests (hematology and serum chemistry)
11. Collect urine for urinalysis.
12. Collect blood or urine for pregnancy test (if female of child-bearing potential).
13. Perform and record spirometry.
14. Schedule subject to return to complete Visit 1.

Pre-Dose Baseline (Day 1)

1. Administer CFRSD-CRISS.
2. Record interval medical history.
3. Record concomitant medications.
4. Record weight.
5. Perform an abbreviated physical examination.
6. Perform and record vital signs
7. Perform and record oximetry.
8. Collect blood for pre-dose gallium and iron concentrations, cytokine and chemokine testing.
9. Collect blood or urine for pregnancy test (for female of childbearing potential).
10. Perform and record spirometry.
11. Collect baseline sputum for culture.

12. Confirm all eligibility criteria when lab results received.
13. Randomize subject.
14. If existing long-term vascular access (e.g., a subcutaneous venous Port) is not available, place a midline catheter, PICC, or peripheral IV.

Post-Dose (Day 1)

1. Initiate infusion and dispense study drug.
2. Observe first six (6) hours of infusion.
3. Administer and dispense subject diary. Dispense CFRSD-CRISS for Days 2-6
4. Collect blood for gallium and iron levels one (1) hour (± 10 minutes) after initiation of study drug infusion.
5. Collect expectorated sputum for gallium and iron levels one (1) hour (± 10 minutes) after initiation of study drug infusion.
6. Record any AEs.
7. Instruct the subject to drink two liters of fluid per day during the infusion.
8. Schedule subject for Visit 2 and remind them to return all study drug bags, the subject diary, and CFRSD-CRISS at the next visit.

10.2 Phone Call (Day 3)

1. Review and record any AEs.
2. Review subject's fluid intake.
3. Review the status of the subject's study drug infusion and pump use.
4. Remind the subject to return all study drug bags, the subject diary, and CFRSD-CRISS at the next visit.

10.3 End of Treatment/Visit 2 (Day 6)

With the exception of the CFRSD-CRISS administration and the blood and sputum samples for gallium and iron levels, the other Visit 2 procedures can be performed in any order in relation to the stop of the study drug infusion.

1. If not already completed for the day, administer CFRSD-CRISS.
2. Review subject diary.
3. Collect and review CFRSD-CRISS from days 2-6.
4. Record any AEs.
5. Record changes to concomitant medications.
6. Record weight.
7. Perform abbreviated physical examination.
8. Perform and record vital signs.
9. Perform and record oximetry.
10. Perform and record spirometry.
11. Assess infusion of study drug and stop infusion.
12. If applicable, remove the midline catheter, PICC, or peripheral IV.

13. Collect urine for urinalysis.
14. Collect blood for laboratory tests (hematology and serum chemistry) and, within 10 minutes of the stop of the infusion, for gallium and iron levels.
15. Collect expectorated sputum for gallium and iron levels within 10 minutes of the stop of the infusion.
16. Collect returned study drug supplies and perform accountability
17. Instruct the subject to drink two liters of fluid per day through Day 8.
18. Dispense subject diary and remind subject to complete the diary through Day 8.
19. Schedule subject for Visit 3.

10.4 Visit 3 (Day 14 +/-2 days)

1. Administer CFRSD-CRISS.
2. Review subject diary.
3. Record any AEs.
4. Record changes to concomitant medications.
5. Record weight.
6. Perform abbreviated physical examination.
7. Perform and record vital signs.
8. Perform and record oximetry.
9. Perform and record spirometry.
10. Collect blood for clinical laboratory tests (hematology and serum chemistries) and for gallium and iron levels.
11. Collect urine for urinalysis.
12. Collect expectorated sputum for gallium and iron levels.
13. Schedule subject for Visit 4

10.5 Visit 4 (Day 28 +/- 3 days)

1. Administer CFRSD-CRISS.
2. Record any AEs.
3. Record changes to concomitant medications.
4. Record weight.
5. Perform complete physical examination.
6. Perform and record vital signs.
7. Perform and record oximetry.
8. Perform and record spirometry.
9. Collect blood for clinical laboratory tests (hematology and serum chemistries), gallium and iron levels, and cytokine and chemokine testing.
10. Collect urine for urinalysis.
11. Collect blood or urine for pregnancy test (if female of child-bearing potential).
12. Collect expectorated sputum for culture and gallium and iron levels.

