A PHASE I, OPEN-LABEL STUDY TO ASSESS LUNG PHARMACOKINETICS AND SAFETY OF A SINGLE DOSE OF APRAMYCIN ADMINISTERED INTRAVENOUSLY IN HEALTHY ADULT SUBJECTS

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STATEMENT OF COMPLIANCE

The clinical trial will be carried out in accordance with Good Clinical Practice (GCP) and as required by the following:

- United States (U.S.) Code of Federal Regulations (CFR) applicable to clinical trials (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312).
- International Council for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use E6(R2) Good Clinical Practice (ICH E6 GCP): Integrated Addendum to ICH E6(R1) Guidance for Industry, published in the Federal Register (83 Federal Register 8882 (2018)), including the latest finalized National Institutes of Health (NIH) Clinical Terms of Award, as applicable.

Compliance with these standards provides public assurance that the rights, safety, and well-being of subjects are protected, consistent with principles that originate in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of the trial) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of the protocol and its attachments and provides the necessary assurances that the trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Site Investigator: William B. Smith, MD

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Name/Title (Print)

Signature: _____

Date: _____

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LIST OF ABBREVIATIONS

AAC(2)-Ia	Acetyltransferase (2) subtype Ia
AAC(3)-IV	Acetyltransferase (3) subtype IV
ACE	Angiotensin-Converting Enzyme
AE	Adverse Event
AII	Angiotensin II
ALT	Alanine Aminotransferase
AM	Alveolar Macrophages
AMR	Alliance for Multispecialty Research
ARA	Angiotensin II-receptor antagonists
APTT	Alpha Partial Thromboplastin Time
AP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
AUC	Area Under the Concentration – Time Curve
$AUC_{(0-t)}$	Area under the concentration time-curve from time zero to time t
$AUC_{(0-24)}$	Area under the concentration time-curve from time zero to 24 h
$AUC_{(0-\infty)}$	Area under the concentration time-curve from time zero to infinity
$AUC_{(0-last)}$	Area under the concentration time-curve from time zero to the last concentration above the lower limit of quantitation
β-HCG	Beta Human Chorionic Gonadotropin
BAL	Bronchoalveolar Lavage
BMI	Body Mass Index
BP	Blood Pressure
bpm	Beats per Minute
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention
CKD – EPI	Chronic Kidney Disease Epidemiology collaboration equation for estimating GFR
CFR	Code of Federal Regulations
CHEM	Chemistry Panel
CI	Confidence Interval
C_{max}	Maximum Concentration

CL_T	Total Clearance
CMS	Clinical Materials Services
ConMed(s)	Concomitant Medication(s)
COAG	Coagulation
COX-2	Cyclooxygenase 2
CPM	Clinical Project Manager
CROMS	Clinical Research Operations and Management Support
CSR	Clinical Study Report
CTU	Clinical Trial Unit
ΔΔQTcF	Time-matched, baseline-adjusted difference in QTcF interval
dB	Decibel
DBP	Diastolic Blood Pressure
DVC	Dynport Vaccine Company, LLC, a GDIT company
dL	Deciliter
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
DPOAE	Distortion product otoacoustic emissions
eCRF	Electronic Case Report Form
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic Data Capture
FEV1	Forced Expiratory Volume in the first second
FIH	First-in Human
FH	Family History
fT3	Free triiodothyronine (T3)
fT4	Free thyroxine (T4)
FVC	Forced Vital Capacity
ELF	Epithelial Lining Fluid
ET	Early Termination
EU	European Union
FDA	Food and Drug Administration
FH	Family History
FSH	Follicle-Stimulating Hormone

FWA	Federalwide Assurance
g	Gram(s)
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
eGFR	Estimated GFR
h	Hour(s)
HAP	Hospital Acquired Pneumonia
HBsAg	Hepatitis B Surface Antigen
Hct	Hematocrit
HCV	Hepatitis C Virus
HEENT	Head, Eyes, Ears, Nose, and Throat
HEM	Hematology Panel
Hgb	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HPF	High-Powered Field
HIPAA	Health Insurance Portability and Accountability Act
HLGT	High Level Group Term
HR	Heart Rate
IATA	International Air Transport Association
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonization
ICMJE	International Committee of Medical Journal Editors
IM	Intramuscular
IMP	Investigational Medical Product
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
IUD	Intrauterine Devise
IV	Intravenous
kg	Kilogram(s)

KIM	Kidney Injury Molecule
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LLN	Lower Limit of Normal
LQTS	Long QT Syndrome
MBC	Minimal Bactericidal Concentration
MDMA	3,4-methylenedioxy-methamphetamine
MDR	Multiple Drug Resistant
MedDRA®	Medical Dictionary for Regulatory Activities
MRSA	Methicillin Resistant *Staphylococcus aureus*
MM	Medical Monitor
µg	Microgram(s)
mg	Milligram(s)
MH	Medical History
MI	Myocardial Infarction
MBC	Minimal Bactericidal Concentration
MIC	Minimal Inhibitory Concentration
min	Minute(s)
mL	Milliliter(s)
MM	Medical Monitor
mmHg	Millimeters of Mercury
mmol	Millimole(s)
MOP	Manual of Procedures
msec	Milliseconds(s)
N	Number (typically refers to the total number of subjects)
n	Number (typically refers to a subset of the total number of subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NOAEL	No Observable Adverse Effect Level
NSAID	Nonsteroidal Anti-Inflammatory Drug
OAEs	Otoacoustic emissions
OER	Office of Extramural Research, NIH
OTC	Over the Counter

PAE	post-antibiotic effect
PBF	Post-bronchoscopy Fever
PD	Pharmacodynamic(s)
PE	Physical Examination
PI	Principal Investigator
PK	Pharmacokinetic(s)
POC	Point of Contact
Protime	Prothrombin time
PVG	Pharmacovigilance Group
QTc	Corrected QT Interval of the ECG
QTcF	Corrected QT interval of the ECG using Fridericia's Formula
RAAS	Renin – Angiotensin - Aldosterone System
RNA	Ribonucleic Acid
rRNA	ribosomal ribonucleic acid
RBC	Red Blood Cell
RP	Research Pharmacist
RR	Respiratory Rate
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SDCC	Statistical and Data Coordinating Center
sec	Second(s)
SMC	Safety Monitoring Committee
SNHL	Sensorineural Hearing Loss
SOC	System Organ Class
SSP	Study Specific Procedures
STH6	Score Threshold
T	Temperature
TCA	Tricyclic Antidepressant
T_{max}	Time to Maximum Concentration
TEAE	Treatment-emergent Adverse Event

TSH	Thyroid Stimulating Hormone
UA	Urinalysis
ULN	Upper Limit of Normal
US	United States
UTMC	University of Tennessee Medical Center, Knoxville
$t_{1/2}$	Terminal Elimination Half-life
TLF	Tables, Listing and Figures
TN	Tennessee
VAP	Ventilator-associated Pneumonia
Vd	Volume of Distribution
VS	Vital Signs
Vz/F	Apparent volume of distribution
WBC	White Blood Cell
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	A Phase I, Open-Label Study to Assess Lung Pharmacokinetics and Safety of a Single Dose of Apramycin Administered Intravenously in Healthy Adult Subjects.
Phase:	1
Population:	Approximately 20 evaluable healthy male or female subjects 18 to 45 years of age (both inclusive) at the time of dosing.
Number of Sites:	One (Alliance for Multispecialty Research [AMR] Phase 1 clinical trial unit [CTU], Knoxville, Tennessee [TN] [(AMR-Knoxville]).
Study Duration:	Approximately 10 months from study activation to last subject last visit.
Subject Participation Duration:	Up to 58 days (from Screening Visit to Final Visit). The trial will consist of a Screening Period of up to 26 days as outpatient (Day -28 to Day -3); a Check-in Day (Day -2); Baseline Day (Day -1); a Treatment Period of 3 days (Days 1 to 3) as inpatient, with dosing on Day 1 and follow-up on Days 2 and 3; and an out-patient Follow-up Period of 27 days (Days 4 to 30) with two site visits, on Day 14 (± 3 days) and Day 30 (± 4 days) (Final Visit).
Description of Agents:	Apramycin is a mono-substituted 2-deoxystreptamine compound that represents a subclass of aminoglycoside antibiotics. Apramycin is provided in 20 mL vials as an aqueous solution of 150 mg/mL apramycin base, with pH adjusted to 5.5 to 6.0 with sulfuric acid. The filling volume is 19 mL, and the extractable volume is 18 mL. A single dose of 30 mg/kg will be administered intravenously (IV) in a total volume of 30 mL over a period of 30 (± 5) min as an open label. Sterile

	normal saline will be used to adjust the volume to the desired Investigational Medical Product (IMP) concentration.
Objectives:	**Primary:** • To assess plasma pharmacokinetic (PK) profile of apramycin and lung penetration of apramycin in epithelial lining fluid (ELF) and alveolar macrophages (AM) after single intravenous (IV) apramycin dose of 30 mg/kg in healthy subjects. **Secondary:** • To assess the safety of single IV administration of 30 mg/kg apramycin in healthy subjects. • To assess changes in otoacoustic testing. **Exploratory:** • To assess changes in kidney function biomarkers.
Outcome Measures:	**Primary:** • Lung concentration and PK parameters of total apramycin: o ELF: C_{max}, $AUC_{(0-24)}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, T_{max}, $t_{½}$ o AM: C_{max}, $AUC_{(0-24)}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, T_{max}, $t_{½}$ • Plasma concentration and PK parameters of total apramycin: o $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, $AUC_{(0-24)}$, C_{max}, T_{max}, $t_{½}$, CL_T, V_d central • Ratio of exposure parameters of Lung PK to Plasma PK: o ratio of C_{max} in ELF over C_{max} in plasma o ratio of C_{max} in AM over C_{max} in plasma o ratio of $AUC_{(0-24)}$, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ in ELF over $AUC_{(0-24)}$, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ in plasma o ratio of $AUC_{(0-24)}$, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ in AM over $AUC_{(0-24)}$, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ in plasma

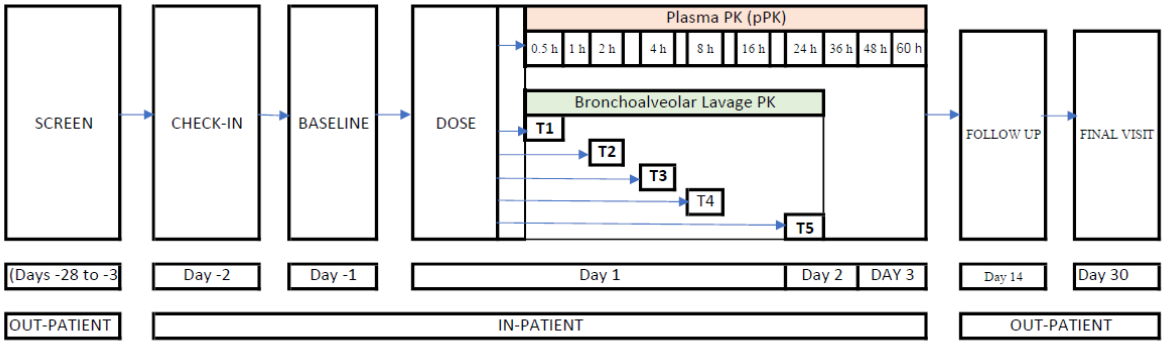
	Secondary: • Type and incidence of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) to Day 30 (± 4 days) after dosing. • Frequency of clinically significant TEAEs in physical examination (PE) findings or changes from baseline in vital signs (VS) and clinical laboratory parameters to Day 14 (± 3 days) after dosing. • Frequency of changes in electrocardiographic (ECG) intervals (QTcF, PR, QRS, HR) and morphology from baseline until 24 h ± 1 h after dosing. • Type and incidence of TEAEs related to auditory (cochlear) function tests (pure-tone audiometry and distortion product otoacoustic emissions [DPOAEs]) to Day 30 (± 4 days) after dosing. **Exploratory:** • Changes from baseline in urinary kidney injury molecule 1 (KIM-1) and serum cystatin C concentrations until Day 30 (± 4 days) after dosing.
Description of Study Design:	Open label study of a single dose of 30 mg/kg of apramycin administered intravenously (IV) over 30 (± 5) minutes. Twenty subjects will be enrolled in the study to one of 5 cohorts, T1-T5, each corresponding to a timepoint after initiation of infusion at which a single fiberoptic bronchoscopy with bronchoalveolar lavage (BAL) is performed. There will be 4 subjects per cohort. Cohort T5 will be enrolled after plasma and lung apramycin concentrations and preliminary PK data analysis are completed in cohorts T1-T4. Enrollment and dosing will be determined by bronchoscopy schedule. For each cohort, if 2 subjects are scheduled to receive study drug on the same day, the dose will be administered sequentially at least 2 hours apart. Bronchoscopy with BAL will be performed under sedation or light anesthesia and oxygenation support. Apramycin concentrations after IV infusion will be measured in BAL and alveolar

	macrophages (AM) prepared by centrifugation of BAL samples. Apramycin concentrations in epithelial lining fluid (ELF) will be corrected for dilution using the urea method. BAL samples will be collected at the following timepoints after the start of the infusion in each T cohort: 30 min ± 5 min (T1), 2 h ± 5 min (T2), 4 h ± 10 min (T3), 8 h ± 15 min (T4) or 24 h ± 1 h (T5). The timing of T5 is nominal; final time will be determined after analysis of apramycin concentrations in the BAL (ELF and AM) in cohorts T1-T4. Apramycin concentrations (total) will be measured in plasma samples prepared from blood samples collected pre-dose, and at the following timepoints after the start of the infusion: 30 ± 5 min, 1 h ± 5 min, 2 h ± 5 min, 4 h ± 10 min, 8 h ± 15 min, 16 h ± 15 min, 24 h ± 1 h, 36 h ± 1h, 48 h ± 1 h and 60 h ± 1 h after dosing. An additional plasma PK sample may be collected at the end of the BAL sample collections if bronchoscopy with BAL is delayed. Free apramycin concentration will be measured at 30 ± 5 min (immediately after the end of infusion) and at 36 h ± 1h. Urea concentration will be measured in plasma and time-matched BAL samples as a volume dilution marker of BAL ELF. All concentration measurements for apramycin and urea will be performed with validated bioanalytical assays. Safety will be evaluated by standard clinical assessments, VS measurements, safety clinical laboratory tests, and 12-lead standard ECGs. Potential nephrotoxicity will be evaluated with creatinine concentrations and estimated glomerular filtration rate (eGFR) and exploratory biomarkers in plasma (Cystatin C) and urine (kidney injury marker 1 [KIM-1]). Potential ototoxic effects will be evaluated by cochlear otoacoustic tests (audiometry and DPOAEs).

	Changes in assessments after administration of the IMP and comparison with the pre-dosing baseline will be reported. TEAEs and SAEs will be collected and graded from the time of dosing to completion of the study (Day 30 ± 4 days). A Safety Monitoring Committee (SMC) will oversee the conduct of the study.
Estimated Time to Complete Enrollment	Up to 6 months (from dosing of first subject to Final Visit of last subject).

