Protocol

Title: IV Colistin for Pulmonary Exacerbations: Improving Safety and Efficacy

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PROTOCOL SYNOPSIS

TITLE	IV Colistin for Pulmonary Exacerbations: Improving Safety and Efficacy
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FUNDING ORGANIZATION	Cystic Fibrosis Foundation Therapeutics, Inc.
NUMBER OF SITES	1
RATIONALE	When patients with cystic fibrosis (CF) develop acute pulmonary exacerbations (APE) secondary to *Pseudomonas aeruginosa* airway infection, their treatment regimens frequently include IV tobramycin or IV colistin. When we reviewed approximately 500 CF subjects receiving IV tobramycin or IV colistin when being treated for APE, patients receiving tobramycin had significantly more episodes of acute kidney injury. We propose to study conventional administration of tobramycin and colistin prospectively, in addition to a novel colistin dosing schedule which is based on serum levels of the drug, to assess how regimens compare for efficacy and safety.
STUDY DESIGN	This is a prospective randomized single center interventional trial enrolling adult CF subjects who are diagnosed with APEs by their treating physician and who, according to their treating physician, will receive either tobramycin or colistin for the treatment of *Pseudomonas aeruginosa* or other appropriate gram-negative rods, such as *Achromobacter xylosoxidans*. The study is designed to evaluate a) whether pharmacokinetically adjusted colistin dosing, given on a twice daily schedule (**BID**), provides a more favorable efficacy and safety profile versus standard colistin three times daily (**TID**) dosing, with no serum levels analyzed, and b) whether pharmacokinetic-adjusted twice daily colistin dosing compares favorably to standard once daily tobramycin dosing, based on efficacy and safety, during treatment of APE. The three treatment arms are as follows: standard colistin TID dosing (based on product insert and

	evidence based recommendations for CF[1]) b) pharmacokinetic-adjusted BID colistin dosing or c) standard tobramycin dosing once daily[2].
PRIMARY OBJECTIVE	1. To evaluate the efficacy (based on FEV_1 improvement) and safety (based on development of AKI) of standard colistin TID dosing [1] versus PK-adjusted BID colistin dosing in adult CF subjects being treated for APE. 2. To evaluate the efficacy and safety of PK-adjusted BID colistin dosing versus standard tobramycin once daily dosing (8-10 mg/kg once daily[2]) in adult CF subjects being treated for APE.
SECONDARY OBJECTIVE	1. To evaluate for time to achievement of steady state with PK-adjusted colistin therapy. 2. To evaluate longitudinal differences in exacerbation rates and antimicrobial resistance between tobramycin and colistin use. 3. To evaluate differences in neurotoxicity and ototoxicity related side effects between study arms. 4. To evaluate the pharmacokinetics of colistin's active metabolites in a broad CF population. 5. To evaluate plasma pharmacokinetics of colistin's active metabolites with levels achieved in the sputum, in order to calculate epithelial lining fluid concentrations. 6. To evaluate for novel markers of nephrotoxic AKI, prior to serum creatinine increases, based on urine protein:creatinine ratios and the urine biomarker Nephrocheck® point-of-care assay.
NUMBER OF SUBJECTS	90
SUBJECT SELECTION CRITERIA: INCLUSION CRITERIA	CF patients 18 years or over who are diagnosed with APE based on CFF guidelines, and are chronically infected with *P. aeruginosa*, or other gram-negative bacteria, can produce sputum, undergo phlebotomy and provide written consent, will be eligible for participation, once their treating physician has determined that they should receive one of the two agents for APE treatment while hospitalized. Subjects able to receive either tobramycin or colistin as part of their IV antibiotic regimen will be randomized to one of three arms. If a treating physician deems that a subject cannot receive tobramycin due to vestibular or ototoxicity or bacterial resistance, the subject will be randomized to either standard or PK-adjusted colistin.

SUBJECT SELECTION CRITERIA: EXCLUSION CRITERIA	Presence of chronic renal insufficiency, with abnormal baseline creatinine >1.2.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Dosing of IV colistin will be determined based on serum concentrations of the drug on a BID dosing schedule.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Dosing of IV tobramycin once daily will occur based on consensus guidelines and will be adjusted based on serum levels. Dosing of IV colistin TID will occur based on product insert (Perrigo Company).
STANDARD OF CARE TREATMENT	All study arms will be receiving APE treatment as a multi-drug therapeutic regimen, based upon CFF guidelines.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for up to14 days, or for the duration of their IV antibiotic treatment for APE while hospitalized. The study will enroll for 36 months.
CONCOMITANT MEDICATIONS	Allowed: Standard therapy for CF pulmonary exacerbations. Prohibited: none
PRIMARY ENDPOINT	1. Absolute change in FEV_1 % predicted with standard colistin TID dosing versus PK-adjusted colistin BID dosing, with APE treatment. 2. Absolute change in FEV_1% predicted with standard tobramycin daily dosing versus PK-adjusted colistin BID dosing, with APE treatment. 3. Absolute change in FEV_1% predicted with standard tobramycin daily dosing versus standard colistin TID dosing. 4. Safety of standard colistin TID dosing versus PK-adjusted colistin BID dosing in APE treatment, based on assessments of clinical symptoms, AKI occurrences during treatment (based on KDIGO criteria), change in urine protein:creatinine ratios, and an FDA approved renal injury biomarker test, Nephrocheck®, which measures: TIMP2 and IGFBP-7 (Astute Medical). 4. Safety of standard tobramycin daily dosing versus PK-adjusted colistin BID dosing and standard colistin TID dosing in APE treatment, based on assessments described in #3.

SECONDARY ENDPOINTS	1. Time to therapeutic dosing and number of therapy interruptions due to changes in creatinine with standard colistin, modified colistin, and standard tobramycin dosing. 2. Total number of IV doses delivered over the course of APE treatment. 3. Change in sputum microbiology profile following treatment with colistin versus tobramycin. 4. Number of exacerbations in the ensuing year and time to first pulmonary exacerbation following APE treatment. 5. Absolute change in Cystic Fibrosis Questionnaire-Revised (CFQ-R) respiratory domain scorebefore and after APE treatment. 6. Number of episodes of neurotoxicity and ototoxicity in study arms, as noted on case-report forms. 7. Colistin peak, mid-point and trough during APE, for calculations of area under the curve (AUC).
PLANNED INTERIM ANALYSIS	When approximately 1/3rd of patients have completed the study, an interim analysis for safety and outcome measures will be conducted. Serious adverse events will be monitored by the Medical Monitor on an ongoing basis throughout the study.
STATISTICS Primary analysis plan	Statistical Methods: A detailed statistical analysis report will be written that will describe all analyses, tables, figures, and data listings generated for this study. All analyses will be performed using SAS ® (SAS Institute, Cary, NC, USA). **Analysis Populations**: All analyses will be performed using an intent-to-treat (ITT) population, which is defined as all randomized subjects. Subjects who are discontinued from the study temporarily or permanently will remain in the analyses population according to ITT. **Demographic and Baseline Characteristics**: Summary statistics will be calculated for demographic and baseline characteristics (age, sex, genotype, diabetic status, FEV_1 % predicted) time to therapeutic dosing for colistin versus tobramycin, number of therapy interruptions between study arms, and total number of IV antibiotic doses. Study medications and exposure to study drug data will be summarized in contingency tables. Summary statistics and changes from

	admission visit to end of treatment will be presented for FEV_1 % predicted and questionnaires, on an intent-to-treat basis, including all subjects receiving at least three days of colistin or tobramycin, and at least one efficacy assessment. **Analysis of Primary Endpoints**: Efficacy analysis: We will perform equivalence testing to show similarity of colistin treatments, as well as for colistin vs tobramycin treatments. For both comparisons, confidence intervals for difference in FEV_1 changes from pre to post treatment time points will be constructed, and equivalence tests to within 5% will be performed. Times to next pulmonary exacerbation and occurrence of neurotoxicity between standard colistin and PK-adjusted colistin will be analyzed using the Kaplan-Meier method. Subsequent pulmonary exacerbations in the ensuing year for all enrolled subjects, as well as antimicrobial resistance to tobramycin and colistin before and after treatment, will be summarized. Unless otherwise noted, 2-sided tests will be used with $\alpha = 0.05$. Safety analysis: The safety profile of each colistin arm and the tobramycin arm will be assessed in terms of the following: incidence of AKI, incidence of treatment related clinical events (ototoxicity, vestibular toxicity, and neurotoxicity). Adverse event rates will be compared between treatment groups using logistic regression, adjusting for age, medications such as vancomycin, baseline FEV_1, admits in the previous year, exposure to nephrotoxic antibiotics in the previous 5 years, and diagnosis of CF related diabetes (CFRD) as covariates. Repeated measures within subjects will be accounted for by including a random intercept for subjects. Summary statistics and changes from admission will be presented for serum Cr and urine:protein ratios. The urine protein:creatinine ratio will be assessed as a biomarker of drug toxicity. We will analyze Nephrocheck® assay results in a case control design. Subjects who develop AKI within each of the study arms will have urine thawed and analyzed by Nephrocheck® and those results will be compared to age, gender, and FEV_1 matched control urines obtained from subjects

	within the study who did not develop AKI. An adjusted odds ratio will be derived from a model fitted with unconditional logistic regression and adjusted for confounding variables. Estimates of odds ratios and 99% confidence intervals will be presented.
RATIONALE FOR NUMBER OF SUBJECTS	In a retrospective cohort 500 subjects, FEV1% improvement after tobramycin treatment during exacerbation therapy was 3% higher than in those receiving colistin; however, rates of acute kidney injury (rise in serum creatinine $\geq$ 0.3 mg/dL while on therapy) were 60% higher in tobramycin treated subjects versus standard colistin treated subjects. Since no preliminary data was available on less frequent colistin dosing, which theoretically would lead to less frequent episodes of kidney injury, we estimate we are powered to identify a safer method for dosing colistin with samples of 30 subjects per group, though we may be underpowered to find a significant FEV_1 difference between groups.