13. Schedule subject for Visit 5.

10.6 Visit 5 (Day 56 +/- 4 days) – Final Visit

1. Administer CFRSD-CRISS.
2. Record any AEs.
3. Record changes to concomitant medications.
4. Record weight.
5. Perform complete physical examination.
6. Perform and record vital signs.
7. Perform and record oximetry.
8. Perform and record spirometry.
9. Collect blood or urine for pregnancy test (if female of child-bearing potential).
10. Collect blood for gallium and iron levels, and cytokine and chemokine testing.
11. Collect expectorated sputum for culture and gallium and iron levels.

10.7 Early Discontinuation of Study Drug

With the exception of the CFRSD-CRISS administration and the blood and sputum samples for gallium and iron levels, the other procedures can be performed in any order in relation to the stop of the study drug infusion (if subject did not already stop infusion prior to visit).

1. If not already completed for the day, administer CFRSD-CRISS.
2. Review subject diary.
3. Collect and review CFRSD-CRISS.
4. Record any AEs.
5. Record changes to concomitant medications.
6. Record weight.
7. Perform abbreviated physical examination.
8. Perform and record vital signs.
9. Perform and record oximetry.
10. Perform and record spirometry.
11. Assess infusion of study drug and stop infusion (if applicable).
12. If applicable, remove the midline catheter, PICC, or peripheral IV.
13. Collect urine for urinalysis.
14. Collect blood for laboratory tests (hematology and serum chemistry) for gallium and iron levels (If infusion stopped at visit, collect within 10 minutes of the stop of the infusion. If infusion stopped prior to the visit, note the collection time).
15. Collect expectorated sputum for gallium and iron levels (If infusion stopped at visit, collect within 10 minutes of the stop of the infusion. If infusion stopped prior to the visit, note the collection time).
16. Collect returned study drug supplies and perform accountability.
17. Instruct the subject to drink two liters of fluid per day for 2 more days.

18. Dispense subject diary and remind subject to complete the diary for 2 more days.
19. Schedule subject for Visit 2 and proceed with the remaining study visits. At Visit 2, conduct all procedures except for 10,11,15,16, and 17.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An 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. AEs will be recorded in the patient CRF. AEs will be described by duration (start and stop dates), severity, outcome, treatment, serious criteria, and relation to study medication.

The investigator must continue to follow up on the AEs related to study drug until resolution or stability.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03, as modified for CF, 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 AE 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)	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Moderate (2)	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate Instrumental activities of daily living (e.g., preparing meals, using the telephone, managing money)
Severe (3)	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living (e.g., bathing, dressing, feeding self, using toilet, taking medications)
Life-threatening (4)	Life-threatening consequences; urgent intervention indicated
Death (5)	Death related to AE

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.

11.2.1 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 SAE Report Forms will be reviewed by the site investigator and sent to the TDNCC within one business day of the site learning of the event. Sites will scan and email or fax the SAE report to:

Email address: cfsaesfacsys@seattlechildrens.org

Direct dial fax number: 206-985-3278

The site will notify the TDNCC of additional information or follow-up to an initial SAE Report as soon as relevant information is available. Follow-up information is reported on an SAE Report Form.

SAEs will be reported to the DSMB in accordance with the Data Safety Monitoring Plan.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB), the site investigator will report SAEs to the IRB.
The Sponsor will report SAEs to the FDA in accordance with 21 CFR 312.

11.3 Medical Monitoring

The Medical Monitor for the TDNCC should be contacted directly at this number to report medical concerns or questions regarding safety.

Pager : (800) 341-0961

12 EARLY DISCONTINUATION OF STUDY DRUG, WITHDRAWAL AND REPLACEMENT OF SUBJECTS

12.1 Discontinuation of Study Drug

If a subject discontinues study drug early for any reason they should be encouraged to return for an Early Discontinuation of Study Drug Visit (Refer to section 10.6). Subjects will not be withdrawn from the study and will be encouraged to complete all remaining visits and procedures.