Figure 1: Description Schematic of Study Design

NOTE: All subjects who signed the ICF and met screening criteria will have baseline assessments on Day -2 and Day -1. Following a single IV dose of 30 mg/kg of apramycin, blood will be drawn for measuring the concentration and PK parameters of total plasma apramycin starting on Day 1 and finishing on Day 3 at the indicated timepoints. A single bronchoscopy with bronchoalveolar lavage will be performed after initiation of dosing at one of the following timepoints in the respective cohorts: 30 min (T1), 2 h (T2), 4 h (T3), 8 h (T4), and 24 h (T5). Total apramycin concentration will be measured at each BAL timepoint and PK parameters will be estimated in BAL fluid (containing ELF) and BAL AM. Four subjects will be assigned to each T cohort. Enrollment and dosing will be rolling and coordinated with the bronchoscopy service. For each cohort, if 2 subjects are scheduled to receive study drug on the same day, the second subject will be dosed at least 2 hours after the first subject. Safety assessments will be reported from the start of the IMP infusion on Day 1 to the last visit on Day 30, and will include physical examination, vital signs, ECG, clinical laboratory tests (hematology, chemistry and urinalysis), audiology examinations (ENT exam, audiometry, DPOAEs), and exploratory markers of nephrotoxicity (serum cystatin C and urinary KIM-1).



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Aminoglycosides are a class of antibiotics established with the introduction of streptomycin in the 1940s for the treatment of tuberculosis and have defended a strong clinical presence ever since and until today.

The common mode of action of aminoglycoside antibiotics is the disruption of bacterial protein synthesis by binding to the 16S ribosomal nucleic acid (rRNA) of the decoding site inside the small ribosomal subunit. Aminoglycosides are characterized by rapid and concentration-dependent killing and demonstrate significant post-antibiotic effect (PAE). All aminoglycosides including apramycin are polar, poly-cationic, and highly water-soluble, a property that limits their ability to cross lipid-rich cellular membranes of the host cells.

Mainly due to the common recognition of the rapid bactericidal activity of aminoglycosides - also at very high bacterial burden - the drug class has established itself as an important therapeutic component in the treatment of serious hospital infections worldwide. Despite the introduction of several new chemical classes of antibacterial agents over the past few decades, aminoglycosides still remain an extremely important therapeutic option. The World Health Organization (WHO) continues to classify aminoglycoside antibiotics as Essential Medicines (WHO 2019a) and as Critically Important Antimicrobials for Human Medicine (WHO 2019b).

The therapeutic efficacy of standard-of-care aminoglycosides currently in clinical use is compromised by significant rates of antimicrobial resistance acquired by horizontal gene transfer. Infections with antibiotic-resistant bacteria caused an estimated 33,000 deaths in Europe in 2015 (Cassini 2018). According to the CDC's *Antibiotic Resistance Threats in the United States, 2019,* more than 2.8 million antibiotic-resistant infections occur in the U.S. each year, and more than 35,000 people die as a result (CDC 2019). Bacterial pathogens highly resistant to various classes of antibiotics are estimated to account for a total of about 700,000 deaths annually worldwide. Without successful intervention, the burden of continued emergence and global spread of antimicrobial resistance is expected to multiply by the year 2050 (O'Neil 2014; CDC 2019). New antibiotics are needed to adequately respond to the increasing prevalence of drug- resistant infections.

2.2 Apramycin

Apramycin is a mono-substituted 2-deoxystreptamine compound that is significantly different from the di-substituted 2-deoxystreptamines which include all the standard-of-care aminoglycoside antibiotics (gentamicin, amikacin, tobramycin, arbekacin, plazomicin and others) and, therefore, represents a subclass of aminoglycosides (O'Connor 1976).

Apramycin was originally approved as an oral and intramuscular veterinary antibiotic in the European Union (EU) for various indications (e.g., colibacillosis and salmonellosis in calves, bacterial enteritis in pigs, or *Escherichia coli* septicemia in poultry (EMA 1999). Its use as a growth-promoter in livestock has been banned in the 1980's and it is authorized only for clinical veterinary targeted therapy in the EU and the USA (EU Commission 2005, FDA 2021).

Antibacterial activity

Apramycin is active against a wide variety of pathogenic Gram-positive and Gram-negative bacteria including pathogens with resistance to gentamicin and all other clinical aminoglycosides of relevance. The primary therapeutic spectrum comprises all relevant *Enterobacterales* including *Escherichia coli, Klebsiella spp., Enterobacter spp., Serratia marcescens, Morganella morganii, Citrobacter freundii, and Providencia spp* (Smith 2016, Juhas 2019). The activity against *Providencia spp*. is of note because of its chromosomal *aac (2')-Ia* gene that causes intrinsic resistance to several aminoglycoside antibiotics including plazomicin, the newest semisynthetic aminoglycoside to receive Food and Drug Administration (FDA) market approval. Apramycin also has significant in vitro activity against multi-drug resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Kang 2017, Juhas 2019). Amongst Gram-positive cocci, both methicillin-sensitive and resistant *Staphylococcus aureus* (MRSA) are sensitive to apramycin (Truelson 2018). The *in vitro* minimum inhibitory concentration (MIC) of apramycin against drug-susceptible clinical isolates at neutral pH tends to be about 4-fold lower than that of gentamicin, and about 2-fold lower than that of amikacin. The potency of apramycin at slightly acidic pH is similar to that of gentamicin and amikacin. The overall MIC90 of apramycin is 8 μg/mL against highly drug- and carbapenem-resistant *Enterobacterales* (IB 2021, Juhas 2019).

Apramycin treatment in mouse models of urinary tract infection (UTI), septicemia, pneumonia, and neutropenic thigh infection significantly reduced the bacterial burden even with bacterial isolates that are multidrug-, carbapenem- and plazomicin-resistant (Becker 2020, IB 2021).

Safety Pharmacology

In vitro and *ex vivo* studies showed no relevant secondary pharmacological effects on receptors or ion channels, which indicates that no off-target effects of apramycin are to be expected. The rat calcium channel N-type appeared to show full inhibition at 2 mM. This receptor interaction has been reported to be associated with the reversible neuromuscular blockade that can be commonly induced by peak aminoglycoside concentrations. *In vitro* investigation of effects on human ether-a-go-go-related gene (hERG) potassium channel assays in Chinese hamster ovary (CHO) cell lines showed $IC_{50} > 40$ mM. High IC_{50}'s were also found for patch recordings of the Nav 1.5 sodium channel (>3 mM) in CHO cells and the Cav 1.2 channel in CHO cells (between 1 to 3 mM), suggesting no potentially clinically significant effects (IB 2021).

Nonclinical Assessment of Safety

Extensive nonclinical safety core battery studies have been conducted in the rat and the Beagle dog to evaluate the effects of apramycin on organ systems (IB 2021).

Repeat-dose toxicity studies identified the kidneys as the main target organ of toxicity. Nephrotoxicity manifested as increases in plasma creatinine and BUN, and histopathological changes in the kidney, primarily tubular necrosis with or without regeneration. Nephrotoxicity was exposure-related, with a lower incidence and severity after the recovery period, suggesting potential reversibility. Nephrotoxicity appeared to correlate better with cumulative exposure than with total $AUC_{(0\text{-}24)}$ (IB 2021).

Ototoxicity is a known risk of aminoglycosides. A repeat, 16-day dose study in guinea pigs showed that apramycin ototoxicity progressed more gradually than under gentamicin treatment, and cochlear function was partly retained even at high concentrations (430 mg apramycin/kg body weight). "No ototoxic effects were observed in the guinea pig model at the 152 mg/kg/day dose level. This would convert to a human equivalent dose (HED) of 33.3 mg/kg, a single dose exposure of 0.8 mMÅ~h, and a cumulated exposure of 13.7 mMÅ~h, all of which are markedly higher than the nephrotoxic no observed adverse effect levels (NOAELs) in both rats and dogs." (Matt 2012) Recent literature data support the finding that apramycin is of lower ototoxicity than gentamicin (Ishikawa 2019).

Besides nephrotoxicity and ototoxicity, no other noteworthy findings in clinical signs and symptoms were observed. Apramycin showed no effects on the CNS, cardiovascular, or respiratory systems (IB 2021).

No secondary pharmacological effects were observed. No mutagenic and no genotoxic effects were observed. Therefore, no adverse effects or risks related to CNS, cardiovascular system, respiratory or off-target effects are to be expected. No special precautions regarding exposure to ultraviolet light have to be taken, as apramycin has no phototoxic potential. No signs of hepatotoxicity were observed in nonclinical safety pharmacology or toxicology studies. No fertility studies or data on embryofetal toxicity of apramycin are available to date. From veterinary toxicology data, no adverse effects on reproductive performance or evidence of maternal toxicity, fetal toxicity, or teratogenicity have been reported. Overall, the results of the nonclinical safety studies and toxicology studies are in line with the safety profiles reported for other aminoglycosides, e.g., gentamicin, amikacin and plazomicin (IB 2021).

Nonclinical Pharmacokinetics

Pharmacokinetic (PK) data for apramycin is available for mouse, rat, guinea pig and dog. In animal models, the PK of apramycin was equivalent to benchmark aminoglycosides tested (e.g., gentamicin). Aminoglycosides are minimally absorbed in the gastrointestinal tract and thus must be administered intravenously (IV), subcutaneously (SC), or intramuscularly (IM) in order to treat systemic infections. Based on the volume of distribution, apramycin showed no wide tissue distribution. Protein binding was low, with fractions unbound (fu) of 90% in human and animal plasma. Apramycin was not metabolized. No interactions with cytochrome P450 (CYP) isoenzymes were found. Almost all of the administered apramycin was excreted via the kidneys and recovered in urine. In mouse models, apramycin content in kidneys increased with

increasing doses and duration of treatment. At higher doses and with longer duration of treatment, apramycin exposure increased due to a decrease in renal excretion (IB 2021).

2.3 Rationale for the Development of Apramycin for Human Use

Apramycin is a mono-substituted 2-deoxystreptamine comprising a unique bicyclic octadiose moiety. It represents a subclass of aminoglycoside antibiotics significantly different from the clinically well-established di-substituted 2-deoxstreptamines gentamicin, amikacin, tobramycin, arbekacin, plazomicin and others (Mingeot-Leclercq 1999).

Based on its chemical structure, it was predicted that apramycin evades known aminoglycoside resistance mechanisms (Böttger & Vasella 2012). This was further confirmed by screening a panel of engineered *E. coli* strains expressing isolated aminoglycoside resistance genes under equipotent constitutive promoter control, which showed the evasion of almost all known aminoglycoside-modifying enzymes involved in resistance (aminoglycoside acetyltransferases, phosphotransferases, and nucleotidyltransferases). The only exception is the acetyltransferase (3) subtype IV (AAC(3)-IV) - a resistance gene that was previously described to confer apramycin resistance in animals but is of low clinical prevalence when compared to all other aminoglycoside resistance mechanisms (Plattner 2020). Apramycin further retains binding to target sites methylated by ribonucleic acid (RNA) methyltransferases. In summary, cross-resistance to clinically approved antibiotics has been demonstrated to be minimal (Böttger 2020).

The antibacterial potency of apramycin against wild-type and drug resistant clinical isolates, has been endorsed by several independent investigators in the scientific literature. An extensive data set on antibacterial potency of apramycin based on more than 1,200 clinical isolates has been generated by the IMI ENABLE project (Juhas 2019) The MIC distribution and *in vivo* efficacy of EBL-1003 against highly drug-resistant *A. baumannii* isolates and lung infections in mice has been particularly encouraging (Becker 2020).

The aim of the clinical development program for apramycin is to allow a full assessment of safety and efficacy as well as ensuring that the product is rapidly made available to fulfill an unmet medical need in the treatment of infections due to multi-drug resistant (MDR) bacteria.

2.4 Clinical Experience

2.4.1 First-in-Human Single Dose Escalation Study

In the First-in-Human (FIH) single dose escalation trial, single doses of 0.3, 1.2, 3.6, 10.8 and 30 mg/kg apramycin were investigated in healthy volunteers. The subjects were randomized to either apramycin (6 per dose level) or placebo (2 per dose level).

Safety

Overall, single IV doses of EBL-1003 (apramycin) up to 30 mg/kg were safe and well tolerated in healthy subjects in this clinical trial.

There were no deaths or discontinuations due to TEAEs during the study. The 2 SAEs observed during the study (1 in Dose Group 0.3 mg/kg: emotional disorder of moderate intensity; 1 in Dose Group 30 mg/kg: ligament rupture of moderate intensity) were both not related to the IMP.

Overall, incidence of TEAEs was low; with 9 subjects (22.5%) reporting altogether 13 TEAEs, mostly not (9 TEAEs), or unlikely (3 TEAEs) related to the IMP. Only for 1 TEAE (mild back pain in Dose Group 30 mg/kg), a causal relationship to the IMP was considered possible. The events were mainly mild (10 TEAEs) or moderate (3 TEAEs); no TEAEs of severe intensity were observed. Except for 1 TEAE for which outcome could not be ascertained (SAE: torn ligament) as the subject was lost to follow-up, all TEAEs were recovered or resolved at the end of the study.

One subject in Dose Step 1 had a TEAE of special interest (pure tone audiometry with sensorineural hearing loss [SNHL] >3 dB). The SNHL was not confirmed upon re-measurement and therefore, this TEAE/AESI was assessed as unlikely related to the study drug.

No effects on safety laboratory parameters, vital signs, ECG parameters or exploratory biomarkers of renal toxicity were observed. No medically relevant effects were seen in otological examinations.

EBL-1003 (apramycin) was well tolerated locally at the infusion site.

There were 2 serious adverse events (SAEs):

- One subject (0.3 mg/kg apramycin) had admitted himself to hospital due to acute emotional decompensation 10 days after treatment. This was recorded as SAE. The event was not related to the study treatment but to a personal crisis. The subject completed the entire course of the study up to the Day 90 visit.
- One subject (30 mg/kg apramycin) was admitted to hospital after tearing the ligament of his right ankle joint. The event was not related to the study treatment (the subject had played soccer). He had completed the study up to Day 14. The subject was lost to follow-up, so the outcome and end date of the SAE could not be verified.

Pharmacokinetics

In the 30 subjects who received apramycin, the plasma concentration profiles of apramycin were similar to those observed by other aminoglycosides, such as gentamicin, after IV infusion.

Exposure characteristics ($AUC_{(0-t)}$ and C_{max}) increased with increasing doses with mean values (arithmetic and geometric) increasing by approximately the same factor as the dose increases. Variability of AUC was small, with coefficients of variation <20%. Variability of C_{max} was slightly larger. Observed mean exposure characteristics were in line with the predictions based on the population PK model for gentamicin (Xuan 2004) and the observed kidney function in the healthy volunteers.