Title: IV Colistin for Pulmonary Exacerbations: Improving Safety and Efficacy

1. Background

As the median projected survival of the CF population increases, a growing challenge for providers will be the strategic management of the comorbidities associated with chronic illness. An aging CF population chronically infected with *Pseudomonas aeruginosa* (PA) faces increased risk of diminishing *in vivo* clinical response to tobramycin, following years of aminoglycoside therapy for acute pulmonary exacerbations (APE). Results of CF antibiotic utilization surveys among accredited care centers have demonstrated the vital importance of tobramycin in pediatric APE treatment (98.5% of centers)[3]. Nearly ubiquitous use of tobramycin during childhood, together with the anticipated need for further antimicrobial treatment over 4 to 5 decades of life, necessitates the need to identify alternative strategies which are equally as potent, as tobramycin resistance and toxicities develop. Not surprisingly, CF sputum microbiology trends in the US have shown significant increases in aminoglycoside resistance (30.4% in 2008 versus 11.8% in 1995)[4]. While it is important to identify potent regimens against PA and other resistant gram-negative rods, the long-term use of nephrotoxic intravenous antibiotics increases the population's risk of renal disease[5]. Strategies to reduce toxicities from treatment, such as the establishment of optimal dosing intervals for tobramycin in the TOPIC trial[6], have been an important advance in decreasing APE treatment toxicities.

Colistimethate sodium or colistin, a member of the polymyxin family of antibiotics, is a powerful treatment option for APE, particularly with multi-drug resistant PA. While it has excellent antimicrobial activity against PA[5], there has classically been a reluctance to use the drug due to its reported nephro- and neurotoxicity. More recent studies in ICU and CF populations suggest that nephrotoxicity secondary to colistin is less common than previously thought and is reversible. Studies comparing nephrotoxicity of intravenous colistin and aminoglycosides in CF actually demonstrate reduced toxicity with colistin[3,6]. A major limitation to its use, however, is the lack of human *in vivo* data regarding its pharmacokinetics (PK) and pharmacodynamics (PD)[7]. There is no widespread consensus on best dosing intervals to optimize pathogen eradication and reduce renal toxicity; partly because the drug never underwent PK modeling studies prior to its FDA approval in the 1950s. Today, though colistin is frequently substituted for tobramycin during exacerbations in patients who have developed resistance to tobramycin, it is unclear how to dose the regimen for both safety and maximal efficacy—*i.e.* once daily versus three times daily, similar to questions posed with tobramycin prior to the TOPIC trial.

Currently, acute kidney injury (AKI), formerly known as acute renal failure, in CF is far greater than the background rate in the general population. In pediatric CF patients, AKI occurs at 100 times the general rate of AKI in children, and is primarily related to nephrotoxic antibiotic use[8]. In adults with chronic *Pseudomonas* airway infection, indices of renal impairment have been detected in 31-42% of the population[5]. Thus, the challenge remains to devise antimicrobial treatment strategies which are powerful enough to minimize antimicrobial resistance but also avoid longitudinal harm.

One limitation to improving safety of antimicrobial drug monitoring has been the lack of availability of clinical assays to measure levels of colistin from the serum. Highly accurate analytical techniques, now available through the University of Colorado School of Pharmacy, now make pharmacokinetic (PK) and pharmacodynamic (PD) studies of colistin possible in order to establish optimal dosing regimens for colistin. This technology

will allow optimization of colistin dosing in order to maximize efficacy and reduce toxicity associated with this agent.

1.1 Overview of clinical studies

Pulmonary exacerbation guidelines and outcomes. Acute pulmonary exacerbations are a sentinel event in CF lung disease progression, increasing mortality, reducing lung function and quality of life[9,10], as well as a major driver of health care costs[11]. Despite aggressive treatment, 25% of exacerbations result in a loss of lung function, for multiple reasons including pathogen virulence, host response, social, and demographic factors[9,10]. Although CFF guideline-driven, evidence-based treatment strategies for chronic bacterial pulmonary infection have been a great success in CF outpatient care, substantially less progress has been made in improving APE outcomes. CF APE treatment guidelines lack high quality data to make specific recommendations for critical aspects of care, such as choice, number, and duration of antibiotics and dosing strategies. There are no Grade A recommendations, and for a majority of questions, there is insufficient evidence to make any recommendations at all (Figure 1).

TABLE 2. EVALUATION OF THE EVIDENCE

Question	Studies	N	Certainty	Magnitude of Benefit	Grade of Recommendation	Recommendation
Site of treatment*	1 RCT(7)	17	Low		I	Insufficient evidence that hospital and home treatment are equivalent
Chronic therapies	**	**	Moderate	Moderate	B	Continue current practices
Simultaneous use of inhaled and IV antibiotics	0	0	Low		I	Insufficient evidence to recommend for or against simultaneous use
Airway clearance therapies	**	**	Moderate	Moderate	B	Continue current practices
Number of antibiotics to treat *Pseudomonas**	17 RCT(25–41) 1 RXO(42) 1 QRT(43)	768	Low		I	Insufficient evidence that a single antibiotic is equivalent to a combination antibiotics
Aminoglycoside dosing*	4 RCT(29, 51–53) 1 RXO(54)	349	Moderate	Small	C	Once-daily dosing is acceptable for treatment of *Pseudomonas*
Continuous infusion beta-lactam antibiotics	1 XO(56)	5	Low		I	Insufficient evidence to recommend continuous infusion
Duration of antibiotics*	0	0	Low		I	Insufficient evidence to define optimal duration of antibiotics
Synergy testing (routine)	1 RCT(24)	132	Low	Zero	D	Routine use not recommended
Systemic steroids	2 RCT(63, 64)	44	Low		I	Insufficient evidence to recommend use of corticosteroids

Definition of abbreviations: N = number of patients evaluated; RCT = randomized controlled trial; RXO = randomized crossover trial; QRT = quasi-randomized trial; XO = crossover trial.
* Cochrane Review exists on this topic.
** Previous recommendations (10, 11).

Figure 1: Cystic Fibrosis Pulmonary Guidelines, Treatment of Pulmonary Exacerbations. Specific treatment recommendations and level of evidence, from AJRCCM (180), pp 802-808, 2009

Rationale for studies of APE treatment strategies: Recent findings have demonstrated that many centers are not following recommended dosing strategies, despite published recommendations[3]. Execution of well-designed PK studies during APE and broad distribution of their findings will be critically important for future CF care, which promises to require a coordinated approach to complex longitudinal management of resistant infectious diseases[4]. It will not be an uncommon scenario that future APE treatment will require coordination with a patient's chronic antimicrobial agents, such as regimens for non-tuberculous mycobacteria, which also have incipient toxicities.

Colistin as an agent to treat *Pseudomonas aeruginosa* and gram negative infections: Though it has been available for clinical use for over 50 years, colistin, also known as polymyxin E, is seeing a reemergence in its use, based on bacterial resistance to other antibiotics and a decline in discovery of novel antibiotic classes in the last 20 years. Colistin is administered parenterally as colistin methanesulfonate

(CMS), an inactive prodrug with less nephrotoxicity than colistin[7,12]. Given its longevity, colistin was never subjected to modern drug development procedures, including pharmacokinetic (PK) and pharmacodynamic (PD) evaluation to guide dosage selection and minimize resistance. Colistin dosing, as recommended by the FDA, is not based on modeling studies, increasing the risk of treatment failures and antimicrobial resistance in what is potentially a "last-line" drug. The drug's antimicrobial activity occurs at the cell membrane of gram-negative species[13], where they bind the lipid A component of LPS. Since the drug is amphipathic, with both lipophilic and hydrophilic groups, it is taken up at the outer membrane and mediates fusion of the inner leaflets of the outer membrane and the outer leaflet of the cytoplasmic membrane, in effect permeabilizing the bacteria[12,14]. It demonstrates concentration dependent killing (AUC:MIC) based on its disruption of bacterial cell membranes.