If a subject discontinues study drug due to a related AE, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

12.2 Withdrawal of Subjects from the Study

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. This may include subjects who discontinue study drug early and who decline to continue to come in for the remaining study visits

Reasonable attempts will be made by the investigator to provide a reason for a subject's withdrawal. 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 encouraged to come in for a final visit as follows:

- If a subject withdraws prior to Day 6, they should come in for an Early Discontinuation of Study Drug Visit (Section 10.6).
- If a subject withdraws on Day 6, they should come in for Visit 2 procedures (Section 10.2)
- If a subject withdraws after Day 6, they should come in for Final Visit Procedures (Section 10.5)

12.3 Replacement of Subjects

Subjects who discontinue study drug early or withdraw from the study will not 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
- Significant non-compliance with study drug regimen
- Failure to perform spirometry at Day 1 or Visit 4
- Use of a prohibited concomitant medication

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The lead Principal Investigator with consultation with the DSMB 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 representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files. The site will report the violation to their IRB in accordance with their IRB reporting requirements.

14 DATA SAFETY MONITORING

A DSMB will be established to monitor safety throughout the study, review interim safety analyses, and to ensure the continued scientific validity and merit of the study. A DSMB Charter will be established for this protocol. Summary reports tabulating SAEs by treatment group will be provided on a quarterly basis to the DSMB. Comprehensive safety interim reports will be provided twice yearly to the DSMB and will include detailed summaries of all safety endpoints. The DSMB will recommend continuation or discontinuation of the study based on these interim reviews.

15 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed SAP will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below. Except where otherwise noted, all tests will be two-sided and statistical significance will be determined at the 0.05 level.

15.1 Data Sets Analyzed

Intent-to-Treat Population: All subjects who are randomized and has study drug infusion started will comprise the intent-to-treat (ITT) population. The ITT population will be analyzed for the primary and secondary endpoint analyses and will comprise the safety population used for all safety analyses.

Per Protocol Population: Subjects in the ITT population who have no protocol violations, received no bisphosphonates or inhaled chronic antibiotics from 7 days prior to Day 1 through

Day 28, and received gallium infusion for at least five sequential days. The per protocol population will be used in the sensitivity analyses of the primary and secondary endpoints.

15.2 Demographic and Baseline Characteristics

Treatment groups will be described and compared with respect to baseline demographic and clinical characteristics such as age, gender, race, ethnicity, genotype, height, weight, body mass index, microbiology, use of concomitant medications (hypertonic saline, dornase alpha, azithromycin) and pulmonary function. Continuous measures will be analyzed using t-tests and categorical variables will be analyzed using chi-square,Fisher's exact, or Cochran-Mantel-Haenszel tests.

15.3 Analysis of Primary Endpoint

The primary endpoint is the difference between treatment groups in the proportion of subjects with a 5% or greater relative change from baseline to Day 28 of FEV_1 measured in liters, calculated as:

$$\frac{(\text{Day 28 } FEV_1 - \text{Day 1 } FEV_1)*100}{\text{Day 1 } FEV_1}$$

To test the null hypothesis that there is no difference between the treatment groups, a two-sample test of proportions will be performed adjusted for baseline FEV_1 strata. The difference between treatment groups will be provided with associated 95% CI. Additional analyses of the relative change in FEV_1 through Day 56 will include the development of a mixed-effect model for repeated measures (MMRM). Adjusted means and 95% CIs will be presented for each visit. Treatment comparisons of the average response at each visit will be generated within the MMRM model based on the linear contrast of the average using the estimated least square means (LSMeans) at Days 14, 28, and 56.

Missing data methods will be described in the SAP.

15.4 Analysis of Secondary Endpoints

Summary statistics (mean, standard deviation, median and range) of lung function parameters, sputum density, CFRSD-CRISS, and blood inflammatory markers for each visit and changes from baseline will be presented by treatment group. Descriptive statistics will be used to summarize the proportion of subjects initiating acute antibiotics (by route) and differences in proportions between the study arms will be estimated with accompanying 95% CIs.