2.5 Rationale

2.5.1 Study Rationale

In a study of apramycin in a mouse lung infection model demonstrated ≥90% lung penetration and multilog CFU reduction at doses of ≥5 mg/kg for an XDR *Acinetobacter baumannii* (*A baumannii)* isolate with an MIC of 8 mg/L (Becker 2020). This finding warrants particular interest in determining the ability of apramycin to penetrate human lung tissue. It has previously been shown that the lung penetration of plazomicin or amikacin in mice is not a good indicator for the ELF targets in humans. Since apramycin has shown lung penetration in mice higher than that of plazomicin, it remains to be confirmed whether it also penetrates well into human ELF.

The current Phase I lung pharmacokinetic study intends to administer apramycin in healthy subjects by intravenous infusion to assess primarily pulmonary drug penetration. It is the first step in assessing the pharmacokinetics of apramycin in the body compartments relevant for diseases associated with bacterial respiratory infections including hospital-associated pneumonia/ventilator-associated pneumonia (HAP/VAP). Secondarily, this study will continue assessing the overall safety and tolerability of apramycin and exploring specifically ototoxic and nephrotoxic effects. The effects on ECG will also be evaluated.

2.5.2 Rationale for Dose Selection

A single dose of 30 mg/kg had been selected for this study based on the favorable safety and tolerability profile observed in the FIH clinical trial in healthy subjects (IB 2021). In addition, based on the translational PK-PD modeling study to assess efficacy, 30 mg/kg of EBL-1003 (apramycin) is the anticipated therapeutic dose for future clinical investigations in patients (Tomas Sou 2021).

2.6 Identified and Potential Risks and Benefits Associated with the Study

2.6.1 Risks and Benefits Associated with the Study Drug, Apramycin

Identified Risks

There are no identified risks associated with apramycin to date. Single apramycin doses ranging from 0.3 to 30 mg/kg body weight were generally safe and well-tolerated in the FIH Phase 1 clinical trial conducted in healthy subjects (IB 2021).

Potential Risks

Based on the knowledge on other aminoglycosides, the following contraindications will be assumed for apramycin:

- Known hypersensitivity to aminoglycosides
- Myasthenia gravis

Potential Drug Interactions

There are no clinical data on drug interactions with apramycin.

Based on the knowledge on other aminoglycosides, concomitant administration of the following drugs should be avoided:

- Drugs affecting renal function, e.g., renin–angiotensin aldosterone system [RAAS] inhibitors: angiotensin-converting enzyme [ACE]-inhibitors, angiotensin II [AII]-receptor antagonists, spironolactone, epleronone; non-steroidal anti-inflammatory drugs [NSAIDs] including cyclooxygenase 2 [COX-2] selective inhibitors).
- Drugs with potential ototoxic effects, e.g., vancomycin, loop diuretics, quinine and quinidine derivatives including mefloquine.
- Neuromuscular blocking agents. e.g., nondepolarizing (steroidal [e.g., d-tubocurarine, rocuronium, vecuronium, pancuronium) and benzylisoquinolinium [e.g., mivacurium, atracurium, cisatracurium]).
- Other aminoglycosides, e.g., gentamicin, tobramycin, amikacin, plazomicin, streptomycin, neomycin, and paromomycin.

Use During Pregnancy and Lactation

- Aminoglycosides pass the placenta barrier and may cause fetal harm. Therefore, apramycin should not be administered to pregnant women.
- Aminoglycosides have been reported to be detected in maternal milk. Therefore, apramycin should not be administered to breastfeeding women.

Undesirable Effects

Until clinical experience with apramycin is available, similar undesirable effects as observed for other aminoglycosides should be anticipated. Special attention should be paid to signs and symptoms of nephrotoxicity, ototoxicity, and vestibular toxicity.

If symptoms are observed after the IMP has been administered, therapy has to be guided by the predominant symptoms. Subjects will have to remain under medical surveillance until all relevant symptoms have subsided. In case of adverse events, the investigator has to decide if the subject has to be withdrawn from the trial. Emergency drugs and equipment have to be available at the study site.

Overdose

There is no specific antidote in case of apramycin overdose. The same measures as recommended for other aminoglycosides (treatment cessation, adequate hydration, dialysis, calcium salts) should be considered until further clinical data are available for apramycin (IB 2021).

Drug Abuse and Dependency

No clinical experience is available.

2.6.2 Bronchoscopy and Bronchoalveolar Lavage Risks

Bronchoscopy with bronchoalveolar lavage has a proven safety record in both clinical applications as well as human research during more than 40 years of clinical use of this procedure in hundreds of thousands of subjects, without any serious lung troubles and side effects. However, like all procedures, there are some risks associate with it (Zhang 2020).

The most common side effect related to the procedure is transient hypoxemia, which may require administration of oxygen, and transient tachycardia and premature contractions, which may require no treatment or appropriate intervention.

Other side effects that could be related to the procedure include nausea and dizziness, due to the drugs used for sedation or anesthesia, and nasal stuffiness and irritation, throat irritation, hoarseness and cough, due to the passage of the bronchoscope and the bronchoalveolar lavage (BAL).

A post-bronchoscopy fever (PBF), typically less than 40°C / 100°F is rare and reported with variable rate, ranging from 0% to 10% in some series. It may occur approximately 8 h (range 4 to 24 h) after BAL and lasts about 14 h. It is not accompanied by bacterial infection or pneumonia with abnormal chest X-ray. Antibiotics are not used for prevention and treatment targets the symptoms (drugs to lower fever, good hydration). Additional tests may be needed to guide treatment (Um 2004).

Rare complications, with incidence 0.1 to 0.3%, are perforation of the bronchus with pneumothorax, atelectasis, and infection. Bleeding has been described for bronchoscopy with biopsy of the lung but is very rare (0.1% or less) if only BAL, and is related to mechanical trauma, especially in subjects who have a disorder of blood coagulation or take anticoagulants (heparin, warfarin, new anticoagulants) and non-prescription non-steroidal anti-inflammatory drug (such as aspirin, and ibuprofen).

These risks are mitigated by adherence to the protocol eligibility criteria. During the bronchoscopy, subjects will be placed on supplemental oxygen and monitored continuously for oxygen saturation and ECG. Topical anesthetic, sedative or anesthetic dosages will be administered according to standard medical practices by experienced and hospital-certified staff. Pre- and post-bronchoscopy care will be provided and will include adequate nursing staff and standard post-sedation recovery care with direct investigator oversight. Finally, the procedure will be performed by experienced pulmonologists that are hospital-certified to perform this procedure and assisted by experienced and certified personnel in specially equipped hospital rooms equipped to handle emergencies.

2.6.3 Audiometry and Otoacoustic test Risks

Generally, there are no risks associated with the planned procedures to examine the ear and assess cochlear (hearing) function.

2.6.4 Venipuncture and IV Catheter Placement Risks

Venipuncture: Venipuncture causes transient discomfort and may cause fainting. Bruising at the site of venipuncture may occur but can be prevented or lessened by applying pressure for several minutes. Infection at the site is possible but highly unlikely as aseptic technique will be used.

IV catheter placement: An indwelling catheter may be placed in an arm vein (preferably antecubital) for frequent blood drawing for PK measurements. The catheter may cause phlebitis with signs of redness and warmth at or near the IV insertion site, and thrombophlebitis with a hard area palpable near the IV insertion site. These risks are minimal as the IV catheters, when used, are only used briefly after dosing. Careful inspection of the catheter site, including visualization of blood return, and withdrawal of the catheter if needed will minimize this risk. There is a risk of infection; however, this is a small risk as aseptic technique will be used.

2.6.5 Additional Risks

ECG: Possible side effects from ECG patches include a rash or minor irritation of the skin.

Blood draws: The amount of blood drawn is about 25.7 mL at Screening Visit, 22.2 mL on Day -2, 188.5 mL during the inpatient period (Days 1 to 3), 22.2 mL at Day 14 Visit, 3.5 mL at Day 30 Visit, and 262.1 mL during the entire trial (Appendix C, Table 5). Additionally, small amounts of blood loss may occur if an IV catheter is used or additional blood samples are collected (for repeat laboratory testing, TEAE evaluation, etc.). Overall, the amount of blood that may be drawn during the trial is within the amount that are considered safe to be drawn during short or extended periods, respectively, and not excessive for the safety and PK assessment requirements of Phase 1 trials. However, there is a small risk that some subjects may develop mild symptoms of hypovolemia or anemia during the trial. These are reversible with specific treatments (e.g., fluid replacement, good nutrition, vitamins, or iron supplementation).

2.7 Known Potential Benefits

The trial has no direct benefit for subjects participating in the trial. Knowledge gained in the trial could be of future benefit to public health and to individuals with infections, who might benefit if the study drug is licensed.

In accordance with the Health Insurance Portability and Accountability Act (HIPAA) regulation, subjects will be promptly notified of any clinical test results that would have an impact on their health. Screening evaluations may detect previously unknown abnormalities that could be clinically significant. This could benefit a subject, as it may lead to earlier diagnostic evaluation and treatment of an underlying disorder.

2.8 Risk/Benefit Ratio

This ratio cannot be calculated by available data. The risk for an SAE occurring in a subject treated with single doses of 30 mg/kg body weight is considered to be very low. Subjects will be

treated in an inpatient unit and closely monitored during the trial, so that appropriate emergency care can be provided immediately if an acute event occurs.

Data collected in the clinical development of apramycin to date, support further development of apramycin. The benefit of a potential new addition to the drugs available for the treatment of antibiotic-resistant bacteria and of using a new generation aminoglycoside with lower toxicity than licensed preparations, outweighs the known risks of the medication and the procedures to be used in this trial.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

3.1.1 Primary

- To assess plasma pharmacokinetic (PK) profile of apramycin and lung penetration of apramycin in epithelial lining fluid (ELF) and alveolar macrophages (AM) after single intravenous (IV) apramycin dose of 30 mg/kg in healthy subjects.

3.1.2 Secondary

- To assess the safety of single IV administration of 30 mg/kg apramycin in healthy subjects.
- To assess changes in otoacoustic testing.

3.1.3 Exploratory

- To assess changes in kidney function biomarkers.

3.2 Study Outcome Measures

3.2.1 Primary

- Lung concentration o and PK parameters of total apramycin:
  - ELF: C_{max}, $AUC_{(0-24)}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, C_{max}, T_{max}, t½
  - AM: C_{max}, $AUC_{(0-24)}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, C_{max}, T_{max}, t½
- Plasma concentration and PK parameters of total apramycin:
  - $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, $AUC_{(0-24)}$, C_{max}, T_{max}, t½, CL_T, Vd central,
- Ratio of exposure parameters of Lung PK to Plasma PK:
  - ratio of C_{max} in ELF over C_{max} in plasma
  - ratio of C_{max} in AM over C_{max} in plasma
  - ratio of $AUC_{(0-24)}$, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ in ELF over $AUC_{(0-24)}$, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ in plasma
  - ratio of $AUC_{(0-24)}$, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ in AM over $AUC_{(0-24)}$, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ in plasma

3.2.2 Secondary

- Type and incidence of treatment-emergent adverse events (TEAEs) and serious AEs (SAEs) to Day 30 (± 4 days) after dosing.

- Frequency of clinically significant TEAEs in physical examination (PE) findings and of changes from baseline in vital signs and clinical laboratory parameters until Day 14 (± 3 days) after dosing.
- Frequency of changes in ECG intervals (QTcF, PR, QRS, HR), and of morphological changes from baseline until 24 ± 1 h after dosing.
- Type and incidence of TEAEs related to auditory (cochlear) function tests (pure-tone audiometry and distortion product otoacoustic emissions [DPOAEs]) to Day 30 (± 4 days) after dosing.

3.2.3 Exploratory

- Changes from baseline in kidney injury molecule 1 (KIM-1) and serum cystatin C concentrations until Day 30 (± 4 days) after dosing.

4 STUDY DESIGN

This is a Phase 1, single center, open-label, study to evaluate the plasma and lung pharmacokinetics (PK), safety and tolerability of a single intravenous (IV) dose of apramycin in twenty healthy male and female subjects 18 to 45 years of age (inclusive).

Each subject will be enrolled to one of five cohorts (T1-T5) before dosing on Day 1. Four subjects will be allocated to each cohort. Each cohort corresponds to the timepoint after dosing when a single bronchoscopy with bronchoalveolar lavage (BAL) will be performed: 0.5 h (± 5 min) (Cohort T1), 2 h (± 5 min) (Cohort T2), 4 h (± 10 min) (Cohort T3), 8 h (± 15 min) (Cohort T4), and 24 h (± 1 h) (Cohort T5). The timing of bronchoscopy and BAL in cohort T5 is nominal. The final timepoint will be determined after analysis of apramycin concentrations in BAL. If apramycin is detectable in BAL collected from cohorts T1-T4, then T5 will be enrolled. If it is not detectable, then cohort T5 will not be enrolled. Blood will be collected at several timepoints to 60 hours after dosing (see collection schedule in Section 8.5.1) and will include samples that will coincide with BAL collections. The concentration and PK exposure parameters (C_{max} and $AUC_{(0\text{-}t)}$) of total apramycin in epithelial lining fluid (ELF) and alveolar macrophages (AM), collected through BAL, and in plasma collected at the scheduled timepoints to 8 h after dosing, will be evaluated from the first four cohorts, T1-T4, to determine if apramycin penetrates into the lung tissues and dosing of the T5 cohort is warranted. If cohort T5 is dosed, the final concentration and PK analysis of apramycin in BAL samples and plasma will include data from all T1-T5 cohorts.

Subjects will participate in the study for approximately 58 days, including a 28-day screening period, consisting of an out-patient period of 26 days (Day -28 to Day -3) with 1 to 2 site visits and 2 inpatient days, Day -2 and Day -1, to confirm eligibility, enroll and complete baseline assessments; a 3-day in-patient treatment period, with treatment administered on Day 1, bronchoscopy with BAL done within 24 h after dosing, and in-patient follow up completed on Days 2 and 3; and a 27-day out-patient follow-up period with site visits on Day 14 (± 3 days) and Day 30 (± 4 days), the last follow up visit. It is anticipated that the duration of the study will be about 6 months to enroll Cohorts T1-T4 and 10 months to complete Cohort T5, if enrolled, following an interim data analysis to review lung PK data.

Enrollment will be rolling and will be coordinated with the bronchoscopy service. Tentative reservations will be made and confirmed once subject eligibility is determined. Enrollment and treatment of subjects will be staggered and no more than two subjects will be dosed on a single day not less than 2 hours apart. Additional subjects may also be admitted to the Clinical Trial Unit (CTU) before dosing and may serve as back-up study subjects. A subject assigned to one of the T1 to T4 cohorts may be reassigned to another cohort depending on the availability of a bronchoscopy time slot. (See Section 5.3.1)

Apramycin will be reconstituted in sterile normal saline and a 30 mg/kg dose will be administered as a single IV infusion in a forearm vein over 30 min (± 5 min) using a syringe or infusion pump. (See Section 6.2 for DP preparation and administration).