Data on the use of colistin in CF treatment is relatively limited. There have been studies demonstrating efficacy and tolerability of colistin in CF patients[15-18], however PK/PD studies are few[15,19,20]. Reed *et al* reported no relationship between the occurrence of colistin-associated adverse effects and plasma concentrations, however utilized older methods which were not as adept at separating active drug from pro-drug to quantify drug levels[15]. A recent "State of the Art" review on use of colistin in CF APE reinforces the need for further studies of colistin PK and clearance rates in order to achieve better dosing strategies[1]. In the last 4 years, advances in mass spectrometry, with robust validations, have vastly improved the analysis of colistin concentrations in plasma, using minimal volumes of 100µl. Rapid sample preparation methods make it possible to analyze samples without getting falsely high colistin concentrations from hydrolysis of colistin pro-drug. The inter-day variability of interspersed samples is low, demonstrating that the method is reliable and robust [21]. Improvements in measurements have brought previous studies into question, due to methodological issues such as large sample volume requirements, tedious sample pretreatment, long run times and questionable extract cleanliness[22]. Terminology: Colistimethate sodium (CMS) is the pro-drug of colistin and the full name of the agent available for parenteral usage in the US. It is hydrolyzed to colistin (A and B components) *in vivo*, which is the active moiety of the drug. We will refer to its active component or colistin throughout the rest of the proposal.

Current trends in colistin use in CF clinical practice: Dosing is expressed in terms of colistin base, or active drug[23]. Thus, as the manufacturer recommends a peak dose of 480 mg daily for pro-drug and since roughly 1/3rd of pro-drug is estimated to be converted to active drug, most Centers aim for approximately a 160 mg maximum daily dose, in 2-4 divided doses, or a maximum dosing of 5 mg/kg/day, based on product insert[24]. An informal survey of colistin dosing at a sampling of large US CF Centers reveals that most centers are utilizing three times daily dosing, based on a pharmacy Listserv query. The most commonly employed dosing range was 60 to 70 mg IV every 8 hours. A recent evidence-based dosing summary of colistin administration in pediatric and adult CF patients supports every 8 hour dosing, based on 8 publications[1]. As these recommendations were not supported by modeling studies, there is no understanding of how much prodrug is being converted to active colistin and the drug accumulation which is occurring between doses.

Successful assay development for quantification of serum colistin levels. Previous studies have demonstrated the accuracy of liquid chromatography-tandem mass spectrometry (LC/MS-MS) technology to measure pharmacologically active colistin A and B from serum[21,22]. Drs. Ty Kiser, PharmD and Michael Wempe, PhD, (Medicinal Chemistry Core), at the University of Colorado Skaggs School of Pharmacy, have developed an LC/MS-MS assay to quantify levels of colistin prodrug (CMS) and active colistin products (**Figure 2**) and have recently performed a pilot study quantifying concentrations in a cohort of CF adults in the first week of treatment for APE. The LC-MS/MS assay measures colistin in human serum and sputum by using polymyxin B1 as the internal standard, with a 24 hour turnaround time. This capability is significant, because very few assays for CMS and colistin A and B are available in the United States.

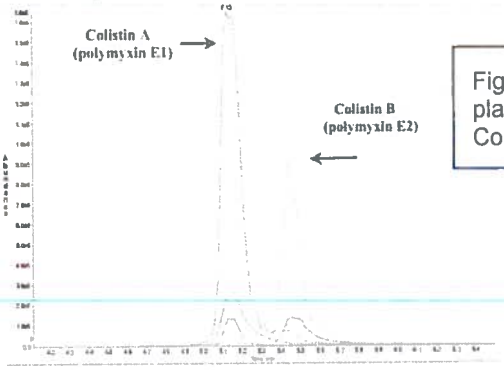

Figure 2: Representative chromatogram of active colistin products in plasma as measured by LC-MS/MS assay in the Medicinal Chemistry Core, University of Colorado School of Pharmacy

Pilot study of PK parameters in an adult CF cohort with APE: We assayed steady-state pharmacokinetic and pharmacodynamic properties of intravenous colistin in CF subjects >18 years old admitted for APE treatment, during the first week of admission. The primary objective of the study was to determine PK parameters, specifically clearance (CL), volume of distribution (VD), area under the curve (AUC), and maximal concentrations (Cmax). A total of 16 CF subjects were evaluated. All subjects received IV colistin on a standard dosing schedule, in conjunction with other anti-Pseudomonal antibiotic agents, dosed three times daily. Patient characteristics are depicted in **Table 1**. Steady state plasma concentrations were obtained after ≥ 2 days of therapy during week 1 of admission, and subjects underwent phlebotomy at 0, 0.5, 1, 2, 4, and 6 hours after administration. Peak, midpoint, and trough colistin A and B concentrations (active drug) were measured utilizing liquid chromatography-mass spectrometry (LC-MS) technology[21,22]. As seen in Table 1, the mean study age of 32 is older than mean ages in prior studies of IV colistin in CF subjects[19,20]. Mean FEV_1 % predicted was 40% predicted at onset of exacerbation, demonstrating that the drug can be given safely with severe airflow limitation. There were no reported side effects of chest tightness that would limit its use. Two subjects experienced SCr rises of ≥0.3 mg/dL, necessitating discontinuation of drug. Mean colistin dosing was 3.42 mg/kg/d over 15 days. A PK analysis of

Table 1: Characteristics of colistin PK pilot study subjects

No. of subjects	16
Gender- no. (% female)	8 (50)
Age (mean ± SD years)	32 ± 13
Sputum culture	
Pseudomonas aeruginosa only	8 (50)
Pseudomonas aeruginosa, Staphylococcus aureus, and/or other GNR	8 (50)
Concomitant systemic antibiotic therapy- no. (%)	
β-lactam, carbapenem, monobactam, or quinolone	16 (100)
vancomycin or linezolid	5 (31)
FEV_1 (mean +/- SD % predicted)	40 ± 15
Mean dose colistin- mg/kg/d (mean ± SD)	3.42 ± 0.6
Mean duration of therapy-days (mean ± SD)	15 ± 9
Baseline SCr- mg/dL (mean ± SD)	.77 ± .27
Number with AKI (% with > 0.3 mg/dL increase in SCr)	2 (13)

SCr- serum creatinine
AKI- acute kidney injury

steady state serum samples was performed, in addition to PD modeling of the area under the curve to minimum inhibitory concentration (AUC_{0-24}:MIC) of the bacteria. The AUC: MIC is a better predictor of successful outcomes in *P. aeruginosa* treatment, with optimal efficacy occurring at ratios >60[25,26].

Colistin PK/PD analysis of standard colistin dosing. Active colistin components were combined for PK/PD analysis. The mean ± SD colistin Cmax, Cmin, half-life, VD, CL, and AUC_{0-8} were 1.1 ± 0.7 mg/L, 0.5 ± 0.5 mg/L, 5.7 ± 2.0 hours, 226 ± 220 L, 30.5 ± 32.6 L/hr and 3.8 ± 3.1 mg*hr/L, respectively. Monte Carlo simulations were conducted to determine the dosing models required to achieve AUC/MIC ratios between 48-60, as these cut points have demonstrated 1-2 log kill in murine models of thigh and pulmonary infections (**Table 2**)[25,26]. MIC values were obtained from historical Colorado Adult Program CF patients' microbiology culture data. Seventy percent of isolates had MICs ranging from 0.12- 1.0. Different dosing strategies were employed until >80% and >90% target attainment was achieved at an AUC/MIC ratio >60. Total daily colistin dosages required to achieve pharmacodynamics targets are illustrated in Table 2. Based on MIC distributions, our PD model suggests that the average daily dosing achieved in the pilot study (3.4 mg/kg/day) is effective at achieving PD targets in approximately 25% of isolates in the first week of therapy. Higher daily dosages of colistin are required to achieve therapeutic targets. We estimate that doses of 5 mg/kg/day are more optimal and within the realm of achievable dosing without increasing toxicity.

Table 2: Probability of achieving pharmacodynamics targets (AUC/MIC > 60) with varying colistin dosing strategies in patients with normal renal function.

MIC	0.06	0.12	0.25	0.5	1
6.85 mg/kg/d	100.0	85.3	38.8	15.0	4.7
5 mg/kg/d	100.0	64.9	23.4	8.5	1.3
3.42 mg/kg/d	100.0	41.8	12.9	4.7	0.0
3 mg/kg/d	77.8	33.4	11.6	1.7	0.0

(data are presented as percentages)

As an additional consideration, a concentration curve (**Figure 3**) demonstrates that many subjects had detectable colistin prodrug in the plasma at the end of the dosing interval, indicating significant accumulation and potentially increasing toxicity to patients. Thus, higher dosing at less frequent intervals appears to be a reasonable approach to optimizing attainment.

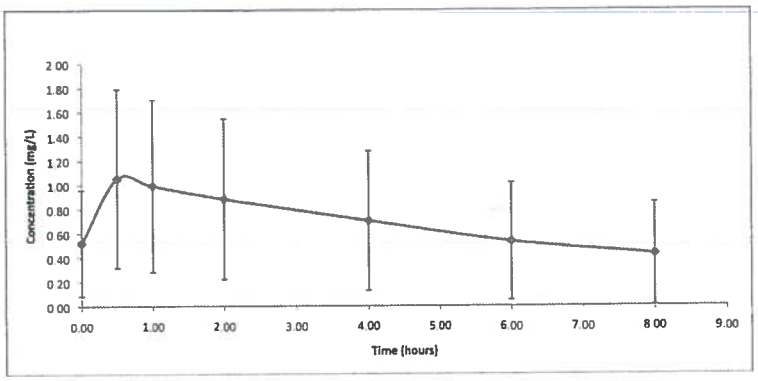

Figure 3: Colistin A&B concentrations plotted against time in week 1 of APE admission (n=16). Subjects had considerable pharmacokinetic variability and most had detectable prodrug both at end of dosing interval (8 hours).