Changes in lung function parameters (FVC in liters, FVC % predicted and FEV_1 % predicted), sputum bacterial density, CFRSD-CRISS, and inflammatory markers will be analyzed using MMRM. Adjusted means and 95% CIs will be presented for each visit. Treatment comparisons of the average response at each visit will be generated within the MMRM model based on the linear contrast of the average using the estimated LSMeans at Days 14, 28, and 56.

A log transformation will be used in all analyses of quantitative sputum culture counts and blood inflammatory markers. Change in bacterial density will include only those subjects with the pathogen at baseline. Additional descriptive analyses will be performed to summarize the disappearance and emergence of the pathogens and their susceptibility to gallium.

For those subjects randomized to receive IV gallium, we will determine of the Css and $t_{1/2\beta}$ by analyzing Days 1, 6, 14, 28 and 56. Clearance will be determined as the ratio of the infusion rate to the steady state concentration immediately prior to completion of the infusion. The $t_{1/2\beta}$ will be determined as 0.693/K where K is the elimination rate constant determined by the slope of the concentration/time curve after the infusion has been discontinued. To avoid unblinding, samples will only be assayed at the end of the completion of the trial.

15.5 Analysis of Safety Endpoints

All reported SAEs and AEs will be coded using MedDRA and grouped by body system. SAEs and AEs will be tabulated by treatment group using standard coding terms sorted by body system. The incidence of AEs in each treatment arm will be tabulated by seriousness, severity, and relationship to study drug. If an AE is reported more than once during the study period for a given subject, the greatest severity and the worst-case relationship will be presented in tables. The number of SAEs and AEs will be summarized for each treatment group as follows: (i) The proportion of subjects with at least one (S)AE, (ii) The average number of (S)AEs per patient, and (iii) The rate of (S)AEs per patient week of follow-up. Histograms showing the frequency of the number of (S)AEs in each treatment group will be included. Rates of (S)AEs by System Organ Class (SOC) will be presented by treatment group. Poisson regression modeling will be used to derive rate ratios and 95% CIs for each SOC. The rate ratios will be compared using a two-sided 0.05 level test for Poisson count data.

Safety lab data at each study visit and changes from baseline will be summarized by treatment group. In addition, the following clinical laboratory summaries will be presented by treatment group: (i) the incidence of clinically significant abnormalities at each study visit; and (ii) tables summarizing the frequencies of subjects below, within, and above the normal reference ranges at baseline and end of study; and (iii) tables displaying baseline to end of study shifts in each laboratory value (shifts between below, within or above normal range).
The proportion of subjects permanently discontinuing study drug will be tabulated by treatment group. Drug discontinuation events will be categorized as: (1) Permanently discontinued study drug and (2) Permanently discontinued study drug and withdrew from study. The reason for permanent drug discontinuation will be summarized.

The number of hospitalization events and proportion of subjects hospitalized from baseline to Day 56 will be summarized and compared by treatment group. Poisson regression modeling will be used to derive rate ratios and 95% CIs to compare hospitalization rates between treatment groups.

15.6 Interim Analysis

Comprehensive safety interim reports will be provided twice yearly to the DSMB, along with abbreviated quarterly reports. Quarterly reports will include SAE tabulations by treatment group. Comprehensive, twice yearly safety reports will include detailed summaries of all SAEs, AEs, laboratory parameter listings, drug discontinuations and withdrawals as well as other pertinent safety data. The DSMB will recommend continuation or discontinuation of the study based on these interim reviews. Any interim analyses with respect to efficacy or futility will be outlined in the DSMB charter.