Safety monitoring during the inpatient period will include: (a) Scheduled assessments per protocol: a physical examination (PE), vital signs (VS; including HR, BP, RR and T); 12-lead standard ECG (in triplicate at scheduled timepoints on Day-1 matching planned timepoints on Day 1); clinical safety laboratory tests (Hematology [HEM], Coagulation [COAG], Chemistry [CHEM] including estimated Glomerular Filtration rate [eGFR], and Urinalysis [UA]; and audiology testing (middle ear examination and cochlear testing [hearing testing, consisting of pure-tone audiometry and DPOAEs). Exploratory plasma and urinary nephrotoxicity biomarkers (plasma cystatin C and urinary KIM-1) will also be measured. In addition, (b) Unscheduled assessments after bronchoscopy will be performed according to orders by the attending pulmonologist and as needed. Subjects with on-going TEAEs will be followed until resolution or stability of the TEAE as assessed by the PI.

Safety Monitoring Committee (SMC) Role

A SMC will be appointed to oversee the safe conduct of the trial, review safety data and provide recommendations on safety monitoring in the current and future clinical trials with apramycin. A scheduled interim SMC meeting will be held after all subjects in Cohorts T1-T4 complete all assessments. If the last cohort (T5) will be enrolled for dosing, a final SMC will be held to review cumulative data on all subjects. If Cohort T5 is not dosed, the interim meeting will be considered final.

If criteria for halting the trial (as listed in Section 9.5.1) are met, enrollment and dosing of new subjects will be suspended, and an *ad hoc* SMC meeting will be held to review all available safety data. Study procedures in subjects already enrolled and dosed will continue per schedule. A suspended clinical trial will resume upon recommendation by the SMC and decision by DMID, NIAID (the sponsor).

4.1 Sub-studies

No sub-studies are planned.

5 STUDY ENROLLMENT AND WITHDRAWAL

Only subjects who meet all inclusion criteria and no exclusion criteria will be eligible for enrollment into the trial. No exemptions are granted on Inclusion/Exclusion Criteria in DMID-sponsored trials.

Twenty healthy male and female subjects, aged 18 to 45 years inclusive, will be enrolled in the trial.

5.1 Subject Inclusion Criteria

All must be answered YES for the subject to be eligible for study participation:

1. Subject reads and signs the Informed Consent Form (ICF) and agree to have bronchoscopy with bronchoalveolar lavage under sedation or light anesthesia and comply with study procedures.
2. Healthy male or non-pregnant, non-lactating female subjects 18 to 45 years of age (both inclusive) at the time of dosing.
    a. Note 1: Determined by medical history (MH), medication use, physical examination (PE), and vital signs, clinical laboratory tests and 12-lead ECG within reference ranges at Screening and Day-2. (See Sections 8.1 and 8.2, Appendix B, Table 2, Table 3 and Table 4 and the study-specific MOP.)

    Exceptions to BP, HR and laboratory test values being with normal ranges are:
    - Abnormal HR and BP on first measurement may be repeated twice more with the subject resting between measurements for at least 5 min according to Section 8.1.6.
    - Subjects with baseline HR ≥ 45 to 50 bpm may be accepted if otherwise healthy adults with known history of asymptomatic bradycardia.
    - Subjects with baseline SBP up to 140 mmHg and DBP up to 90 mmHg may be accepted if otherwise healthy.
    - A laboratory value that is Grade 1 will be allowed if not considered to be clinically significant by the investigator, with the exception of ALT, AST, AP, BUN, urine protein, serum creatinine or estimated glomerular filtration rate (eGFR) <70 mL/min /1.73 m^2 by the Chronic Kidney Disease Epidemiology collaboration (CKD-EPI) equation.
3. Female subjects of childbearing potential should use highly effective methods of contraception from the time of screening to 30 days after dosing.
    - Note 1: A female is considered of childbearing potential unless post-menopausal (defined as history of ≥1 year of spontaneous amenorrhea and a FSH level >40 IU/L), or permanently surgically sterilized.

- Note 2: Highly effective contraceptive methods include: (a) surgical sterilization methods, such as tubal ligation, bilateral oophorectomy, salpingectomy, hysterectomy, or successful tubal obliteration (e.g., Essure®) with documented radiological confirmation test at least 90 days after the procedure, or (b) long-acting reversible contraception, such as progestin-releasing subdermal implants, copper intrauterine devices (IUDs), levonorgestrel-releasing IUDs.
- Note 3: A subject who is not sexually active and abstains from sexual intercourse can be enrolled and abstinence documented.

4. Males, including vasectomized men, having sexual intercourse with women of childbearing potential must agree to consistent use of condoms from IMP administration through at least 30 days after dosing, and must also agree to not donate sperm during this same time period.
   - Note: A subject who is not sexually active and abstains from sexual intercourse can be enrolled and abstinence documented.
5. BMI 18.0 to 32.0 kg/m^2 (inclusive) and body weight not less than 50 kg.
6. Subjects with normal hearing, i.e., symmetric hearing with air conduction thresholds no worse than 20 dB hearing loss for the frequencies 0.5-1-2-4-6-8 kHz bilaterally.
7. Normal (reproducibility 70% or better) of distortion product otoacoustic emissions (DPOAEs).
   Note: Absence of DPOAEs at no more than two consecutive or non-consecutive DPOAEs in each ear is acceptable.
8. Normal otoscopic findings in the ears, normal tympanic membrane mobility and stapedial reflex present.
9. From the signing of the informed consent until the last follow-up visit, subjects must be willing to avoid exposure to loud music or noise.
   - Note: Noise avoidance to include continuous usage of earpieces at high volume, attending loud concerts or dance events, or using firearms or attending fireworks.
10. Normal lung function with Forced Expiratory Volume in the first second (FEV1) predicted ≥ 80% and FEV1/Forced Vital Capacity (FVC) > 70%.
11. Subjects must be willing to avoid excessive physical exercise within 48 h prior to dosing until discharge from the CTU on Day 3, and 24 h before each follow-up visit (Day 14 ± 3 days and Day 30 ± 4 days).
12. No history of acute febrile or infectious illness for at least 7 days prior to the administration of the IMP.
13. No history of lower respiratory tract infection within 4 weeks prior to screening.

14. Have adequate venous access for infusion and blood draws.

5.2 Subject Exclusion Criteria

All must be answered NO for the subject to be eligible for study participation:

1. Lactating females.

Medical and surgical history:

2. Any history of hypersensitivity to aminoglycosides.
3. Any history of drug hypersensitivity, asthma, urticaria or other severe allergic diathesis.
4. Any history of seasonal allergies with ongoing symptoms for more than a week prior to dosing requiring glucocorticoids and/or frequent use of antihistamines for treatment.
5. Any history of a chronic condition that may increase risk to subject or interfere with endpoint assessment, or any unstable chronic disease.
   - Note 1: Unstable chronic disease is defined by need for frequent medical interventions that lead to a change in medications and/or required hospitalization, surgery or an invasive procedure or emergency department/urgent care visit.
   - Note 2: Any chronic disease, that has been diagnosed within 90 days of screening is excluded.
6. History of any psychiatric medical condition that has required hospitalization in the last 5 years or subject is considered psychologically unstable by the investigator.
7. History of acute or chronic problems with hearing and/or balance in the last 24 months.
   - Note: These include but not limited to use of hearing aid, head injury leading to otologic damage, tumor of the head or neck, autoimmune disease of the inner ear, tinnitus, vestibular disease, auditory neurinoma, endolymphatic hydrops and/or Meniere's disease, perilymphatic fistula, otitis media, labyrinthitis, sudden hearing loss, known retrocochlear hearing impairment, conductive hearing loss exceeding 10 dB at any frequency, ear canal and/or middle ear disease including inflammation or effusion, pathological tympanometry.
8. Past injury or surgery to the middle or inner ears.
   - Note: Myringotomy or tympanic tube insertion in childhood with complete healing and normal hearing test are excluded.
9. Family history of hearing loss before the age of 60.
10. Subjects who have had previous intolerance or contraindications to medications applied for sedation or anesthesia during bronchoscopy.

- Note: These include benzodiazepines or topical anesthetic agents (lidocaine or xylocaine) including reversal agents such as flumazenil.

Laboratory examinations:

11. Positive serum pregnancy test for women at screening or urine pregnancy test at check-in.
12. Positive test for HIV antibodies, hepatitis B-virus surface antigen (HBsAg), or anti-hepatitis C-virus antibodies (anti-HCV).

Prior medication:

13. Use of any prescription or non-prescription medication prior to the dose of IMP as described in Section 6.6.
    - Note: Exceptions are hormonal contraceptives, which are permitted throughout the study, and solitary doses of up to 1,000 mg paracetamol.
14. Use of any investigational drug product within 30 days or 5 half-lives (whichever is longer) before dosing.
15. Planned participation in a clinical research study that requires treatment with a study drug or blood draws or other invasive assessments during the study period (screening until final visit).

Lifestyle restrictions:

16. More than low-risk alcohol consumption (men: ≥ 24 g of pure alcohol regularly per day; women: ≥12 g of pure alcohol regularly per day) for the previous 3 months.
17. Any history of alcohol or drug abuse or positive alcohol breathalyzer test.
18. Suspicion of illicit drug use / abuse or positive urine drug screen test (See Section 6.7.4 and Section 8.2.5 for prohibited substances).
19. "History of ≥10 pack-years smoking, or history of any nicotine use in the 6 months before check-in (Day -2) or positive urine cotinine screen at check-in.
    - Note 1: Nicotine products include cigarettes, e-cigarettes, pipe, cigar, chewing tobacco, nicotine patch.
    - Note 2: A positive urine cotinine at screening is allowed if negative at check-in (Day -2).
20. Caffeinated beverages/foods are prohibited within 48 hours before dosing to Day 3 of the trial. During the follow up period, consumption is restricted to not more than 3 cups or equivalent per day.
21. Judged by the investigator to have occupational noise exposure of high risk during the trial (e.g., construction site workers, military workers, etc.).

22. Blood or plasma donation of 500 mL within 3 months or more than 100 mL within 30 days before signing informed consent or planned donation prior to completion of this trial.

5.3 Treatment Assignment Procedures

5.3.1 Enrollment Procedures

Twenty healthy subjects who consent to participate in the trial and meet the eligibility criteria will be enrolled following admittance to the CTU and confirmation of eligibility. Subjects will be registered using Advantage eClinical®, a web-based application developed by The Emmes Company, LLC, the DMID Statistical and Data Coordinating Center (SDCC) for the trial. Reasons for screen failure or no enrollment and dosing of an eligible subject will be listed in the MOP and also be entered in the system. Instructions for using the enrollment module are included in the Advantage eClinical® User's Guide.

Since this is an open-label study of a single dose of IMP, subjects will be enrolled on a rolling basis. Four subjects will be assigned to each one of five cohorts, T1-T5 (Section 4), for lung PK with equal allocation (1:1:1:1:1) and registered into the Advantage eClinical®. Each cohort corresponds to a single Lung PK sampling timepoint. It is planned to enroll subjects into cohorts T1 to T4 according to availability of the bronchoscopy suite. However, on the day of bronchoscopy, a subject may be reassigned to another cohort than planned on the same day, depending on emergencies at the bronchoscopy suite at the time originally planned, or cancelled.

Appointments for the bronchoscopy procedure will be made in advance and every effort will be made to admit, dose and perform bronchoscopy with BAL according to the schedule. However, if a scheduled bronchoscopy cannot be performed according to schedule on a dosing day, the following steps may be taken according to the time of cancellation before or after dosing:

- *If the procedure is cancelled before administration of study drug*, an effort will be made to reschedule the procedure.
- *If the procedure is cancelled after administration of the study drug*, bronchoscopy may be either rescheduled if it can be performed at a later time that can accommodate an available Lung PK timepoint up to 8 h after dosing or cancelled.
    - If bronchoscopy is rescheduled, the registration in the Advantage eClinical® will be updated to indicate the cohort that the subject completed and a future subject would be assigned in the registry to complete the missed Lung PK timepoint.
    - If it is not feasible to reschedule bronchoscopy, the subject will be terminated from participation in the study and will be replaced. The subject will be asked to remain in the study to complete safety and otoacoustic testing until Final Visit (Day 30 ± 4 days). If the subject declines to remain in the study for safety assessments,

appropriate early termination assessments will be completed prior to discharge from the CTU. (See Section 5.3.3 and Section 7.7)

5.3.2 Reasons for Withdrawal and Discontinuation of Study Drug Administration

A subject may withdraw from the trial at any time for any reason, without any consequences.

A subject will be discontinued from the trial if any of the following occur before dosing:

- Request by the subject to terminate participation.
- Failure to receive the study drug due to difficulty initiating or maintaining an intravenous infusion line.

A subject may be removed from the trial after dosing for the following reasons; however, whenever possible, the subject will be followed for safety per protocol to Day 30 (± 4 days):

- Failure to adhere to protocol requirements.
- Loss to follow-up.
- Request of primary care provider.
- Request of the Institutional Review Board (IRB)/Ethics Committee (EC), FDA or DMID.
- The subject's well-being, based on the opinion of the investigator.
- The occurrence of an SAE or TEAE warranting withdrawal.
- Failure to receive the entire volume of apramycin solution during the designated infusion time.

5.3.3 Handling of Withdrawals and Discontinuation of Administration

- Subjects who are withdrawn before dosing may be replaced with a subject assigned to the same lung PK timepoint group (T1-T5).
- Subjects who did not receive the entire infusion volume of apramycin will be replaced and withdrawn from bronchoscopy and associated procedures and from PK analysis but will be encouraged to continue follow up for safety.
- Subjects who withdraw after receiving the entire study treatment but before the scheduled bronchoscopy and associate procedures will be replaced and withdrawn from the study and data analysis but will be followed for safety.
- Subjects who withdraw after receiving the entire study treatment and completed scheduled bronchoscopy and assessments will not be replaced unless more than 50% of subjects in a Lung PK timepoint cohort are withdrawn from the trial.
- Generally, subjects who received the entire amount of the study drug but withdraw from the trial will be encouraged to continue follow-up (with subjects' consent) for safety assessments and plasma PK sample collection.

- Subjects withdrawing will be asked to complete safety and otoacoustic testing according to the ET schedule (See Section 7.7).

5.3.4 Lost to Follow-up

If subjects fail to appear for a follow-up assessment, extensive effort (i.e., three documented contact attempts via phone calls, e-mail, etc., made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subjects' records.

5.3.5 Termination of Study

Although DMID has every intention of completing the trial, it reserves the right to terminate the trial at any time for clinical or administrative reasons. In addition, the trial may be terminated or suspended at the request of the FDA, SMC or IRB/EC.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Description of Study Product

Product: Apramycin (EBL-1003)

The active drug substance apramycin is chemically a monosubstituted 2-deoxystreptamine. It is manufactured as crystalline free base by purification of the commercially available intermediate apramycin sulfate (CAS 37321-09-8). Apramycin sulfate is manufactured by fermentation (*Streptoalloteichus tenebrarius*) by Shandong Qilu King-Phar Pharmaceutical Co, China and then recrystallized to apramycin free base and purified by Research Institutes of Sweden (RISE). Drug Substance is then formulated and bottled by Patheon Ferentino, Italy (a Thermo Fisher Scientific company) for Juvabis AG, the pharmaceutical sponsor of the study.