Projections for an optimized dosing schedule. Based on our preliminary data evaluating standard dosing of colistin, we hypothesize that PK-adjusted dosing will lead to better attainment of PD targets. Our proposed loading and maintenance dosing in the PK-adjusted colistin study arm are as follows. During Week 1, we will target a 5 mg/kg/day total dose, divided twice daily, instead of 3 times daily. This will allow for higher peaks with less accumulation. Once levels are attained, dosing may be decreased in Week 2 of treatment, given that accumulation will continue to occur, leading to higher concentrations. Thus, maintenance dose for the second week, which will be PK-adjusted, will likely be lower. The proposed study will incorporate an ongoing evaluation of population PK data as patients are enrolled to continue modeling the dosing strategy which best achieves desired concentrations in most patients, per each week of treatment, thus simulating the best approach and applying it prospectively over the course of the trial.

Retrospective efficacy and safety of standard IV colistin dosing in a large CF inpatient population. We retrospectively analyzed IV antibiotic courses for treatment of CF APE amongst patients of the Denver Adult CF Program from 2012-2015. Greater than 240 courses of colistin were administered to 240 adult subjects, and >350 courses of tobramycin in 350 subjects. A dataset of admissions with complete data (n=465) was analyzed for FEV_1 improvement based on treatment with tobramycin or colistin and for the development of acute kidney injury (AKI) based on an absolute increase of serum creatinine by $\geq$ 0.3 mg/dl[27]. Subjects received on average a course of 14.6 days of colistin during APE treatment versus 12.7 days of tobramycin. The average ages of subjects receiving tobramycin and colistin were 31 and 32 years old respectively. The tobramycin group was 57% female, while the colistin group was 60% female. The colistin group consisted of 66% CFRD diagnosed patients, while the tobramycin group had 38% with CFRD. More admissions occurred for the colistin group (average of 2.3/year), versus the tobramycin group, which underwent an average of 1.8 admits per year. Pre-treatment FEV_1 % predicted ranged higher for the group receiving tobramycin (average of 50%), versus the colistin group (average of 40% predicted), reflecting a sicker population receiving intravenous colistin. Of interest, the degree of FEV_1 improvement before and after APE treatment was 12% predicted with tobramycin versus 9% predicted for colistin (p=0.006). However, the small increase in FEV_1 improvement came at a price, since the incidence of AKI was 19% for the tobramycin group and 12% for the colistin group, giving an OR of 3.6 for tobramycin (p=0.002). The analysis was adjusted for prior year admissions, reflecting the reality that CF subjects receive multiple IV antibiotic courses over sequential years. Since mounting evidence

demonstrates that AKI occurrences, defined as a creatinine rise by ≥ 0.3 mg/dL, are associated with increased morbidity, higher one year mortality, and chronic kidney disease, the fact that patients experience this endpoint not infrequently, and repeatedly, is meaningful[28,29].

Summary: Higher doses of colistin administered less frequently may better achieve an AUC:MIC which is optimal for concentration dependent killing of *P. aeruginosa*. PK- guided dosing of intravenous colistin on a twice daily basis may achieve endpoints of reduced antibiotic nephrotoxicity, based on less accumulation, and improved treatment outcomes, including shorter times to therapeutic dosing of colistin in the treatment of APE.

2. Study Rationale:

If our hypotheses are appropriately tested and proven correct, then current practice recommendations for thousands of patients with CF worldwide undergoing treatment for pulmonary exacerbations may be altered. Based on CF registry data, 40,000 patients in the US undergo IV antibiotic treatment yearly. The average number of exacerbations per year in the registry is one. Thus, if patients receive yearly or more frequent IV therapy each year over the course of a lifetime, this adds up to over 30 exposures to nephrotoxic agents prior to reaching age 40. Additional concerns extend to the current standard dosing of IV tobramycin, which appears to have relatively higher toxicity than colistin and is without doubt the mostly widely utilized agent in the treatment of exacerbations worldwide. Furthermore, this study will address how to best administer intravenous colistin. It will produce data now required by the FDA for the approval of any novel antibiotic agent, is conducted under exacerbation conditions in CF subjects, and will directly compare efficacy and safety to IV tobramycin in APE. Its execution will afford the CF community an understanding of toxicities as they relate to blood levels, as occurred with tobramycin a decade ago, utilizing robust and highly accurate technology for its measurements.

There is also a sizable financial impact given the cost of pulmonary exacerbations. The mean length of stay for CF patients hospitalized for pulmonary exacerbation is greater than 10 days, costing approximately $125,000 per event. Episodes of renal toxicity or failure to return to baseline FEV_1% extend hospital stays. Ultimately establishing better IV regimens may also improve quality of life years for these patients as FEV_1% is closely linked to both QOL symptoms and survival in CF.

2.1 Risk/Benefit Assessment: No experimental or new medications are being introduced to subjects in this study. No important drugs in the treatment of CF APE are being withdrawn or held. Subjects will be approached for enrollment in two scenarios: a) if their treating physician is in agreement that *either* a tobramycin-based or a colistin-based regimen will be efficacious to treat their APE or b) if their treating physicians feels that they can only receive colistin due to previous ototoxicity or vestibular toxicity from tobramycin. If a treating physician feels that a subject cannot receive colistin, they may be enrolled only to the tobramycin arm. Since all exacerbations are treated with a multi-drug regimen, the patient's treating physician will decide the other antimicrobial components of the regimen. The study procedures as described below are all current

standard clinical care as it is being delivered on the CF pulmonary floor and is supported by clinical guidelines. The product insert for colistin dosing gives an option for dosing anywhere between twice to four times daily dosing, thus the experimental PK-adjusted colistin dosing schedule is within current product use recommendations. We feel that measurement of serum levels will actually make the use of the drug safer and result in lower rates of kidney toxicity, which is clearly a priority when patients are treated with regimens on multiple occasions over a year's time. In addition, the completion of this study will give important insight into the toxicities of using standard dosing tobramycin in a large adult CF population and how toxicities associated with its repeated use may be better managed longitudinally. Finally, the Denver Adult CF inpatient service is extremely active. For the past 3 academic years, there were >300 admissions per year, on average, for intravenous antibiotic treatment of APE, with a 10-15% increase in admissions per year. Based on the last 4 years of data, our program's use of IV colistin for APE is increasing at a rate of 25% per year. Nationally, there is no clear consensus on use of colistin. Completion of this trial will create a widely standardized and prospectively studied protocol for safest treatment practices with this important drug.

3. STUDY OBJECTIVES

3.1 Primary Objectives

1. To evaluate the efficacy (based on FEV_1 improvement) and safety (based on development of AKI) of standard colistin TID dosing[24] versus PK-adjusted BID colistin dosing in adult CF subjects being treated for APE.
2. To evaluate the efficacy and safety of PK-adjusted BID colistin dosing versus standard tobramycin once daily dosing (8-10 mg/kg once daily) in adult CF subjects being treated for APE.
3. To evaluate the efficacy and safety of standard colistin TID dosing versus standard tobramycin once daily dosing in adult CF subjects being treated for APE.

3.2 Secondary Objectives

1. To evaluate for time to achievement of steady state with PK-adjusted colistin therapy.
2. To evaluate longitudinal differences in exacerbation rates and antimicrobial resistance between tobramycin and colistin use.
3. To evaluate differences in neurotoxicity and ototoxicity related side effects between study arms.
4. To evaluate the pharmacokinetics of colistin's active metabolites in a broad CF population.
5. To evaluate plasma pharmacokinetics of colistin's active metabolites with levels achieved in the sputum, in order to calculate epithelial lining fluid concentrations.
6. To evaluate for novel markers of nephrotoxic AKI, prior to serum creatinine increases, based on urine protein:creatinine ratios and the urine biomarker Nephrocheck® point-of-care assay.

4. STUDY DESIGN

4.1 Study Overview

This is a prospective randomized single center interventional trial enrolling adult CF subjects who are diagnosed with acute pulmonary exacerbations (APE) by their treating

physician and who, according to their treating physician, will receive either tobramycin or colistin for the treatment of *Pseudomonas aeruginosa* or other appropriate gram-negative rods, such as *Achromobacter xylosoxidans*. The study is designed to evaluate a) whether pharmacokinetically adjusted colistin dosing, given on a twice daily schedule, provides a more favorable efficacy and safety profile versus standard colistin three times daily dosing, with no serum levels analyzed, b) whether pharmacokinetic-adjusted twice daily colistin dosing compares favorably to standard once daily tobramycin dosing, based on efficacy and safety, and c) how standard colistin dosing compares to standard once daily tobramycin dosing, based on efficacy and safety, during treatment of APE. The three treatment arms are as follows: 1) standard colistin TID dosing (based on product insert and evidence based recommendations for CF[5]), 2) pharmacokinetic-adjusted twice daily colistin dosing, or 3) standard tobramycin dosing once daily. The premise of the study is that newer, highly accurate analytical techniques, available through the University of Colorado School of Pharmacy have allowed pharmacokinetic (PK) and pharmacodynamic (PD) studies of colistin, in a cohort of adult CF subjects receiving the agent during APE. This has allowed us a new understanding of how the drug is metabolized in CF and how we can optimize its administration.