15.7 Sample Size and Randomization

The primary endpoint is the difference between treatment groups in the proportion of subjects with a 5% or greater improvement in the relative change in FEV_1 in liters from baseline to Day 28. A 5% or greater improvement in the relative change is well established as a minimum clinically important change by CF physicians based on the choice of the non-inferiority margin of 4% in a recent Phase 3 clinical trial (46) and the effect size of a number of key therapies utilized in CF (43;47-52). A responder-based endpoint is also an established approach for identifying efficacy on an individual patient level, as opposed to highly variable average changes across patients within a treatment group. In the recently completed pilot study of gallium, 8 of 11 (73%) experienced a 5% or greater relative change in FEV_1 from baseline to Day 14 and 5 of 11 (45%) experienced a 5% or greater relative change in FEV_1 from baseline to Day 28. There was no placebo group in the study; however, data from a completed randomized trial of azithromycin in subjects chronically infected with *P. aeruginosa* was used to estimate the expected responder rate among those receiving placebo (43). In this study, the proportion of placebo subjects with a 5% or greater relative improvement in FEV_1 ranged from 28% to 33% across study visits from Day 28 through Day 84. Thus, the expected treatment difference in this study is between approximately 15% and 45% based on the observed response rates from the gallium pilot study. However, it is felt that the minimum clinically important difference between groups is based on clinician opinion is 30% given the treatment burden of an intravenous therapy (e.g., increasing the responder rate from 30% to 60%). With a sample size of 60 per group and assuming a responder rate of 30% in the placebo group, the study has 90% power to detect a 28.3% or greater improvement in the responder rate. Accounting for an attrition rate of 10% and interim analyses, the study will still have 90% power to detect a treatment difference of 30.0% or greater between groups.

A key secondary endpoint is the difference between treatment groups in the average relative change in FEV_1 from baseline (Day 1) to Day 28. Based on estimates from prior CF clinical trials among CF patients chronically infected with *P. aeruginosa*, the expected standard deviation of the relative change over a 28 day period ranges from 9 – 23 (43;53). A two-sided .05 level two-sample t-test will be used to test for differences between treatment groups with respect to the 28 day relative change. With a sample size of 60 per group and a standard deviation as low as 9 or as high as 23, the study would have 90% power to detect a minimum of 5.4% or 13.7% difference in the 28 day relative change between treatment groups, respectively. Additionally, the study has 90% power to detect a 0.78 log difference between treatment groups with respect to change in bacterial density assuming a standard deviation of 1.3 based on prior studies in CF(43).

Study personnel at the investigative site will use the Medidata Rave® and Balance™ systems to randomize each subject. Medidata Balance™ is a dynamic randomization and trial supply management solution that is fully unified with the Rave® EDC system. An adaptive randomization (dynamic allocation based on minimization) (54) will be employed with the goal of ensuring equal representation in each study arm based on lung function (FEV_1). Subjects will be randomly assigned in a 1:1 ratio to one of two arms: (1) IV placebo infusion, or (2) IV gallium nitrate solution. Subject randomization will be stratified by baseline FEV_1 group (FEV_1 < 50% of predicted, 50-70% of predicted and >70% of predicted). The dynamic allocation algorithm seeks to optimize randomization balance by minimizing a weighted average of the marginal imbalance of treatment allocation for each factor and for the study overall (55). A

random element is added to the otherwise deterministic minimization algorithm to reduce allocation predictability by using a biased coin (56) to include a chance of allocation to a treatment arm other than the arm that optimizes balance. Access to the randomization code will be strictly controlled and limited to select members of the Biostatistics and Clinical Data Management unit at the TDNCC and the central pharmacy.

16 DATA COLLECTION, RETENTION AND MONITORING

16.1 Data Collection Instruments

An electronic data capture system will be utilized for collection of study data. The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject who signs informed consent.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific CRFs when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the sponsor (or designee), but will be identified by a site number, subject number and initials.

If a correction is required for a CRF, the time and date stamp tracks the person entering or updating CRF data and creates an electronic audit trail.

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. A copy of the CRF will remain at the Investigator's site at the completion of the study.

16.2 Data Management Procedures

The data will be entered into a validated database. The Biostatistics and Clinical Data Management group will be responsible for data processing, in accordance with agreed procedures. Database lock will occur once quality assurance procedures have been completed.

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. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented in an audit trail.

16.4 Security and Archival of Data

The EDC system is hosted by Medidata; the data are stored at Medidata's primary data center in Houston, Texas, with fail-safe data centers in New Jersey. Data are regularly backed up by Medidata and stored with Iron Mountain.