The diluent for apramycin will be 0.9 % sodium chloride injection, USP. The USP grade 0.9% Sodium Chloride Injection, or normal saline, is a sterile, nonpyrogenic, isotonic solution; each mL contains sodium chloride 9 mg. It contains no bacteriostatic agent, antimicrobial agent, preservatives, or added buffer and is supplied only in single-dose vials. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3, range 4.5 to 7.0). This product should be used to dilute apramycin to the desired concentration. 0.9% sodium chloride for injection, USP is clear in color.

6.1.1 Acquisition

Product: Apramycin (EBL-1003)

Upon request by DMID, study product will be shipped for clinical labeling from a German location supplied by CSM (a Clinigen company) to the following address:

DMID-Clinical Materials Services (CMS)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876

Tel: 240-477-1350
Fax: 240-477-1360

E-mail: DMID.CMS@ThermoFisher.com

The diluent, 0.9% Sodium Chloride Injection, USP, will make up the infusion volume (30 mL) and will be provided by the clinical research site. Details will be provided in the protocol-specific Manual of Procedures (MOP).

The IMP will be shipped from DMID-CMS to the CTU upon request and approval by DMID. Details will be provided in the protocol-specific MOP.

Ancillary supplies required for IV administration (including syringes or infusion bag, tubing and IV catheter and line-filter) will be supplied by the clinical research site as described in the protocol-specific MOP.

6.1.2 Formulation, Packaging, and Labeling

Product: Apramycin (EBL-1003)

Apramycin for Infusion, the IMP, is formulated as 150 mg free base /mL in sterile aqueous solution, pH 5.5 to 6.0 adjusted with sulfuric acid. The study product is supplied in 20 mL glass vials. A volume of ≥18 mL can be withdrawn from each vial. To protect the solution from light, the vials are stored in folding boxes.

The clinical research site will provide the diluent, 0.9% sodium chloride injection, USP. Commercial source, product information and packaging will be described in the MOP.

Each product vial and diluent will be labeled in compliance with applicable regulatory requirements, including the FDA-required cautionary statement, *"Caution – New drug – Limited by Federal (or United States) Law to Investigational Use".*

6.1.3 Product Storage and Stability

Product: Apramycin (EBL-1003)

Apramycin for Infusion will be shipped refrigerated at +5 ± 3°C (35.6 to 46.4°F) and should be stored refrigerated at 2 to 8°C (35.6 to 46.4°F) within the packaging until time of preparation. Protect from freezing. The reconstituted solution must be stored at 2 to 25°C (storage at 2 to 8°C if possible) and used within 5 hours or discarded. If not used, it must be quarantined and maintained for study product accountability as per Section 6.4.

The diluent, 0.9% NaCl for injection, USP, will be stored per manufacturing instructions. Product storage and stability will be described in the MOP.

The clinical supplies storage area at the CTU will be monitored by its staff for temperature consistency with the acceptable storage temperature range specified in this protocol or in the MOP. Documentation of temperature monitoring will be maintained.

6.2 Dosage, Preparation, and Administration of Study Intervention/ Investigational Products

The site Research Pharmacist (RP) will prepare the study product on the same day as administration. The study drug will be inspected for damage, contamination, discoloration, or

particulate matter before use. Any study drug that fails inspection will be quarantined at appropriate temperature and labeled 'Do Not Use' until further notice. The Site Principal Investigator (PI) or responsible person will immediately contact the DMID Clinical Project Manager (CPM) and study clinical team for further instructions before administering any additional study drug infusions. Based on the information collected, DMID and/or Juvabis AG will determine whether the affected study drug can be used. If it cannot be used, the CTU will receive specific instructions on how to return it to DMID CMS or destroy it on site.

Preparation of the product will be performed using aseptic technique under a sterile environment (e.g., Biologic Safety Cabinet or laminar flow hood). Each subject will receive 30 mg/kg of apramycin in a volume of 30 mL as a single IV infusion over 30 (± 5) minutes in a hand or forearm vein using a syringe or infusion pump. Based on the subject weight, the appropriate weight-based apramycin for infusion dose will be calculated and the appropriate number of vials will be removed from storage to prepare the infusion. Sterile normal saline will be used to adjust the volume.

The subjects will be admitted to the Phase 1 unit 2 days before the planned infusion. Verification that the subject still meets all inclusion criteria and does not have any exclusion criteria must be made on the morning of Day 1 prior to dosing. The site Research Pharmacist will prepare the infusion as described in the protocol-specific MOP. Apramycin for infusion should be administered as a single IV infusion over 30 min (± 5) using a syringe or infusion pump via a hand or forearm vein. The drug should not be administered as a bolus. The IV administration set must contain a 0.20 µm in-line filter. Blood for plasma PK sampling will be drawn from a catheter inserted in a vein in the other forearm.

Refer to protocol-specific MOP for detailed information on apramycin dose and volume calculations, as well as preparation and administration of the study product and handling of infusion interruptions.

6.3 Modification of the Study Intervention/ Investigational Products

Not applicable for single-dose study drug trial. See Study Halting Criteria, Section 9.5.1. Handling of infusion interruptions will be reported in the MOP.

6.3.1 Overdose

An overdose is defined as a dose greater than the high-dose level evaluated in the trial. All overdoses will be reported as a deviation; if the overdose is associated with a TEAE, then the TEAE will also be reported. In the event of an overdose of apramycin, the Investigator will use clinical judgment in treating the overdose and contact the DMID Medical Monitor (MM). There is no specific antidote in case of apramycin overdose. The same measures as recommended for other aminoglycosides (treatment cessation, adequate hydration, dialysis, administration of

calcium salts for neuromuscular blockade) would be considered until further clinical data are available for apramycin. (IB 2021) The investigator will refer to the relevant document(s) for detailed information regarding warnings, precautions, contraindications, TEAEs, and other significant data pertaining to apramycin. Such documentation may include but not be limited to the IB.

6.4 Accountability Procedures for the Study Intervention/ Investigational Products

The Site PI is responsible for the distribution and disposition of the study drugs and has ultimate responsibility for accountability. The Site PI may delegate this responsibility to the Site RP. If delegated, the Site RP will be responsible for maintaining complete records and documentation of the study drug's receipt, accountability, dispensation, temperature monitoring, storage conditions, and final disposition. Time of study drug administration to the subject must be recorded on the appropriate data collection form (DCF).

All study drugs, whether administered or not, will be documented on the appropriate study drug accountability record or dispensing log. Used and unused apramycin vials will be retained until monitored. Upon completion of the trial and after the final monitoring visit, any remaining unused study drugs will either be returned or destroyed appropriately at the CTU as per DMID requirements and instructions that will be communicated to the CTU by the DMID CPM. Unused apramycin vials will be released for disposition per DMID requirements. DMID does not require used containers of study product to be maintained at the research pharmacy, except when the local institution's SOP/policy mandates retaining used IV vials. If local SOPs allow destruction of used study product containers, the used vials can be destroyed per the site's SOPs; a second staff member must observe the destruction and sign verification (two signatures) that the used vials were discarded.

Any unused solution left in the IV infusion syringe or bag or the IV administration tubing after administration to the subject should be discarded as biohazardous waste. If a container of study product is unusable due to breakage, or any other reason, this explanation should also be noted on the Study Product Accountability Record (e.g., broken – dropped on floor). Details will be provided in the MOP.

6.5 Assessment of Subject Compliance with the Study Intervention/ Investigational Products

Since each dose of apramycin will be administered by site personnel, subject compliance is not anticipated to be an issue. Complete information regarding any partial or interrupted dosing will be documented. Subjects unable to receive the full volume of apramycin infusion will be withdrawn from data analysis and followed up for safety as described in Section 5.3.3.

6.6 Prior and Concomitant Medications/Treatments

Medications include the following: prescription drugs, birth control hormonal preparations, non-prescription medication, herbs, vitamins, nutritional supplements, and illicit and recreational substances.

Medications taken before or after dosing will be reported as Prior Medications or Concomitant Medications (ConMeds), respectively.

Prior prescription medications will be recorded at Screening Visit. All prior medications are not allowed during the study period with the exception of oral contraceptives, which are permitted throughout the study, and solitary doses of up to 1,000 mg acetaminophen (paracetamol).

The following medications are prohibited for the indicated periods prior to dosing:

- Use of any investigational drug product within 30 days or 5 half-lives (whichever is longer) before dosing or planned use during the study period (screening until Final Visit).
- Use of aminoglycosides within 3 months prior to dosing.
- Use of neuromuscular blocking agents within 1 week or 5 half-lives (whichever is longer) prior to dosing.
- Use of potentially nephrotoxic medication 2 weeks prior to dosing: Renin– angiotensin-aldosterone system [RAAS] inhibitors: angiotensin-converting enzyme [ACE]-inhibitors, angiotensin II [AII]-receptor antagonists (enalapril, lisinopril, ramipril captopril, benazepril), spironolactone, eplerenone; non-steroidal anti-inflammatory drugs [NSAIDs] (aspirin, ibuprofen, naproxen, indomethacin, etc.) including cyclooxygenase 2 [COX-2] selective inhibitors (Bextra, Celebrex, Vioxx).
- Use of potentially ototoxic medication 3 months prior to dosing: vancomycin, loop diuretics (bumetanide, ethacrynic acid, furosemide), quinine and quinidine derivatives including mefloquine with the exception of occasional intake of quinine containing beverages.

Non-prescription medications, herbs, vitamins, and nutritional supplements will not be taken within 15 days before dosing and during the trial. *Exceptions*: vitamins and OTC medications taken for < 48 h for the treatment of common symptoms (e.g., headache, indigestion, muscle pain) may be allowed if approved by the designated study clinician.

Blood/blood products (RBCs, WBCs, platelets, and plasma) donation of 500 mL within 3 months or more than 100 mL within 30 days before signing informed consent to this trial is not allowed, and it is prohibited during the course of this trial.

Following dosing, each new ConMed and changes to existing medications will be recorded. Subjects will be required not to utilize non-study medications during the trial except those deemed necessary by the Site PI or sub-investigator.

Any drug (e.g., non-prescription medications, herbal supplements, vitamins, or prescription medications) or vaccines or blood/blood products used by the subject during the trial will be recorded in the subject's source documents and on the appropriate electronic case report form (eCRF), and the PI or authorized study clinician (listed on FDA Form 1572) will note whether the use was medically indicated and immediately necessary. Any use of medications not authorized by the study PI or authorized clinician will be recorded as a deviation.

6.7 Subject Restrictions and Precautions

From signing informed consent until end-of-study, the subjects have to follow the instructions of the study site. They have to adhere to the following restrictions during participation in the study.

6.7.1 Smoking

Smoking is prohibited during the in-patient period of the study (Day -2 to discharge on Day 3) and should be avoided during the out-patient follow-up period until the last visit (Day 30 ± 4 days).

6.7.2 Physical Activity

Subjects will be counseled to refrain from rigorous physical activity starting 2 days before dosing until discharge from the CTU on Day 3, and 24 h before each follow-up visit (Day 14 ± 3 days and Day 30 ± 4 days).

6.7.3 Food and Fluid intake

Subjects will be provided food and non-alcoholic beverages by the CTU during the dosing period of the trial. Subjects will fast at least 6 hours prior to blood draw on days of clinical laboratory testing (screening, Day -2, Day 1 [pre-dose], Day 2, Day 3 and Day 14 [± 3 days]). Subjects will fast for at least 6 h before dosing on Day 1 and 2 h after dosing. In addition, subjects will fast from 4 hours before to at least 2 hours after bronchoscopy. A light nutritional broth or beverage may be served if bronchoscopy is scheduled 4 h to 8 h after dosing on Day 1. Water can be taken *ad lib* during those periods of fasting. Water consumption will be at least 2 L daily from the time of dosing to 48 h (Day 2) after dosing, and the time and amount consumed will be recorded.

6.7.4 Alcohol, Marijuana and Illicit Drugs

Subjects will have to agree to consume not more than 24 g (men)/ 12 g (women) pure alcohol per day from Screening to enrollment (Day -2) and from the day of discharge from the CTU on Day 3 to Day 30 ± 4 days (final visit). Alcohol will not be served during the in-patient period

from Day -2 check-in to Day 3. Marijuana and illicit drugs are prohibited during the course of the trial.

6.7.5 Caffeinated beverages

Caffeinated beverages or foods containing caffeine are prohibited within 48 hours before dosing to Day 3 of the trial. On other days, consumption is restricted to not more than 3 cups or equivalent per day.

6.7.6 Exposure to loud noise

Subjects have to avoid exposure to loud noise (e.g., construction sites, visiting concerts or dance events, fireworks, listening to music or playing videogames with headphones) from screening until Day 30 ± 4 days (final visit).

7 STUDY SCHEDULE

The Schedule of Study Procedures and Evaluations is included as Appendix A.

7.1 Recruitment

The subject population will be recruited from the local population surrounding the CTU, utilizing the CTU subject database and IRB-approved advertisements and social media. IRB-approved, prescreening questionnaires will be used to determine if subjects meet study requirements before scheduling screening visits.

7.2 Screening Visit (Day -28 to Day -3)

This will be an out-patient visit to complete the following within 28 days before study drug dosing:

Note: A second screening visit may be scheduled to complete Otoacoustic screening tests to accommodate subjects' personal schedule.

- Obtain informed consent.
- Assign a study ID number to subjects who consent to participate.
- Record demographics including age, gender, race, and ethnicity.
- Obtain contact information.
- Obtain height and body weight, and calculate Body Mass Index (BMI; wt [kg] / ht [m^2])
- Take VS (supine and resting for at least 5 min systolic and diastolic BP [SBP and DBP], heart rate [HR], respiratory rate [RR], and oral temperature [T]).
- Obtain Medical History (MH).
- Review history of Prior Medications.
- Perform complete Physical Examination (PE).
- Obtain blood and urine samples for screening clinical laboratory tests that include a calculation of Glomerular Filtration Rate (GFR) by the CKD-EPI equation.
- Obtain blood samples for viral serology (HIV antibody, HBsAg, HCV antibody).
- Obtain serum for β-HCG pregnancy test from all women.
- Obtain serum for FSH level from only post-menopausal women.
- Obtain urine sample for illicit drugs and drugs of abuse (urine drug screen), and cotinine.
- Perform alcohol breathalyzer test.
- Obtain a single 12-lead ECG with 10-sec rhythm strip.
- Perform spirometry.
- ENT Examination that includes:
  - Ear Otoscopy.
  - Valsalva test.

  - Tympanometry.
  - Stapedial Reflexes.
- Cochlear (Hearing) Examination that includes:
  - Ear Audiometry (pure tone audiometry).
  - Distortion Product Otoacoustic Emissions (DPOAEs).
- Counsel on use of contraception (males and females), avoidance of pregnancy (women of childbearing potential), avoidance of prohibited medication, illicit drugs, alcohol, nicotine products, vigorous exercise, and exposure to loud noises).
- Confirm eligibility.