1. Adult patients will be enrolled at the time therapy is initiated for the treatment of a pulmonary exacerbation of CF.
2. Subjects will be block randomized based on gender, FEV_1%, and time of enrollment, into one of three treatment arms.
3. Patients receiving standard colistin dosing will be treated according to product insert and evidence based recommendations for CF[2]. Patient demographics, medications, and results of standard laboratory tests will be recorded.
4. Patients receiving standard tobramycin dosing will be treated according to evidence based recommendations for CF[5]. Patient demographics, medications, and results of standard laboratory tests will be recorded. Dose adjustment will be made by the PI and treating physician and will occur based on CF treatment guidelines, with the assistance of the National Jewish Health | Saint Joseph Hospital inpatient pharmacists.
5. Patient receiving PK-adjusted colistin dosing will be treated with twice daily dosing. Patient demographics, medications, and results of standard laboratory tests will be recorded. Subjects in this group will undergo drug level monitoring on study visits 2 and 3. The assay will be performed at the Medicinal Chemistry Core Facility at the University of Colorado Skaggs School of Pharmacy and has a turn-around time of 36 hours. Dose adjustment will be made by the PI and treating physician and based on levels, according to recommendations from the University of Colorado School of Pharmacy faculty, in collaboration with the National Jewish Health | Saint Joseph Hospital inpatient pharmacists.

5. CRITERIA FOR EVALUATION

5.1 Primary Endpoints

1. Absolute change in FEV_1 % predicted with standard colistin TID dosing versus PK-adjusted colistin BID dosing, with APE treatment.
2. Absolute change in FEV_1% predicted with standard tobramycin daily dosing versus PK-adjusted colistin BID dosing, with APE treatment.

3. Absolute change in FEV1% predicted with standard tobramycin daily dosing versus standard colistin TID dosing, with APE treatment.
4. Safety of standard colistin TID dosing versus PK-adjusted colistin BID dosing in APE treatment, based on assessments of clinical symptoms, AKI occurrences during treatment (based on KDIGO criteria), change in urine protein:creatinine ratios, and an FDA approved renal injury biomarker test, Nephrocheck®, which measures: TIMP2 and IGFBP-7 (Astute Medical).
5. Safety of standard tobramycin daily dosing versus PK-adjusted colistin BID dosing or standard colistin TID dosing in APE treatment, based on assessments described in #4.

5.2 Secondary Endpoints

1. Time to therapeutic colistin dosing and number of therapy interruptions due to changes in creatinine with standard colistin, modified colistin, and standard tobramycin dosing.
2. Total number of IV doses delivered over the course of APE treatment.
3. Change in sputum microbiology profile following treatment with colistin versus tobramycin.
4. Number of exacerbations in the ensuing year and time to first pulmonary exacerbation following APE treatment.
5. Absolute change in Cystic Fibrosis Questionnaire-Revised (CFQ-R) respiratory domain score before and after APE treatment.
6. Number of episodes of neurotoxicity and ototoxicity in study arms, based on case-report form.
7. Colistin peak, mid-point, and trough during APE, for calculations of area under the curve (AUC).

6. SUBJECT SELECTION

6.1 Study population

Subjects with a diagnosis of CF who meet all the inclusion and none of the exclusion criteria will be eligible for participation in this study.

6.2 Inclusion criteria:

1. Male or female ≥ 18 years of age at Visit 1.
2. Documentation of CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype and one or more of the following criteria:
   - Sweat chloride equal or greater than 60 mEq/L by quantitative pilocarpine iontophoresis test.
   - Two well-characterized mutations in the CFTR gene
   - Abnormal nasal potential difference (change in NPD in response to a low chloride solution and isoproterenol of less than -5 mV).
3. Documentation of the presence of an acute pulmonary exacerbation, based on CF Foundation guidelines, as diagnosed by a faculty member of the Denver Adult CF Program.
4. Respiratory culture(s) demonstrating evidence of *Pseudomonas aeruginosa* or *Achromobacter species* airway infection.
5. Subject is able to produce sputum, undergo phlebotomy, and provide written consent.
6. The subject's treating physician has determined that they should receive either tobramycin or colistin intravenously as one of the designated agents for their APE

treatment. Subjects who are able to receive either tobramycin or colistin as part of their antibiotic regimen will be randomized into one of three arms. If a treating physician deems that a subject cannot receive tobramycin due to vestibular toxicity, ototoxicity or bacterial resistance, the subject will be randomized to either standard or PK-adjusted colistin.

6.3 Exclusion criteria:

1. Presence of chronic renal insufficiency, with abnormal baseline creatinine >1.2mg/dL.
2. Presence of a condition or abnormality that in the opinion of the investigator would compromise the safety of the patient or the quality of the data.
3. Inability to perform reproducible spirometry.
4. Inability to expectorate sputum.

6.4 Study specific tolerance for inclusion/exclusion criteria

Subjects who fail to meet one or more of the inclusion criteria or meet any of the exclusion criteria will not be enrolled in this study. Waivers of any of the above study entry criteria will not be granted.

7. CONCURRENT MEDICATIONS

All subjects who are treated for acute pulmonary exacerbations are treated with antibiotic combinations, utilizing a minimum of 2 agents. Intravenous tobramycin and intravenous colistin are never utilized simultaneously, due to their combined nephrotoxicity. Thus, the choice of accompanying antibiotics will be made by the subject's treating physician. Subjects will only be randomized to either tobramycin or colistin.

7.1 Allowed medications and treatments

Standard therapy for CF APE is allowed. Subjects will receive nebulized bronchodilators, mucolytics, airway clearance treatments, nutritional support, diabetes management, and pancreatic enzymes, based on current standard recommendations for CF exacerbation treatment.

8. STUDY TREATMENTS

8.1 Method of assigning subjects to treatment groups

All subjects will enter the study at the time they are diagnosed with an APE and designated for hospital admission to treat with a drug regimen which includes either intravenous tobramycin or colistin. Block randomization will be utilized based on gender, FEV_1% predicted, and time of enrollment to ensure relatively equal treatment allocation based on these variables.

8.2 Blinding

Subjects and investigators will not be blinded to the use of tobramycin versus colistin. Measurement of tobramycin pharmacokinetics and adjustment of doses appropriately is standard clinical practice and cannot be modified for the study.

8.3 Packaging and labeling of study drugs to site

Intravenous tobramycin (X-gen Pharmaceuticals) and intravenous colistin (Perrigo Company), which are administered to patients with CF APE, are purchased and formulated as solutions for intravenous administration by the Pharmacy Department at

National Jewish Health | Saint Joseph Hospital. There will be no change to the formulation or supplier of these products from current standard practice. The central study pharmacy is synonymous with National Jewish Health | Saint Joseph Hospital's inpatient pharmacy.

8.4 Handling and dosing regimens for study drugs.

8.4.1 Pre-treatment hydration: On the date of admission, all subjects will receive an initial normal saline (NS) 1 liter bolus. All subjects are initiated on the non-nephrotoxic component of their APE treatment on admission day one, as directed by their treating physician (beta lactam, carbapenem, monobactam, etc), based on infecting pathogen profile, previous treatment response, antimicrobial resistance, and drug allergies [30-32]. On the second hospital day, once a baseline serum creatinine (SCr) has been confirmed and the patients' attending physician and clinical pharmacist assess that they are ready to initiate treatment with either tobramycin or colistin, one of the three treatment arms will be implemented as directed by randomization. As per institutional protocol, all subjects receiving tobramycin or colistin receive 1 liter NS IV daily.

8.4.2 Specific drug treatment arms

Standard colistin arm: Subjects initially receive IV colistin 2.5 mg/kg/d divided into three times daily (TID) dosing (Perrigo Company) [1,15, 24]. Previous literature has supported upward dose titration to minimize the incidence of side effects associated with parenteral administration[15]. Subjects receiving colistin will undergo a 2 day up-titration of dose to an ultimate dose of 4-5 mg/kg/day, for a total treatment of 14 days[15]. The drug is infused over 30 minutes on a TID dosing schedule. There are no pharmacokinetic measurements to guide standard dosing; however, plasma, sputum, and urine will be collected and analyzed from the subjects in this treatment arm on the same schedule as the modified colistin arm to serve as study controls. Treatment is currently guided by evidence of neurotoxicity or nephrotoxicity, based on clinical exam and on serum and urine studies.