Medidata maintains 21 CFR Part 11-compliant electronic systems, with procedures in place to safeguard against unauthorized acquisition of data. Any authorized communication with the

Medidata servers at the Houston Data Center is conducted via SSL (128-bit) encryption. Robust password procedures, consistent with 21 Part 11, are in place. Robust physical security procedures are in place at the Houston Data Center to prevent unauthorized personnel physical access to the server rooms. EDC account access is maintained and monitored by the Biostatistics and Clinical Data Management unit at the TDNCC

Other databases will be stored on Seattle Children's servers and are safeguarded against unauthorized access by established security procedures. Network accounts are password protected and maintained and monitored by the Seattle Children's Information Services group. Data is backed up regularly according to the Information Technology group's procedures.

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, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent and HIPAA Authorization 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 (e.g., patient files, signed informed consent forms, copies of CRFs, Study Essential Documents) 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

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Part 312 and ICH Guidelines for GCP (E6) and to ensure investigator compliance to 21 CFR Parts 50, 56 and 312 and to GCP. 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 identify all study subjects on CRFs and other documentation submitted to the Sponsor. If specific consent is given, the subject's CFF patient registry number will also be collected. 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 evaluation forms, reports and other records will be identified by a coded number and initials only. All laboratory specimens will be identified by a coded number.

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. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to subjects. A protocol amendment intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards

The protocol and consent form will be reviewed and approved by the IRB of each participating center prior to study initiation. SAEs regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB. The Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning subject recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's 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's unconditional approval statement will be transmitted by the Investigator to the Sponsor 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 approval except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB 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 subjects or 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 and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB-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 (or legal representative) prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

During the course of the study, if modifications are made to the consent form that impact the subject, the subject will be re-consented as described above.

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.

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18 APPENDIX 1. SCHEDULE OF EVENTS

Combined Screening/Baseline Schedule of Events

Task/Procedure	Visit 1[a] Day 1		Phone Call Day 3	Visit 2 Day 6[b]	Visit 3 Day 14 (+/- 2 days)	Visit 4 Day 28 (+/- 3 days)	Visit 5 Day 56 (+/- 4 days) (FINAL VISIT) [c]	Early Discontinuation of Study Drug [d]
	Screening/ Pre-Dose Baseline	Post-Dose			Follow-up			
Informed Consent	X							
CFRSD-CRISS Administration [e]	X			X	X	X	X	X
Medical History and Demographics	X							
Adverse Events Review		X	X	X	X	X	X	X
Concomitant Medication Review	X			X	X	X	X	X
Height	X							
Weight	X			X	X	X	X	X
Complete Physical Exam	X					X	X	
Abbreviated Physical Exam				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
Serum chemistry and Hematology Performed at Site Lab	X			X	X	X		X
Blood Collection for Gallium and Iron Levels	X[f]	X[f]		X[g]	X	X	X	X[h]

Task/Procedure	Visit 1[a] Day 1		Phone Call Day 3	Visit 2 Day 6[b]	Visit 3 Day 14 (+/- 2 days)	Visit 4 Day 28 (+/- 3 days)	Visit 5 Day 56 (+/- 4 days) (FINAL VISIT) [c]	Early Discontinuation of Study Drug [d]
	Screening/ Pre-Dose Baseline	Post-Dose			Follow-up			
Blood Collection for Cytokines and Chemokines	X					X	X	
Pregnancy Test (urine or serum)	X					X	X	
Urine Collection for Urinalysis Performed at Site Lab	X			X	X	X		X
Collect Expectorated Sputum for Microbiology	X[i]					X	X	
Collect Expectorated Sputum for Gallium and Iron Levels		X[f]		X[g]	X	X	X	X[h]
Randomize subject	X							
Obtain venous access [j]	X							
Study drug administration [k]		START		STOP				STOP[l]
Six-Hour Observation		X						
Dispense Subject Diary [m]		X		X				X
Review Subject Diary [M]				X	X			X
Review fluid intake and assess status of study drug infusion and pump use			X					
Remove Venous Access				X				X
Collect/Count Returned Study Drug Supplies				X				X