Subjects who meet the eligibility criteria of the otoacoustic assessments will be contacted by CTU personnel and asked to return on Day -2 to complete check-in assessments and be admitted to the CTU if they continue to meet eligibility criteria.

Subjects who fail screening due to a medical condition or abnormal laboratory tests including pregnancy test and positive tests for HIV, HBV, and HCV will be informed of the findings and counseled to seek medical care for further evaluation and treatment. If subjects test positive for HIV antibody, HBsAg, and/or HCV antibody, they will be informed that test results may be reported to the local health authorities according to state or local law.

Subjects who fail the otoacoustic screening will be informed of the findings and counseled to seek medical care by an otolaryngologist or audiologist for further evaluation and treatment.

Subjects who fail screening assessments on either one of these visits will not be rescreened unless they had an inter-current, short-term medical illness. Subjects who meet eligibility criteria but could not be enrolled within permissible window may be rescreened.

7.3 Check-in (Day -2)

Subjects meeting all inclusion and no exclusion criteria at Screening Visit will check into the CTU on Day -2 and the following procedures will be performed:

- Review inclusion/exclusion criteria to confirm the subject remains eligible for admission.
- Update MH.
- Update Prior Medications.
- Obtain VS.
- Obtain body weight.
- Perform complete PE.
- Obtain blood and urine samples for clinical laboratory tests that include a calculation of GFR by the CKD-EPI equation (for determining eligibility).

- For all women, a urine β-HCG pregnancy test will be done, and negative results confirmed before dosing.
- Obtain urine sample for illicit drugs and drugs of abuse (urine drug screen) and cotinine.
- Perform alcohol breathalyzer test.
- Obtain sample for serum Cystatin-C.
- Obtain sample for urinary KIM-1.
- Obtain a single 12-lead ECG with 10-sec rhythm strip.
- Perform otoacoustic assessments.
  - Ear otoscopy.
  - Audiometry (pure tone audiometry including high frequencies).
  - DPOAEs.

Note: Day -2 otoacoustic assessments may be completed on Day -4 or Day-3 as if needed.

- Admit eligible subjects in the CTU.
- Counsel to avoid rigorous physical activity, encourage good hydration, avoid caffeinated beverages and exposure to loud noises.

7.4 Baseline (Day -1)

Admitted subjects will have the following assessments:

- Review inclusion/exclusion criteria, before 12-lead ECGs, to confirm the subject remains eligible for enrollment.
- Update MH.
- Update Prior Medications.
- Obtain VS.
- Perform abbreviated PE.
- Assign subject to Lung PK timepoint cohorts (T1-T4) and enroll in Advantage eClinical®.
- Obtain baseline 12-lead standard ECG with 10-sec rhythm strip in triplicate at the same timepoints as the projected timepoints for ECGs to be recorded before and after dosing on Day 1 (within 30 min before and 1 h [± 5 min], 4 h [± 10 min], and 16 h [± 15 min] after start of dosing).
- Counsel to avoid rigorous physical activity, encourage good hydration, avoid caffeinated beverages.

7.5 Inpatient Treatment Period (Days 1 to 3)

7.5.1 DAY 1: Administration of Study Drug – Bronchoscopy (Cohorts T1 – T4)

Before Dosing:

- Withhold breakfast or food at least 4 hours before scheduled post-dose bronchoscopy, according to subject assignment into one the Lung PK cohorts (T1-T5).
- Allow access to water.
- Review inclusion/exclusion criteria to confirm the subject remains eligible for dosing.
- Update MH.
- Update Prior Medications and review any new medication.
- Obtain VS within 30 min before dosing (baseline).
- Perform symptom-directed PE.
- Obtain 12-lead standard ECG with 10-sec rhythm strip in triplicate within 30 min before dosing.
- Insert IV catheter for blood collection into a hand or forearm vein.
- Insert IV line for study drug infusion into a vein in the other forearm.
- Obtain blood and urine samples for clinical laboratory tests that include a calculation of GFR by the CKD-EPI equation (for baseline).
- Obtain sample for serum Cystatin-C (for baseline).
- Obtain sample for urinary KIM-1 (for baseline).
- Obtain pre-dose blood (plasma) PK sample for total apramycin within 30 min before starting administration of study drug.
    - Measure pre-dose urea concentration in an aliquot of the blood 9plasma) PK sample collected within 30 min before starting administration of study drug.

Note 1: *If 12-lead standard ECGs, VS assessments, and PK blood draws to be done at the same timepoint before or after dosing, these procedures will be performed in this order: 12-lead standard ECG, VS, and PK blood draw.*

Note 2: *In cohort T3, obtain ECG, VS and PK draws at the 4 h timepoint before administration of sedation or anesthetic for BAL.*

Note 3: *CTU staff may complete pre-dosing procedures in the bronchoscopy ward instead of the CTU to enable timely initiation of bronchoscopy at 0.5 h* (± 5 min) *after initiation of the dosing in subjects assigned to cohort T1.*

Dosing:

- Administer a single 30 mg/kg body weight dose of apramycin as a single IV infusion with a syringe or infusion pump over 30 (± 5) minutes in a forearm vein, as described in Section 6.2.
- Initiate TEAE assessments (excluding laboratory events) and SAE assessments.
- Perform focused PE (for TEAE assessments).

- Initiate recording of ConMeds.
- Withhold food until 2 h after end of dosing or 2 h after end of scheduled bronchoscopy according to subject assignment into one of the Lung PK cohorts (T1-T5).
- Allow access to water.

After Dosing:

- Bronchoscopy with BAL once per subject at the following nominal timepoints after starting IMP infusion according to the assigned cohort: 0.5 h (± 5 min) (T1), 2 h (± 5 min) (T2), 4 h (± 10 min) (T3) and 8 h (T4) (± 15 min).
  - Transfer subject to the UTMC bronchoscopy suite accompanied by CTU staff.
    - Note: Subjects in T1 may start receiving study drug in the bronchoscopy suite.
  - Collect scheduled safety information before and after the procedure in the bronchoscopy suite and during the recovery period.
  - Monitor subject before and after the procedure per orders by the bronchoscopy and anesthesia services.
  - Transfer subject to the CTU from the UTMC bronchoscopy suite accompanied by CTU staff.
  - Collect BAL samples for the measurement of total apramycin concentration and estimate of PK parameters once per subject at the assigned timepoint above, as described in Section 8.5, corresponding to the assigned cohort: 0.5 h (± 5 min) (T1), 2 h (± 5 min) (T2), 4 h (± 10 min) (T3) and 8 h (T4) (± 15 min) after initiation of dosing. Details on processing the BAL samples and preparation of ELF and AM samples for apramycin concentration measurements and PK analysis will be provided in the MOP.

    Note 1: Collect plasma samples for total apramycin concentration within 5 min of the corresponding BAL procedure at 0.5 h (± 5 min), 2 h (± 5 min), 4 h (± 10 min), or 8 h (± 15 min) after initiation of the infusion. If collection of BAL samples is delayed, plasma samples will be collected at the indicated nominal timepoint (and allowable windows), as described above, and an additional plasma PK will be collected within 5 min after the last BAL sample.
    Note 2: Measure urea concentration at the same timepoint as the nominal timepoint of plasma PK or in the plasma sample that is collected within 5 min after the last BAL sample if BAL is delayed.
  - Obtain the bronchoscopy Procedure Record. Data to be transcribed from procedure record onto study CRF will be described in the MOP.
- Check infusion site (during and end-of-infusion, and 1 h (±5 min) and 16 h (± 15 min) after the infusion for infusion site reactions. Details will be provided in the MOP.
- Obtain 12-lead standard ECG with 10-sec rhythm strip in triplicate at 1 h (±5 min), 4 h (±10 min) and 16 h (±15 min) after dosing. Record ECG before VS and PK draws and, at 4 h, before administration of sedation or anesthetic for BAL in cohort T3.

- Obtain VS at 1 h (±5 min), 4 h (±10 min) and 16 h (± 15 min) after dosing:
  - Note: *More frequent monitoring will be at the PI's discretion based on subject's clinical status after bronchoscopy.*
- Obtain blood (plasma) PK samples for total apramycin at 0.5 h (±5 min, immediately at the end of infusion), 1 h (±5 min), 2 h (±5 min), 4 h (±10 min), 8 h (±15 min), and 16 h (±15 min) after initiating the IMP infusion.
  - Note 1: An aliquot of plasma PK collected at 0.5 h (±5 min; immediately after the end of infusion) after starting the IMP infusion will be used for the protein binding test (for measurement of free apramycin).
  - Note 2: An aliquot of plasma PK collected at 0.5 h (±5 min) after starting the IMP infusion will be used for the measurement of urea.
- Monitor subject closely until fully recovered per bronchoscopy instructions.
  - Provide oxygenation as needed.
- Perform TEAE assessments (excluding laboratory events) and SAE assessments.
- Document ConMeds.
- Do not provide solid food until at least 2 h after bronchoscopy and the subject is able to swallow.
- Encourage water intake at least 2 L daily – Document time and volume consumed.
- Counsel to avoid rigorous physical activity and avoid caffeinated beverage**s.**

7.5.2 DAY 2: Inpatient Follow-up – Bronchoscopy T5

For a subject assigned to lung PK timepoint group T5:

- Perform bronchoscopy with BAL on Day 2 at 24 h (± 1 h) after dosing as described for subjects in cohorts T1-T4 (see Section 7.5.1, Day 1, *After Dosing*).
  - Collect BAL samples for PK analysis once per subject at the assigned timepoint, 24 h (±1 h) (T5) after initiation of dosing, as described in Section 8.5, corresponding to the assigned cohort T5.

    Note 1: Collect plasma sample for total apramycin concentration within 5 min of the corresponding BAL procedure at 24 h (± 1 h) after initiation of the infusion. If collection of BAL samples is delayed, plasma sample will be collected at the indicated nominal timepoint (and allowable windows), and an additional plasma PK will be collected within 5 min after the last BAL sample. (See Section 8.5.1)
    Note 2: Urea will be measured in plasma collected at the same nominal timepoint as the plasma PK sample or in the plasma PK sample that is collected within 5 min after the last BAL sample.
- Monitor subject closely until fully recovered per bronchoscopy instructions.
  - Provide oxygenation as needed.

For all subjects:

- Obtain single 12-lead standard ECG with 10-sec rhythm strip at 24 h (±1 h) and 36 h (±1 h) after dosing.
- Obtain VS at 24 h (±1 h) and 36 h (±1 h) after dosing.

- Obtain blood (plasma) PK samples for total apramycin at 24 h (±1 h) and 36 h (± 1 h) after dosing.
  - An aliquot of plasma PK collected at 36 h (±1 h) after starting the IMP infusion will be used in the protein binding test (for the measurement of free apramycin).
- Obtain blood and urine samples for safety clinical laboratory tests that include a calculation of GFR by the CKD-EPI equation at 24 h (±1 h) after dosing.
- Check on infusion site at 24 h (±1 h) and 36 h (±1 h) after dosing.
- Perform TEAE and SAE assessments.
- Perform symptom-directed (focused) PE for evaluation of TEAEs as needed.
- Document ConMeds.
- Encourage water intake at least 2 L daily – Document time and volume consumed.
- Counsel to avoid rigorous physical activity and avoid caffeinated beverages.

7.5.3 DAY 3: Inpatient Follow-up

- Obtain 12-lead standard ECG with 10-sec rhythm strip at 48 h (±1 h) and 60 h (±1 h) after dosing.
- Obtain VS at 48 h (±1 h) and 60 h (±1 h) after dosing.
- Obtain blood (plasma) PK samples for total apramycin at 48 h (±1 h) and 60 h (±1 h) after dosing.
- Obtain blood and urine samples for clinical laboratory tests that include a calculation of GFR by the CKD-EPI equation at 48 h (±1 h) after dosing.
- Obtain blood sample for Cystatin C and urine sample for KIM-1 (exploratory biomarkers of renal injury) at 48 h (±1h) after dosing.
- Perform otoacoustic assessments.
  - Ear otoscopy.
  - Audiometry (pure tone audiometry including high frequencies).
  - DPOAEs.

  Note: Day -2 otoacoustic assessments may be completed on Day 3 (+2 days) if needed.
- Check on infusion site at 48 h (±1 h) and 60 h (±1 h).
- Perform TEAE and SAE assessments.
- Document ConMeds.
- Perform complete PE.
- Counsel on use of contraception (males and females), avoidance of pregnancy (women of childbearing potential), avoidance of prohibited medication, illicit drugs, alcohol, nicotine products, vigorous exercise, and exposure to loud noises.
- Instruct on the next scheduled visit.
- Discharge subject from the CTU after review of clinical laboratory tests, ECGs and other assessments by PI or authorized clinician and audiologist.

Note 1: If the time of discharge is late in the evening, due to delays in starting procedures on Day 1 or personal reasons (such as distance from residence), the subject may stay in the CTU overnight and be discharged the following morning.

Note 2: If otoacoustic assessments could not be performed on Day 3, the subject will be discharged from the CTU on Day 3 after all other assessments are completed. Otoacoustic assessments will be completed up to Day 3 (+2 days) as outpatient.

7.6 Outpatient Follow-up Period (Days 14 and 30)

Subjects will return to the CTU to have the following assessments:

7.6.1 DAY 14 (± 3 days)

- Obtain 12-lead standard ECG with 10-sec rhythm strip.
- Obtain VS.
- Obtain blood and urine samples for clinical laboratory tests that include a calculation of GFR by the CKD-EPI equation.
- Obtain blood sample for Cystatin C and urine sample for KIM-1 (exploratory biomarkers of renal injury).
- Perform otoacoustic assessments.
  - Ear otoscopy.
  - Audiometry (pure tone audiometry including high frequencies).
  - DPOAEs.
- Check on infusion site.
- Perform TEAE and SAE assessments.
- Document ConMeds.
- Perform a complete PE.
- Counsel on use of contraception (males and females), avoidance of pregnancy (women of childbearing potential), avoidance of sperm donation, avoidance of prohibited medication, illicit drugs, alcohol, nicotine products, vigorous exercise, and exposure to loud noises.
- Instruct on the next scheduled visit.

7.6.2 Day 30 (± 4 days) – FINAL VISIT

- Perform TEAE and SAE assessments.
- Document ConMeds.
- Obtain blood sample for Cystatin C and urine sample for KIM-1 (exploratory biomarkers of renal injury).
- Perform symptom-directed (focused PE) for evaluation of TEAEs as needed.

- Perform otoacoustic assessments.
  - Ear otoscopy.
  - Audiometry (pure tone audiometry including high frequencies).
  - DPOAEs.
- Discharge subject from the study.
- Follow up on-going TEAEs including otoacoustic abnormalities according to PI instruction to resolution or until stable.