Modified colistin arm Subjects receiving IV colistin undergo a 2 day up-titration to a maximum dose of 5 mg/kg/day, divided into twice daily (BID) dosing, for a total treatment of 14 days. The drug is infused over 30 minutes BID. Steady state plasma concentrations on visit 2 (generally at the 2nd-3rd dose once at goal dosing) will be measured; specifically, colistin peak (30-60 minutes after infusion), midpoint (4-6 hours after infusion) and/or trough (30-0 minutes prior to next infusion). A steady state sputum sample will be procured at visit 2 to assess epithelial lining concentrations, unless subjects are on inhaled colistin as this will create inaccuracies in colistin sputum concentration measurements. Processing of sputum for colistin measurements will be performed by the standardized method for processing sputum developed by CF Therapeutic Development Network [33,34]. A baseline and steady state urine samples will also be collected. Levels of colistin A and B and pro-drug from plasma, sputum, and urine (following processing) will be quantified within 36 hours to allow for measurement and interpretation. Dr. Kiser will determine the optimal dose and intervals for APE subjects based on PK modeling with feedback from the PI, treating physician, and the National Jewish Health | Saint Joseph Hospital inpatient pharmacists. Colistin concentrations in plasma, sputum, and urine will be re-assessed at visit 3, after PK adjusted optimal dosing of colistin has reached a steady state.

Standard tobramycin arm: Subjects receive IV tobramycin 8-10 mg/kg/day (X-gen Pharmaceuticals) with once daily dosing for 14 days. Peaks and troughs are drawn with the second dose of tobramycin, and the drug is infused over 30 minutes. Dose adjustment will occur to achieve non-detectable troughs and peaks 20-30 mg/dL[2]. Tobramycin concentrations are assessed once weekly during each week of APE treatment.

8.4.3 Dose modification: Subjects active in the study who experience moderate to severe toxicity will undergo dose adjustments as follows. If clinical providers and site investigators believe that it is appropriate to have the subject continue in the study, this will be allowed. If study drug is discontinued, the data acquired prior to its discontinuation will be utilized in the analysis.

1. Tobramycin will be discontinued if ototoxicity occurs (patient complains of ringing in ears and/or hearing loss).
2. Tobramycin will be held with increases of creatinine >0.3 mg/dL. If creatinine increases are not reversible, the drug is discontinued. If they are reversible, the drug may be resumed at lower dosing or longer intervals, at the discretion of the physician and the clinical pharmacist in joint consultation.
3. Colistin will be discontinued for serious episodes of neurotoxicity, such as debilitating headache, seizure, and neuromuscular weakness.
4. Colistin will be held for one dose and then resumed at a lower dose if creatinine rises $\geq$ 0.3 mg/dL from baseline. If creatinine fails to recover or continues to rise, the drug will be discontinued. Patients who do not undergo PK measurements are maintained at the highest dose achievable (between 3-5 mg/kg/day) without renal insufficiency (< 0.3 mg/dl increase in serum creatinine).

8.4.4 Study drug preparation, handling and dispensing: Appropriately trained pharmacy personnel and nursing staff are responsible for the administration of medications. Medications are dispensed and labeled in accordance with federal and local state Board of Pharmacy regulations.

8.4.5 Administration instructions: Instruction of hours of administration, dose, and duration of administration will be transmitted from the National Jewish Health | Saint Joseph Hospital inpatient pharmacists to CF pulmonary floor nursing staff. Drugs will be administered through indwelling peripherally inserted central catheters (PICC) or indwelling ports.

8.4.6 Storage: All study drugs are stored in the National Jewish Health | Saint Joseph Hospital's inpatient pharmacy in a secure area under restricted access at controlled room temperatures, as recommended by the product manufacturer.

8.5 Study Drug Accountability: Subjects will be dispensed study drugs during the time of their inpatient admission and all infusions will be recorded by nursing staff into the electronic medical record. Additionally, the dose and duration of dosing for each subject will be recorded with each subject ID.

8.6 Measures of treatment compliance

All subjects are inpatients with all administered medications being recorded. Missed doses and total amount of doses administered will be recorded for the study. The study monitor will review this data throughout the course of the study.

8.7 Standard of care treatment with tobramycin

Subjects will receive intravenous tobramycin in accordance with standard procedures established through the Department of Pharmacy and Department of Nursing at National Jewish Health | Saint Joseph Hospital. It is administered through a peripheral IV, peripherally inserted central catheter (PICC line), or indwelling port.

8.7.1 Administration instructions: Intravenous tobramycin will be administered as per standard care[35] on a weight-based dosing regimen. The amount to be given to each patient who is prescribed tobramycin is based on standard treatment recommendations of 8-10 mg/kg/day as a one time dose daily. The precise dose is determined by the CF clinical pharmacist and treating physician, based on previous experiences with dosing of the drug and previous doses which achieved levels within treatment guidelines for that individual. Precise administration instructions are as follows:
Tobramycin (X-gen Pharmaceuticals) is reconstituted in a 1.2 gram vial with 30 cc of sterile water for injection with resultant solution of 40 mg/ml. The appropriate dose is drawn up in a syringe and injected into 100 ml normal saline piggy back. The medication is infused over 30 min. Tobramycin peaks and troughs are drawn with the second dose of tobramycin. Dose adjustment will occur to achieve non-detectable troughs, and peaks 20-30 mg/dL[2]. Tobramycin concentrations are assessed once weekly during each week of APE treatment.

8.8 Standard of care treatment with colistin

Subjects will receive intravenous colistin in accordance with standard procedures established through the Department of Pharmacy and Department of Nursing at National Jewish Health | Saint Joseph Hospital. It is administered through a peripheral IV, peripherally inserted central catheter (PICC line), or indwelling port.

8.8.1 Administration instructions: Intravenous colistin will be administered as per standard care [1,24] on a weight-based dosing regimen. The amount to be given to each patient who is prescribed colistin is based on standard treatment recommendations of 3-5 mg/kg/day, divided into 3 doses. The precise dose is determined by the CF clinical pharmacist and treating physician, based on maximal dosing within the reference range tolerated by the individual and without evidence of toxicities. Precise administration instructions are as follows:
Colistin powder in a 150 mg vial is reconstituted with 2 ml of sterile water for injection with resultant solution concentration of 75mg/ml. The desired dose is drawn up in an appropriate syringe and then injected into 50 cc normal saline piggy back. The medication is infuse over 30 minutes. There are no pharmacokinetic measurements to guide standard dosing; however, plasma, sputum, and urine will be collected and analyzed from the subjects in this treatment arm on the same schedule as the modified colistin arm to serve as study controls. Treatment is guided by evidence of toxicity (either

nephrotoxicity or neurotoxicity), based on clinical exam and on serum and urine studies.

8.9 Pharmacokinetic driven treatment with colistin.
Subjects will receive intravenous colistin in accordance with standard procedures established through the Department of Pharmacy and Department of Nursing at National Jewish Health | Saint Joseph Hospital. It is administered through a peripheral IV, peripherally inserted central catheter (PICC line), or indwelling port. The main difference between this study group and the standard colistin group will be the measurements of drug levels and adjustments to dosing based on results.

8.9.1 Administration instructions: Intravenous colistin will be administered as per standard care[1,24] on a weight-based dosing regimen; however, instead of dosing three times daily, dosing will be on a twice daily schedule. The total amount to be given to each patient who is prescribed colistin will still be standard treatment recommendations of 3-5 mg/kg/day, but divided into 2 doses. The precise dose is determined by the CF clinical pharmacist and treating physician, based on previous experiences with dosing of the drug and previous doses which achieved levels within treatment guidelines for that individual, if that information is available. Precise administration instructions are as follows:
Colistin powder in a 150 mg vial is reconstituted with 2 ml of sterile water for injection with resultant solution concentration of 75mg/ml. The desired dose is drawn up in an appropriate syringe and then injected into a 50 cc normal saline piggy back bag. It is infused over 30 minutes. Steady state plasma concentrations on visit 2 of therapy (on 2nd-3rd dose once at goal dosing) will be measured; specifically, colistin peak (30-60 minutes after infusion), midpoint (4-6 hours after dosing) and/or trough (30-0 minutes prior to next infusion). A steady state sputum sample will be procured at visit 2 to assess epithelial lining concentrations, unless subjects are on inhaled colistin as this will create inaccuracies in colistin sputum concentration measurements. Levels of colistin A and B and pro-drug from plasma, sputum, and urine will be quantified in the University of Colorado School of Pharmacy Medicinal Chemistry Core, with a 36 hour turnaround time to allow for measurement and interpretation. Dr. Ty Kiser at the School of Pharmacy will determine the optimal dose and intervals for APE subjects based on PK modeling with feedback from the PI, treating physician, and the National Jewish Health | Saint Joseph Hospital inpatient pharmacists. Colistin concentrations in plasma, sputum, and urine will be re-assessed at visit 3, after PK adjusted optimal dosing of colistin has reached a steady state.