Separate Screening/Baseline Schedule of Events

Task/Procedure	Visit 1[a] (-1 to -6 days)	Visit 1 Day 1		Visits 2 – Visits 5 & Early Discontinuation of Study Drug
	Screening	Pre-Dose Baseline	Post-Dose	Refer to Combined Screening/Baseline Schedule of Events
Informed Consent	X			
CFRSD-CRISS Administration [e]		X		
Medical History and Demographics	X	X[n]		
Adverse Events Review			X	
Concomitant Medication Review	X	X		
Height	X			
Weight	X	X		
Complete Physical Exam	X			
Abbreviated Physical Exam		X		
Vital Signs	X	X		
Oximetry	X	X		
Spirometry	X	X		
Serum Chemistry and Hematology Performed at Site Lab	X			
Blood Collection for Gallium and Iron Levels		X[f]	X[f]	
Blood Collection for Cytokines and Chemokines		X		
Pregnancy Test (urine or serum)	X	X		
Urine Collection for Urinalysis at Performed Site Lab	X			

Task/Procedure	Visit 1[a] (-1 to -6 days)	Visit 1 Day 1		Visits 2 – Visits 5 & Early Discontinuation of Study Drug
	Screening	Pre-Dose Baseline	Post-Dose	Refer to Combined Screening/Baseline Schedule of Events
Collect Expectorated Sputum for Microbiology		X[g]		
Collect Expectorated Sputum for PK			X[g]	
Randomize subject		X		
Obtain Venous Access		X		
Study drug administration [K]			START	
Six-Hour Observation			X	
Dispense Subject Diary [m]			X	

[a] Screening may occur on the morning of Day 1 prior to start of study drug. In this case, Screening, Baseline and Start of Treatment (Day 1) occur on the same day. Alternatively, Screening and Baseline/Day 1 may be separated into two visits as long as Screening takes place within 7 days of Baseline/Day 1**.**

[b] Visit 2 should be scheduled at approximately the same time as Visit 1 as the study drug should be stopped at approximately the same time of day as it was started.

[c] Subjects who withdraw from the study should be encouraged to come in for final visit procedures as described in section 12.2.

[d] Subjects who discontinue the study drug early, should be encouraged to come in for the Early Discontinuation of Study Drug Visit (Section 10.6) and then proceed with all the remaining study visits. At Visit 2, perform all study procedures except for stopping the study drug administration, dispensing the subject diary, and collecting/counting returned study drug supplies (as these procedures will be performed as part of the Early Discontinuation of Study Drug Visit).

[e] The CFRSD-CRISS diary will be completed daily from Day 1 through Day 6 and then again at Visits 3, 4, and 5.

[f] Day 1 blood for gallium and iron levels: pre-dose, and 1 hour (+/- 10 minutes) after initiating study drug infusion and Day 1 expectorated sputum for gallium and iron levels: 1 hour (+/- 10 minutes) after initiating study drug infusion.

[g] Day 6 blood and sputum for gallium levels: prior to infusion stop (+/- 10 minutes)

[h] Early discontinuation of study drug for gallium and iron levels: If infusion stopped at visit, collect within 10 minutes of the stop of the infusion. If infusion stopped prior to the visit, note the collection time.

[i] Sputum for microbiology should be collected on Day 1 prior to the start of study drug. If Screening is done prior to Day 1, do not collect sputum for microbiology at Screening.

[j] If existing long-term vascular access (e.g., a subcutaneous venous Port) is not available, place a midline catheter, peripherally inserted central catheter (PICC), or peripheral IV.

[k] Study drug is initiated on Day 1 in clinic and will continue into Day 6 (for 5 full days of administration) in clinic or outpatient. Whether inpatient, outpatient, or a combination of both, the infusion should continue for 5 days uninterrupted except for brief interruptions. The specifics of administration (pump type, bag vs. syringe, inpatient vs. outpatient) can be determined by each site according to institutional policy.

[l] If infusion was not stopped prior to the visit.

[m] A Subject Diary will be maintained from Day 1 to 8. Infusion start and stop times will be documented from Day 1 to Day 6. Fluid intake will be documented from Day 1 through Day 8.

[n] Medical History review is performed at the pre-dose baseline portion of Visit 1.