7.7 Early Termination (if needed)

- Obtain VS.
- Obtain weight.
- Check infusion site.
- Perform complete PE.
- Perform TEAE and SAE assessments.
- Update ConMeds.
- Obtain single 12-lead standard ECG with 10-sec rhythm strip.
- Obtain blood and urine for clinical laboratory tests that include a calculation of Glomerular Filtration Rate (GFR) by the CKD-EPI equation.
- Obtain blood sample for Cystatin C and urine sample for KIM-1 (exploratory biomarkers of renal injury).
- Obtain blood PK sample for total apramycin if ET occurs within 24 h of dosing.
- Perform otoacoustic assessments (ear otoscopy, audiometry [pure tone audiometry including high frequencies], and DPOAEs).
- Counsel on use of contraception (males and females), avoidance of pregnancy (women of childbearing potential), avoidance of sperm donation.

7.8 Unscheduled Visit (if needed)

A subject may return to the clinic for an unscheduled visit at any time after discharge on Day 3. The following activities at a minimum will be performed:

- Perform TEAE and SAE assessments.
- Update ConMeds.
- Obtain VS.
- Perform symptom-directed (focused) PE, if applicable.
- Obtain blood and/or urine for clinical safety laboratory tests that include a calculation of GFR by the CKD-EPI equation, if applicable.

- Obtain single 12-lead standard ECG with 10-sec rhythm strip as needed for the evaluation of a TEAE, if applicable.
- Perform otoacoustic assessments, if applicable (Ear otoscopy, Audiometry [pure tone audiometry including high frequencies], and DPOAEs).

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Procedures/Evaluations

8.1.1 Informed Consent

The informed consent form (ICF) will be approved by the reviewing IRB/EC and executed before performing any study-related activities.

Informed consent will be obtained for all subjects participating in the trial before performing any screening assessments. Subjects may withdraw consent at any time. Participation in the trial may be terminated at any time without the subject's consent as determined by the Investigator.

8.1.2 Demographics

Demographic information (date of birth, gender, ethnicity and race) will be recorded on the subject's source documents and eCRF at Screening Visit. Name, address, phone number, and emergency contact information will be documented in the source documents only.

8.1.3 Inclusion/Exclusion Criteria

Eligibility screening of healthy subjects will be completed within 28 days before study drug dosing and will be documented on the subject's source documents and eCRF. Review of inclusion and exclusion criteria will be performed on Day -2 to determine eligibility for admission, on Day -1, before 12-lead ECG, to determine eligibility for enrollment, and on Day 1 pre-dose to confirm the subject remains eligible for dosing.

Screening failures and the reason for failure to meet the study eligibility requirements will be documented in the source documents and entered into the study database.

8.1.4 Medical History

For subjects enrolled in the trial, the medical history (MH) will be obtained by direct interview of the subject and recorded on the subject's source document and eCRF. The MH will capture the subject's current disease processes, past disease processes, history of hospitalization, history of surgery, allergies, and prior medications (see Section 6.6). Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat (HEENT), mouth, skin, and the cardiovascular, gastrointestinal, renal, urological, nervous, hematological, lymphatic, endocrine, musculoskeletal, and genital/reproductive systems. A history of hearing abnormalities or hearing loss, especially following administration of aminoglycoside antibiotics or other medications, kidney disorders, cardiovascular disorders, including MI and angina, cardiac arrhythmias including Long QT Syndrome (LQTS), syncope due to cardiac arrhythmias or unexplained, psychiatric illness, and substance abuse will be specifically solicited.

Occupational history of exposure to loud noise will be obtained. Family history of hearing loss, especially on the maternal side, renal disorders, and sudden death will be obtained. The MH will be obtained at Screening Visit and updated upon admission to the CTU at check-in (Day -2), on Day -1, and before dosing on Day 1. After start of study drug dosing, any worsening of pre-dosing MH or new symptoms will be evaluated and reported as TEAEs.

8.1.5 Physical Examination

A complete PE – except genital, breast, and rectal exams – will be performed at the Screening Visit, check-in visit (Day -2), Day 3, and Day 14 (± 3 days) after dosing, or ET Visit, and will assess general appearance, HEENT, heart, lungs, abdomen, skin, musculoskeletal system, and lymph nodes, and a standard neurological exam.

An abbreviated PE will be performed at Day -1. An abbreviated PE differs from a complete PE in that the abdomen and neurological system are not evaluated.

A symptom-directed (focused) PE may be performed before dosing on Day 1 and at any time after dosing on Day 1 to the last visit (Day 30 ± 4 days) for evaluation of TEAEs.

Height and weight will be measured, and BMI calculated, at the Screening Visit. Weight only will be measured on Day -2, and Day 14 (±3 days), or ET.

Refer to the protocol-specific MOP for further details. The findings of each examination will be recorded on the subject's source documents and eCRF. Any new findings on examination or worsening of existing conditions after dosing are to be reported as TEAEs.

8.1.6 Vital Signs

VS include resting (measured after supine for at least 5 min) systolic blood pressure (SBP) and diastolic BP (DBP), heart rate (HR), respiratory rate (RR) and oral temperature (T). VS will be measured at the Screening Visit, on Day -2, on Day -1, on Day 1 (within 0.5 h [30 min] before dosing and at 0.5 h ±5 min, 1 h ±5 min, 4 h ±10 min, and 16 h ±15 min after dosing); on Day 2 (at 24 h ±1 h and 36 h ±1 h after dosing); on Day 3 (at 48 h ±1 h and 60 h ± 1 h after dosing); and on Day 14 (± 3 days), or ET. Normal references ranges are shown in Appendix B, Table 2, and exceptions for enrollment in Section 5.1, Inclusion Criterion #2.

VS that are considered aberrant due to an error in measurement may be repeated. At Screening and after dosing, an abnormal VS measurement may be repeated up to two more times at rest, within 5 min of each other. If the second measurement is abnormal, it will be reported at the highest grade of the two measurements and the subject will be excluded (if at Screening Visit) or the event reported as a TEAE and graded for severity per Appendix B, Table 2 (if after dosing). If the second measurement is normal, a third measurement will be taken at least after 5 min at rest. If the third measurement is still normal, the subject is eligible (if at Screening Visit) or there is no TEAE (if after dosing); if it is abnormal, the subject will be excluded (if at Screening Visit)

or a TEAE will be reported at the highest assessed grade (between first and third measurements) and graded for severity per Appendix B, Table 2 (if after dosing).

8.1.7 12-lead Standard Electrocardiogram (ECG)

Single 12-lead standard ECG and 10-sec rhythm strip will be obtained at the Screening Visit, on Day -2, at 24 ±1 h and 36 ± 1 h (Day 2) and 48 h ±1 h and 60 h ±1 h (Day 3) after dosing, and on Day 14 (± 3 days), or ET.

Triplicate 12-lead standard ECGs will be obtained over a period of 5 minutes on Day -1 (at the same 4 timepoints as projected to be taken on Day 1), and on Day 1 pre-dose (within 30 min) and at 1 h ±5 min, 4 h ±10 min and 16 h ± 15 min after dosing.

ECGs will be performed after the subject rests quietly in a supine position for at least 5 min. The ECGs will be reviewed by the PI or a designated clinician (listed on FDA Form 1572). ECGs will be analyzed for PR, QRS and QT intervals, ventricular rate (based on RR intervals), and for morphological abnormalities. The QT interval will be corrected by the Fridericia (QTcF) formula, recorded and analyzed. To be eligible for participation, the QTcF interval must be within protocol reference range criteria at Screening and Day -2 and there must be no clinically significant ECG abnormalities after enrollment on Day -1 and before dosing on Day 1. If the site PI identifies clinically significant changes in the ECGs after enrollment in Day -1 and before dosing on Day 1, the subject would not be eligible to receive study drug and should be withdrawn and replaced. ECG PR and QTcF intervals after dosing will be reported as TEAEs if they meet toxicity grading criteria (See Appendix B, Table 4). If a question regarding ECG interpretation arises, the study investigators will have the ECG reviewed by a cardiologist.

8.1.8 Spirometry

Spirometry will be performed to evaluate FEV1 and FEV1/FVC at Screening. A subject will be eligible if predicted FEV1 is ≥ 80% and FEV1/FVC is > 70 %.

8.2 Laboratory Evaluations

Venipuncture schedule and blood volumes are shown in Appendix A and Appendix C, respectively.

8.2.1 Clinical Laboratory Evaluations (Hematology, Coagulation, Chemistry, and Urinalysis).

Blood and urine samples for clinical laboratory tests will be collected at the Screening Visit and on Day -2 to determine eligibility, and on Day 1, pre-dose, to determine (Baseline). Clinical laboratory tests will also be collected on Day 2 (24 h ± 1 h after dosing), Day 3 (48 h ± 1 h after dosing), and on Day 14 (± 3 days) or ET. Subjects must be fasting at least for 6 h before any blood draw for clinical laboratory assessments. These tests will include:

- HEM: Hgb, Hct, RBC, platelet count, and WBC count with absolute differential count.
- COAG: INR with prothrombin time, alpha partial thromboplastin time.
- CHEM: electrolytes (sodium, potassium, chloride, total carbon dioxide [CO_2]), calcium, magnesium, creatinine, with estimation of GFR by the CKD-EPI equation, blood urea nitrogen (BUN), glucose (fasting), total bilirubin, direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), total protein, albumin, TSH, free T4, and free T3.
  - The CKD-EPI equation, expressed as a single equation, is GFR = 141 × min(Scr/κ, 1)α × max(Scr/κ, 1)-1.209 × 0.993Age × 1.018 [if female] _ 1.159 [if black], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1 (Levey et al 2009).
- UA: Routine dipstick testing of clean-catch urine for blood, protein, and glucose
  - If urine dipstick is abnormal, urine microscopy will be performed.

Clinical laboratory tests at the Screening Visit and Day -2 should be in the normal reference range with exceptions. (See Section 5.2, Inclusion Criteria #2 and Appendix B, Table 3).

Laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to collection or laboratory error may be repeated once, preferably within 24 h or as soon as subject is available.

Laboratory values will be transferred to Advantage eClinical®.

Abnormal safety laboratory values after dosing will be graded for severity per Appendix B, Table 3 and Table 4 . Abnormal values within the allowable Grade 1 range per Appendix B noted at screening or baseline will only be considered TEAEs if they deteriorate after study drug dosing to Grade 2 or higher.

8.2.2 Viral Serology Testing

Serological testing for HIV antibody, HBsAg, and HCV antibody will be performed at Screening Visit. These tests must be negative for study eligibility. In cases where a false-positive result is suspected, confirmatory testing (e.g., polymerase chain reaction) may be performed.

8.2.3 Pregnancy Testing

In all women, a serum β-HCG pregnancy test will be done at the Screening Visit. A urine pregnancy test will be done upon check in on Day -2 and results must be negative for dosing with study drug.

8.2.4 Serum FSH Testing

A serum FSH level for confirmation of post-menopausal status in female subjects will be measured at the initial Screening Visit only.

8.2.5 Urine Toxicology Screening

A urine toxicology screen will be performed at Screening Visit and on Day -2 to detect the presence of amphetamines, cocaine, barbiturates, benzodiazepines, opiate, methylenedioxymethamphetamine (MDMA), methadone, marijuana (THC), methamphetamine, tricyclic antidepressants (TCAs), and phencyclidine. Results must be negative for study eligibility. Urine creatinine will be measured as part of the profile to assess quality of collected sample.

8.2.6 Alcohol Breathalyzer Test

A breathalyzer test for alcohol will be performed at Screening and Check-in (Day -2). Results must be negative for study eligibility.

8.2.7 Urine Cotinine Testing

Detection of urine cotinine, for recent tobacco products consumption, will be performed at the Screening Visit, and on Day -2. A positive urine cotinine at screening is allowed but results must be negative on Day -2 for study eligibility for enrollment.

8.2.8 Exploratory Biomarkers for Renal Injury

The following tests will be performed on indicated days for exploratory purposes, not to determine eligibility:

8.2.8.1 Serum Cystatin C

The test will be performed in enrolled subjects on Day -2, pre-dose on Day 1 (Baseline), Day 3 (at 48 h ± 1 h after start of dosing), Day 14 (± 3 days), and Day 30 (± 4 days), or ET.

8.2.8.2 Urinary KIM-1

The test will be performed in enrolled subjects on Day -2, pre-dose on Day 1 (Baseline), Day 3 (at 48 h ±1 h after start of dosing), Day 14 (± 3 days), and Day 30 (± 4 days), or ET.

8.3 Bronchoscopy with Bronchoalveolar Lavage

Bronchoscopy with bronchoalveolar lavage is performed to obtain fluid samples from a lung segment following dosing with apramycin to measure the accumulation of the drug in the lung. The procedure will be performed in the Pulmonology Department of the UTMC, which is accredited to perform this procedure, according to established SOPs by licensed and experienced

pulmonologists and support staff. The procedure will be done in eligible and enrolled subjects who were assigned to cohorts T1-T5, at the following nominal timepoints: 0.5 h ±5 min (T1), 2h ±5 min (T2), 4 h ± 10 min (T3), 8 h ± 15 min (T4) and 24 h (nominal) ±1 h (T5) after the start of apramycin infusion. (The exact timing of T5 will be determined after review of BAL apramycin concentrations in cohorts T1 to T4.) Subjects in each cohort will have only one procedure done during the study.

Subjects, who had previously been informed of the procedure and provided informed consent, will be taken to the bronchoscopy suites by CTU staff, will review the procedure with the operator and confirm understanding prior to the procedure. The subject will be monitored during the procedure by VS, pulse oximetry and will receive oxygen as needed. Sedation or light anesthesia will be induced by appropriate intravenous medications. The procedure will be performed according to standard protocol and a study-specific protocol (SSP) for the collection of BAL samples for PK analysis. BAL samples will be stored on ice and then transferred into test tubes to the CTU research lab for measurement of total and differential cell counts and for centrifugation and processing into BAL supernatant (containing ELF) and BAL pellet (containing AM) samples. After the procedure, the subject will be observed in the recovery room for side effects and will be released by the pulmonologist to the care of the CTU staff when stable. The subject will continue to be monitored closely until discharge from the CTU. Procedure records will be obtained by the CTU staff.

8.4 Ear Examination and Otoacoustic Testing

Ear exams and otoacoustic tests will be performed by licensed and experienced audiologists at the Audiology section of the ENT department of the UTMC. The following tests will be performed at the indicated study visits. These tests may be performed at the screening visit, or at a second screening visit due to time restrictions on the initial visit.

- Ear otoscopy, Valsalva test, Tympanometry and Stapedial Reflexes (Screening visit).
- Ear otoscopy only (Day -2 [or Day -4 or Day -3 if needed], Day 3 (+2 days), Day 14 (± 3 days) and Day 30 (± 4 days), or ET).
- Audiometry.
    - Pure tone audiometry at standard frequencies, 0.5 to 8 kHz (Screening visit).
    - Pure tone audiometry at standard (0.5 to 8 kHz) and higher frequencies (Day -2 [or Day -4 or Day -3 if needed], Day 3 (+2 days), Day 14 (± 3 days), and Day 30 (± 4 days), or ET.
- Distortion product otoacoustic emissions (DPOAEs) at Screening Visit, and on Day -2 [or Day -4 or Day -3 if needed], Day 3 (+2 days), Day 14 (± 3 days), and Day 30 (± 4 days), or ET.