9. Study Procedures and guidelines
A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed below.

TABLE 3: Timing of study assessments

	Visit 1	Visit 2	Visit 3	Visit 4
Informed Consent and Eligibility Review	X			
Demographics	X			
Medical History and Concomitant Medications	X			X
Physical Exam and Vital Signs	X	X	X	X
Questionnaires	X			X
Spirometry	X			X
Blood labs	→	→	→	→
Urine labs	→	→	→	→
Sputum microbiology	X			X
Blood collection for pharmacokinetic analysis (PK)		X	X	
Sputum collection for pharmacokinetic analysis (PK)		X	X	
Urine collection for pharmacokinetic analysis (PK)		X	X	
Urine collection for Nephrocheck	X	X	X	X

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 or subject's legal representative.

9.1 Clinical assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented as noted in the Schedule of Events. Dose, route, unit, frequency of administration, and dates of medication will be captured. The total number of IV doses of tobramycin and colistin for each study arm will be recorded.

9.1.2 Demographics

Demographic information (date of birth, sex, race) will be recorded at Visit 1.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying disease will be recorded at Visit 1.

9.1.4 Physical examination

A complete physical examination will be performed by either the investigator or a CF physician as noted in the Schedule of Events. An exacerbation score will be calculated for each subject, in order to quantify severity of exacerbation, using the validated Rosenfeld score. Any new clinically significant abnormal physical exam findings must be documented as adverse events (AEs) and will be followed by the physician rounding on the service at each scheduled daily visit.

9.1.5 Vital signs

Resting measurements of body temperature, blood pressure, pulse, and respirations will be performed and recorded as noted in the Schedule of Events.

9.1.6 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.
Subjects who are in the hospital routinely receive nebulized bronchodilators four times daily. Every effort will be made to check study spirometry within the same window, late morning, after the initial bronchodilator treatment, for study subjects in all arms.

9.1.7 Subject Questionnaire: Cystic Fibrosis Questionnaire-Revised (CFQ-R)
The CFQ-R is a patient reported quality of life (QoL) instrument, regarding symptoms and mood over the preceding 2 weeks. The CFQ-R takes approximately 15 minutes to complete. This questionnaire will be completed by the subject at the beginning and end of study participation.

9.1.8 Pulmonary exacerbation evaluation
The number of subsequent diagnoses of acute pulmonary exacerbations and the amount of time between exacerbations in the year following study enrollment will be documented.

9.1.9 Adverse Events
Information regarding occurrence of adverse events will be captured throughout the study. A case report form (CRF) will be completed by the rounding physician, which will include signs and symptoms related to specific toxicities due to tobramycin or colistin. Severity, seriousness, outcome, treatment, and relation to study participation will be recorded.

9.2 Clinical Laboratory Measurements
9.2.1 Blood chemistry profile
Blood will be obtained as part of standard of care and sent to the clinical chemistry lab for determination of serum sodium, potassium, chloride, bicarbonate, random glucose, BUN, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, direct bilirubin, and albumin. All of the results will be recorded as part of the study.

9.2.2 Pregnancy test
Urine or blood, if necessary, will be collected from females who are of childbearing age for a pregnancy test as part of standard of care and tested in clinic according to site standard procedures. The results will be recorded as part of the study.

9.2.3 Tobramycin Pharmacokinetic Measurements
Blood will be obtained as part of standard of care and sent to the clinical chemistry lab for determination of peak and trough tobramycin concentrations on the second dose, followed by a second peak and trough during the second week of IV tobramycin therapy. Drug concentrations will be determined at National Jewish Health | Saint Joseph Hospital. The results will be recorded as part of the study.

9.2.4 Qualitative bacteriology

Sputum will be collected for culture as part of standard of care. All sputum specimens will be collected in a sterile specimen cup, labeled, and sent to the National Jewish Health | Saint Joseph Hospital's microbiology lab on the day of collection. Culture and sensitivities for typical CF pathogens is performed. The results will be recorded as part of the study.

9.2.5 Urine studies

Urine will be obtained as part of standard of care and sent to the clinical chemistry lab for determination of urine protein and urine protein:creatinine ratio. All of the results will be recorded as part of the study.

9.3 Research Laboratory Measurements

9.3.1 Colistin Pharmacokinetic Measurements (blood, sputum, and urine)

Blood, sputum, and urine will be obtained as noted in the Schedule of Events. Measurements of pharmacologically active colistin levels will be performed by LC-MS/MS in the University of Colorado School of Pharmacy Medicinal Chemistry Core. Prior to mass spectrometry measurement of the sputum specimen, it will be processed as per standard Cystic Fibrosis Therapeutic Development Network protocols.

9.3.2 Nephrotoxic Acute Kidney Injury assay (urine)

Urine will be obtained as noted in the Schedule of Events and will undergo cryopreservation. In patients who develop acute kidney injury (elevation of serum creatinine $\geq$0.3 mg/dL) urine will be thawed and evaluated for neutrophil gelatinase-associated lipocalin (NGAL) and tissue-inhibitor of metalloproteinase 2 (TIMP-2) and insulin-like growth factor binding protein 7 (IGFBP-7). NGAL will be evaluated by enzyme-linked immunosorbent assay (ELISA). TIMP-2 and IGFBP-7 will be measured by the Nephrocheck® assay. Nephrocheck® is a newly FDA approved assay for detection of nephrotoxic acute kidney injury and will be tested for its sensitivity and specificity for detection of acute kidney injury in CF within the study. Both assays will be run by the University of Colorado Renal Division.

10 EVALUATIONS BY VISIT

10.1 Visit 1

1. Review the study with the subject (or subject's legal representative) and obtain written informed consent and HIPAA authorization.
2. Administer QoL questionnaire
3. Review respiratory culture results in the previous 12 months
4. Assign the subject a unique identification number.
5. Record demographics data.
6. Record medical history, including exacerbation criteria based on Rosenfeld score, baseline serum creatinine, and number of exacerbations in the preceding year.
7. Record concomitant medications.
8. Perform a complete physical examination.
9. Obtain and record vital signs.
10. Record oximetry.
11. Record results from blood and urine clinical laboratory tests (chemistry, hematology).

12. Record results from urine or blood pregnancy test (if female of child-bearing potential).
13. Record results from spirometry.
14. Record results from sputum culture microbiology.
15. Collect urine for Nephrocheck®

10.2 Visit 2

1. Administer QoL questionnaire
2. Record serum creatinine from daily clinical laboratory draws.
3. Collect urine for Nephrocheck®
4. Record urine protein from three times weekly clinical laboratory check.
5. Record tobramycin peak and trough from week 1 clinical laboratory draw
   OR
   Collect blood, sputum, and urine for colistin pk measurement.

10.3 Visit 3

1. Administer QoL questionnaire
2. Record serum creatinine from daily clinical laboratory draws.
3. Collect urine for Nephrocheck®
4. Record urine protein from three times weekly clinical laboratory check.
5. Record tobramycin peak and trough from week 2 clinical laboratory draw
   OR
   Collect blood, sputum, and urine for colistin pk measurement.

10.4 Visit 4

1. Administer QoL questionnaire.
2. Record concomitant medications.
3. Record spirometry.
4. Record serum creatinine from daily clinical laboratory draws.
5. Record results from sputum culture microbiology.
6. Record total number of IV antibiotics doses over the preceding 14 days.
7. Collect urine sample for Nephrocheck®.
8. Complete Case Report Form (for toxicities).

10.5 Acute Visit/Early Termination of Study Participation

1. Administer QoL questionnaire
2. Record any adverse events.
3. Review and record subject dosing for colistin or tobramycin since last visit.
4. Record changes to concomitant medications.
5. Perform complete physical examination.
6. Record vital signs.
7. Record spirometry.
8. For episodes of renal toxicity, monitor daily measures of serum creatinine for return to baseline.

11. ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

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

The investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and will record the information in the site's source documents. Adverse events will be recorded in the subject CRF.

11.2 AE severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, as modified for cystic fibrosis, should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. If the experience is not covered in the modified criteria, the guidelines shown in Table 4 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 4. 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.

11.3 AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following guidelines in Table 5.

Table 5. 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' 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.

11.4 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
- 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.

11.4.1 Serious adverse experience reporting

The coordinator 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 Medical Monitor within one business day of the site learning of the event.

The site will notify the Medical Monitor 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.

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

11.5 Medical Monitoring

Milene Saavedra, MD should be contacted directly at these numbers to report medical concerns or questions regarding safety.
Office phone: (303) 398-1318
Cell phone: (303) 809-2306

Edith Zemanick, MD will serve as an independent Medical Monitor for this study. AEs and SAEs will be collected and provided to Dr. Zemanick for review in a timely manner.

12. Discontinuation and replacement of subjects

12.1 Early Discontinuation of the Study Treatment

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

- Subject or subject's legal representative decision
- Adverse event
- Protocol violation
- Death

If a subject is discontinued from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment early will be evaluated for an early discontinuation of study treatment visit as soon as possible and then encouraged to complete all remaining scheduled visits and procedures.

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 withdraw from study treatment early and who decline to continue to come in for remaining follow-up

visits or it may include subjects who completed treatment and decline to come in for remaining follow up visits.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw from the study should be encouraged to come in for a final visit (and the procedures to be followed would include those for an early discontinuation visit).

12.3 Replacement of subjects

Subjects who discontinue or are withdrawn from the study before completing Visit 2 may be replaced at the discretion of the study investigator team.

13. Protocol violations

A protocol violation occurs when the subject, investigator, or the 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
- Use of a prohibited concomitant medication. Subjects requiring additional non-nephrotoxic antibiotics to treat pulmonary exacerbation will be asked to continue in the study and this will not be treated as a protocol violation.

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The sponsor will determine if a protocol violation should result in early discontinuation of study treatment for 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 study regulatory binder and in the Sponsor's files. The site will report the violation to their IRB in accordance with the IRB reporting requirements.

14. Data Safety Monitoring

A Data Safety and Monitoring Plan (DSMP) is in place for this study.

Safety data will be monitored on a continual basis throughout the trial by Edith Zemanick, MD. Efficacy will be evaluated in two planned interim analyses, each of which will occurs after 1/3rd of the data is accrued or 1/3rd of the participants have completed dosing. Safety monitoring will include monitoring of serious adverse events, adverse events, participant withdrawals, safety data, and enrollment summaries.

Serious adverse events will be monitored by Edith Zemanick, MD on an ongoing basis throughout the study. When approximately 50% of patients have completed the study through Visit 4, an interim analysis for safety, futility, and early significance will be conducted

Milene Saavedra, MD will monitor adherence to the protocol on a continual basis. If deviations occur, the PI will discuss with study staff any issues that may be causing these deviations and ways to improve adherence to the protocol. Deviations will be reported to the IRB in accordance with the IRB reporting requirements

Katie Poch will monitor the data collection for accuracy and quality for the study. Data will be collected from subject medical and research records and maintained in electronic Case Report Forms (eCRFs) using REDCap (Research Electronic Data Capture), a secure web-based application that supports data capture for research studies.

15. Statistical Methods and Considerations

15.1 General: Summary statistics will be calculated for demographic and baseline characteristics (age, sex, genotype, diabetic status, FEV_1 % predicted) time to therapeutic dosing for PK-adjusted colistin versus tobramycin, number of therapy interruptions between study arms, and total number of IV antibiotic doses. Study medications and exposure to study drug data will be summarized in contingency tables. Summary statistics and changes from admission visit to end of treatment will be presented for FEV_1 % predicted and questionnaire results, on an intent-to-treat basis, including all subjects receiving at least three days of colistin or tobramycin, and at least one efficacy assessment. Subjects will be enrolled once.

15.2 Efficacy analysis: We will perform equivalence testing to show similarity of colistin treatments, as well as for colistin vs tobramycin treatments. For both comparisons, confidence intervals for difference in FEV_1 changes from pre to post treatment time points will be constructed, and equivalence tests to within 5% will be performed. These will be derived from linear mixed models fitted for FEV_1 that include a random intercept for subjects to account for repeated measures, and the following covariates: age, other medication, admits in the previous year, and diabetes. Nested random terms will be included in the model to account for repeated measures – one for subject and one for visit within subject. CFQ-R domain scores will be analyzed in a similar fashion. Times to next pulmonary exacerbation and occurrence of neurotoxicity between standard colistin and PK-adjusted colistin will be analyzed using the Kaplan-Meier method. Subsequent pulmonary exacerbations in the ensuing year for all enrolled subjects, as well as antimicrobial resistance to tobramycin and colistin before and after treatment, will be summarized. Unless otherwise noted, 2-sided tests will be used with $\alpha = 0.05$.

15.3 Safety analysis: The safety profile of each colistin arm and the tobramycin arm will be assessed in terms of the following: incidence of acute kidney injury (AKI), incidence of treatment related clinical events (ototoxicity, vestibular toxicity, and neurotoxicity). Adverse event rates will be compared between treatment groups using logistic regression, adjusting for age, medications such as vancomycin, baseline FEV_1, admits in the previous year, exposure to nephrotoxic antibiotics in the previous 5 years, and diagnosis of CF related diabetes (CFRD) as covariates. Repeated measures within subjects will be accounted for by including a random intercept for subjects.
Summary statistics and changes from admission will be presented for serum creatinine and urine:protein ratios. The urine protein:creatinine ratio will be assessed as a

biomarker of drug toxicity. There are no published criteria for cessation of therapy or accurate prediction of AKI as a result of the urine protein values. Thus, this data will be analyzed as a predictor for AKI. We will analyze Nephrocheck® assay results in a case control design. Subjects who develop AKI within each of the study arms will have urine thawed and analyzed by Nephrocheck® and those results will be compared to age, gender, and FEV_1 matched control urines obtained from subjects within the study who did not develop AKI. An adjusted odds ratio will be derived from a model fitted with unconditional logistic regression and adjusted for confounding variables. Estimates of odds ratios and 99% confidence intervals will be presented.

15.4 Sample size and power: The original power calculations estimated 90 subjects per arm in order to achieve power to demonstrate efficacy, or higher FEV1 in a tobramycin versus colistin study arm. Since preliminary data demonstrates that colistin is safer than tobramycin in terms of kidney injury, the more appropriate power calculation would evaluate colistin superiority for safety. In a retrospective cohort 500 subjects, FEV1% improvement after tobramycin treatment during exacerbation therapy was 3% higher than in those receiving colistin; however, rates of acute kidney injury (rise in serum creatinine $\geq$ 0.3 mg/dL while on therapy) were 60% higher in tobramycin treated subjects versus standard colistin treated subjects. Since no preliminary data was available on less frequent colistin dosing, which theoretically would lead to less frequent episodes of kidney injury, we estimate we are powered to identify a safer method for dosing colistin with samples of 30 subjects per group, though we may be underpowered to find a significant FEV_1 difference between groups.

16. DATA COLLECTION, RETENTION AND CLINICAL MONITORING

16.1 Data Collection Instruments

The investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject who is enrolled in the study.

Study personnel will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documented to be collected by the Sponsor (or designee), but will be identified by a subject number and initials.

If a correction is required for a CRF, the time and date stamps track the person entering or updating CRF data and create 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.

16.2 Data Management Procedures

Study subjects will not be identified by name in the study database or on any data capture screens but will be identified by initials and a unique subject identification number. Only study personnel will be able to link the study ID to the subject's name. The study coordinator will be responsible for data processing, in accordance with

procedural documentation. 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 for the handling and analysis of data for clinical trials.

All study visits will occur in the context of regular clinical care. Data will be entered and stored in REDCap (Research Electronic Data Capture), a secure web-based application that supports data capture for research studies. A system of computerized data validation checks will be implemented and applied to the database on a regular basis. REDCap is able to log all user activity and all pages viewed by every user. It is capable of monitoring data entry, exporting data, modifying a field, running a report, or add/modifying a user, among a plethora of other activities. Administrators to be able to determine all the activity and all the data viewed or modified by any given user.

16.3 Security and Archiving of Data

Within REDCap, user controls limit access to various functionalities, such as being able to export data, to enter data, to add or modify database fields or survey questions, and to view the logging records.

To secure data, all user- submitted data in REDCap is filtered for any possibly harmful markup tags (e.g. <script>) and is then escaped before ever being displayed on a web page within the application. SQL queries sent to the database server from REDCap are all properly escaped before being sent. If any values used in an SQL query originated from user-defined values, they would have already been sanitized beforehand as well, as described above. User-defined data used within SQL queries also have their data type checked to prevent any mismatching of data types (e.g. making sure a number is really a number). These processes of sanitization, filtering, data type checking, and escaping all help to protect against methods of attack, such as Cross-Site Scripting (XSS) and SQL Injection. To specifically protect against Cross-Site Request Forgery (CSRF), which is another method of attack, REDCap utilizes a "nonce" (a secret, user-specific token) on every web form used in the application. The nonce is generated anew on each web page as the user navigates within REDCap during a session.

Robust password procedures, consistent with 21 Part 11, and physical security procedures are in place to prevent unauthorized personnel physical access to the server rooms.

Network accounts are password protected and maintained and monitored by the National Jewish Health Information Technology group. Data is backed up regularly according to the Information Technology's group procedures.

16.4 Availability and Retention of Investigational Records

The investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization, and 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, Essential Documents and Study Reference Binders) must be secured for a period of 2 years following completion of the study. There may be 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.

16.5 Monitoring

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, if needed. Monitoring visits may be conducted by representatives of the Sponsor according to the U.S. CFR 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.

16.6 Subject Confidentiality

In order to maintain subject confidentiality, only a subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor.

17. ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

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

To maintain confidentiality, all laboratory specimens, evaluation forms, reports, and other records will be identified by a coded number and initials only. Clinical information will not be released without written permission of the subject except as necessary for monitoring by the FDA. The Investigator must 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 primary investigator, Milene Saavedra, M.D., and agreed to by all members of the investigator team. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within 5 working days.

17.2 Institutional Review Boards and Independent Ethics Committees

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

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IEC's written unconditional approval of the study protocol and the informed consent form will be in possession of the Investigator before the study is initiated.

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

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

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25 [a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA), 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 the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB/IEC approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written informed consent form (ICF) will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study. If a subject is unable to sign the ICF and HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form will be given to the subject and the original maintained with the subject's records.

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

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