Note 1: On Day 3, ear otoscopy, audiometry and DPOAEs can be done at any time.

Note 2: If otoacoustic assessments could not be performed on Day 3, the subject will be discharged from the CTU on Day 3 after all other assessments are completed. Otoacoustic assessments will be completed up to Day 3 (+2 days) as outpatient.

Audiology records will be obtained by the CTU staff and results will be recorded as described in the MOP.

8.5 Bioanalytical Assays for Pharmacokinetics (PK)

The following LC-MS/MS methods will be validated before subject enrollment starts for determination of concentration of apramycin in plasma and bronchoalveolar lavage (BAL) samples: Total and Free apramycin in plasma, total apramycin in BAL supernatant (BAL SUP, containing ELF), and total apramycin in BAL pellet (BAL AM). In addition, a validated LC-MS/MS method will be used for the measurement of urea in plasma and BAL SUP.

8.5.1 Assay for Total Apramycin in Plasma

A single dose of apramycin will be administered by 30 (± 5) min IV infusion. Blood (plasma) samples for assay of total apramycin concentration and estimate of PK parameters will be collected at the following study days and timepoints: On Day 1 within 30 min before dosing, and 0.5 h (±5 min, immediately at end of infusion), 1 h (±5 min), 2 h (±5 min), 4 h (±10 min), 8 h (±15 min), and 16 h (±15 min) after dosing; on Day 2 at 24 h (± 1 h) and 36 h (± 1 h) after dosing; and on Day 3 at 48 h (± 1 h) and 60 h (± 1 h) after dosing, or ET if occurs within 24 h of dosing.

Plasma samples for total apramycin concentration should be collected within 5 min of the corresponding BAL procedure at 0.5 h (± 5 min, immediately after the end of infusion), 2 h (± 5 min), 4 h (± 10 min), 8 h (± 15 min) and 24 h (± 1 h) after the start of the infusion. If BAL is delayed, blood (plasma) for apramycin will be collected at the nominal timepoint (including windows) and a second sample will be collected within 5 min of the BAL sample.

Blood samples will be obtained promptly after each corresponding 12-lead standard ECG timepoint and VS assessment.

Sample collections will be scheduled for the nominal time point and actual collection times recorded in source documents. If bronchoscopy/BAL is delayed, an additional sample for plasma apramycin assay should be collected within 5 min after collection of the last BAL sample, to be used for the calculation of Lung PK / Plasma PK ratios.

8.5.2 Protein Binding Test for Apramycin in Plasma

Protein binding of apramycin in plasma will be measured at the following timepoints: 0.5 h (± 5 min; immediately at the end of infusion), and 36 h (± 1 h) after the start of the infusion. The

assay will be performed in plasma PK aliquots collected at the matching time points as described in Section 8.5.1. A separate blood collection is not required.

8.5.3 Assays for Total Apramycin in Bronchoalveolar Lavage Components (ELF and AM)

During bronchoscopy with bronchoalveolar lavage (BAL), samples will be obtained at the following timepoints overall during the study: 0.5 h (± 5 min), 2 h (± 5 min), 4 h (± 10 min), 8 h (±15) min and 24 h (± 1 h) after the start of the infusion. Each subject will have bronchoscopy with BAL at one timepoint only. At each time point, BAL samples will be collected from 4 subjects assigned to one of the cohorts T1-T5. Each BAL collection will be centrifuged for the separation of the supernatant (BAL SUP containing ELF) and the pellet (BAL AM) components, and total apramycin will be measured in BAL SUP and BAL AM. Due to dilution during BAL, the concentration of apramycin in ELF will be corrected using the urea method (Tenero 2013; Gottfried 2017; Rizk 2018). Formulae for the calculations will be provided in the SAP.

8.5.4 Plasma and BAL Urea assay

Urea will be assayed in plasma samples collected at baseline (as part of the total apramycin plasma PK) and at 0.5 h (± 5 min, immediately after the end of infusion), 2 h (± 5 min), 4 h (± 10 min), 8 h (± 15 min) and 24 h (± 1 h) after the start of the infusion), and in BAL SUP samples corresponding to plasma PK timepoints. If BAL is delayed and a second plasma sample is collected within 5 min of the last BAL sample, plasma urea will be measured only in the second sample.

8.5.5 Specimen Preparation, Handling, and Shipping

8.5.5.1 Instructions for Specimens Preparation, Handling, and Storage

Details regarding the specimen preparation, handling, and storage of bioanalytical samples for the measurement of plasma total and free apramycin, total apramycin in BAL SUP (containing ELF) and BAL AM, plasma urea, and urea in BAL SUP (ELF) will be described in the protocol-specific MOP.

Blood samples and BAL samples left after all routine clinical laboratory testing and pharmacokinetic assays are completed will not be stored indefinitely or used for purposes other than those described but will be disposed of per DMID (sponsor) guidance. No genetic testing will be done on collected blood samples and BAL samples.

8.5.5.2 Specimen Shipment

Specimen shipment will occur at intervals during the trial following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage

temperature and documentation as detailed in the central clinical laboratory manual and protocol-specific MOP, as appropriate.

All specimens for clinical screening and safety laboratory evaluations will be transported from the CTU to the local clinical laboratory.

Plasma and Bronchoalveolar BAL SUP (containing ELF) and BAL AM samples for bioanalytical assays will be shipped from the CTU to DMID-CMS at:

Fisher BioServices
c/o DMID Clinical Materials Services (CMS)
20439 Seneca Meadows Parkway
Germantown, MD 20876

Phone: 240-477-1350
Fax: 240-477-1360

Email: DMID.CMS@thermofisher.com

Plasma and Bronchoalveolar BAL SUP (containing ELF) and AM samples will then be provided by DMID-CMS to the bioanalytical lab, KCAS Inc., at:

Test Materials Management (TMM)
KCAS Bioanalytical and Biomarker Services
10830 S. Clay Blair Boulevard
Olathe, KS, 66061

Telephone: 913-248-3006
Fax: 913-248-3106

Email: TMM@kcasbio.com

Further information will be provided in the study-specific MOP.

8.5.6 Long-term Storage of Plasma and BAL (ELF and AM) PK Samples

Any residual plasma and BAL samples (BAL SUP [ELF] and BAL AM) collected for the measurement of total apramycin concentration may be stored at the DMID CMS long-term and used for future exploratory research if collected from subjects who consented to its use for this purpose. (See Section 14.8)

9 ASSESSMENT OF SAFETY

Regulatory requirements including FDA regulations and ICH Guidelines for GCP set forth safety monitoring and reporting responsibilities of sponsors and investigators to ensure the safety and protection of human subjects participating in clinical trials.

Responsibilities:

Investigators participating in this clinical trial are responsible for and will:

- Evaluate subject safety including assessment of treatment-emergent adverse events (TEAEs) for seriousness, severity, and causality (relatedness to study drug).
- Notify DMID of treatment-emergent serious AEs (SAEs) within 24 h of site awareness.
- Provide detailed written reports, including necessary documentation requested by DMID or IRB/EC, promptly following immediate initial reports.
- Inform the IRB/EC of SAEs and TEAEs as required by applicable regulatory requirements.

9.1 Specification of Safety Parameters

Safety will be assessed by the timing, frequency, causality, and severity of:

1. Treatment-emergent AEs and SAEs occurring from time of dosing through Final Visit (Day 30 ± 4 days), or ET.
2. Clinical laboratory TEAEs occurring from time of first dose through Day 14 (± 3 days), or ET.
3. 12-lead standard ECGs performed in the 24-h period after dosing, on Day 3, and Day 14 (± 3 days), or ET.
4. Audiology (Ear examination and otoacoustic testing) TEAEs at Day 3 (+ 2 days), Day 14 (± 3 days) and Day 30 (± 4 days), or ET.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

Definitions

ICH E6 defines an AE as any untoward medical occurrence in a subject or clinical investigation subject who was administered a pharmaceutical product, regardless of its causal relationship to the product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with use of the product,

and are described as treatment-emergent AEs (TEAEs). The occurrence of a TEAE may come to the attention of study personnel during study visits and interviews, or by a subject presenting for medical care.

The FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether it is considered drug-related or not.

Any systemic medical condition, VS and ECG measurement, and clinical safety lab test value that is present on Day 1 prior to dosing will be considered a baseline finding for the purpose of data analysis. For ear and otoacoustic testing, baseline comprises assessments performed on Day -2. However, if the condition increases in severity or frequency after IMP administration at any time during the trial, it will be recorded as a TEAE.

Any medical condition that is reported after screening but before study drug administration will be evaluated and reported as MH update.

All TEAEs will be graded for severity according to Appendix B, Table 2, Table 3, and Table 4 and for relationship to the study drug.

9.2.1.1 Severity of Events

Intensity of TEAEs will be graded as follows, unless otherwise specified in Appendix B:

Mild: Require minimal or no treatment; do not interfere with the subject's daily activities.

Moderate: Result in a low level of inconvenience or concern with therapeutic measures; may cause some interference with functioning.

Severe: Interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment; are usually incapacitating.

9.2.1.2 Relationship to Study Products

TEAEs and treatment-emergent SAEs will be assessed by the investigator to determine relationship to the study drug, using the following two terms. In a clinical trial, the study drug must always be suspect.

- **Related:** There is a reasonable possibility that the study drug caused the treatment-emergent AE/SAE.
- **Not Related**: There is not a reasonable possibility that the study drug caused the treatment-emergent AE/SAE.

The investigator will provide an assessment of association or relationship of each treatment-emergent AE/SAE to the study drug based on:

- Temporal relationship of the TEAE/SAE to study drug dosing.

- Whether an alternative etiology has been identified.
- Biological plausibility.
- Existing therapy and/or ConMeds.

9.2.1.3 Reporting Adverse Events

TEAEs will be captured on the appropriate subject's source document and eCRF. Information collected for TEAEs includes event description, time of onset, investigator assessment of severity and relationship to the study drug, date of resolution of the event, seriousness, and outcome.

All TEAEs will be documented from the time of study drug dosing through the time of last assessment (e.g., Day 14 ± 3 days for clinical safety labs and ECGs) or Final Visit (Day 30 ± 4 days), or ET. All TEAEs including abnormal safety laboratory test results will be followed to resolution or until considered stable in the clinical judgment of the study investigator. Evaluation of TEAEs may require unscheduled visits and clinical and laboratory investigations, according to the clinical judgment of the Site PI and study physicians.

9.2.2 Serious Adverse Events

An SAE is any treatment-emergent AE that meets at least one of the following criteria:

- Death.
- Life-threatening AE*.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability or incapacity, or substantial disruption of the ability to conduct normal life function.
- Congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

*A TEAE is considered "life-threatening" if, in the view of either the investigator or DMID, its occurrence places the subject at immediate risk of death. It does not include a TEAE that, had it occurred in a more severe form, might have caused death.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology by an authorized study physician (listed on FDA Form 1572).

- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution.
- Reviewed and evaluated by the SMC (periodic review unless related), DMID, and the IRB.

9.2.3 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

A licensed study clinician (listed on FDA Form 1572) will determine seriousness, severity, and causality of abnormal laboratory values; provide a medical evaluation of TEAEs; and classify TEAEs based upon medical judgment.

Abnormal laboratory values or clinical findings for all enrolled subjects after dosing will be assessed using the toxicity scales in Appendix B. Out of range values and findings noted at screening or baseline, but allowable for enrollment and dosing if within Grade 1, will only be considered TEAEs if their severity increases to Grade 2 or higher. For abnormalities noted from the time of study drug dosing, any Grade 1 or higher laboratory abnormality listed on the toxicity Table 3 in Appendix B will be entered in the database as a TEAE. Safety laboratory results that are reported as TEAEs will be evaluated by the CTU clinician as clinically significant (CS) or not clinically significant (NCS). Abnormal laboratory values, performed as part of HEM, COAG, CHEM, or UA but not listed in this toxicity table will be evaluated by the study clinicians, recorded in the source document and, if clinically significant, considered TEAEs and graded according to the criteria in Section 9.2.1.

Protocol-specific laboratory normal range values are included in the study-specific MOP and toxicity grades for evaluation of TEAEs are included in Appendix B, Table 3.

Gross blood in urine that is confirmed due to menses is not a TEAE (but is for all other reasons).

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

All SAEs will be:

- Recorded on the appropriate SAE report form and sent to DMID Pharmacovigilance Group (PVG).
- Entered into the appropriate subject source document and eCRF in Advantage eClinical®
- Reported to the IRB.

- Reviewed and followed to resolution or stability by an authorized study physician (listed on FDA Form 1572).
- Collected on each subject until Day 30 ± 4 days (Final Visit), or ET Visit.

Any TEAE that meets a protocol-defined serious criterion will be submitted immediately (within 24 h of site awareness) on an SAE report form to DMID PVG:

DMID Pharmacovigilance Group

Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Drive, Suite 650

Bethesda, MD 20817, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)

SAE Email Address: PVG@dmidcroms.com

In addition to the SAE report form, selected SAE data fields will also be entered into Advantage eClinical®. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the SAE may be requested by DMID PVG and will be provided as soon as possible.

The DMID Medical Monitor (MM) and DMID CPM will be notified of the SAE by the DMID PVG. The DMID MM will review and assess the SAE for regulatory reporting and potential impact on subject safety and protocol conduct.

At any time after completion of the trial, if the Site PI or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study drug, the Site PI or appropriate sub-investigator will report the event to the DMID PVG.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected TEAE that is both serious and unexpected. DMID will report an AE as a suspected AE only if there is evidence to suggest a causal relationship between the drug and the TEAE. DMID will notify FDA and all participating investigators (i.e., all investigators to whom DMID is providing drug under its IND(s) or under any PI's IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after DMID determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal TEAE or suspected life-threatening TEAE as soon as possible, but in no case

later than 7 calendar days after DMID's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to the study drug will be reported to the FDA at least annually in a summary format.

9.3.3 Other Adverse Events (if applicable)

9.3.3.1 Reporting of Overdose

An overdose is defined as a dose greater than the high-dose level evaluated in the trial as described in Section 6.3.1 of the protocol. All overdoses will be reported; if the overdose is associated with a TEAE, then the TEAE will also be reported. In the event of an overdose of study drug, the investigator will use clinical judgment in treating the overdose and contact the DMID MM. There is no specific antidote in case of apramycin overdose. The same measures as recommended for other aminoglycosides (treatment cessation, adequate hydration, dialysis, administration of calcium salts for symptoms of neuromuscular blockade) should be considered until further clinical data are available for apramycin. The investigator will refer to the relevant document(s) for detailed information regarding warnings, precautions, contraindications, TEAEs, and other significant data pertaining to apramycin. Such documentation may include but not be limited to the IB.

9.3.4 Reporting of Pregnancy

Pregnancies that occur in female subjects during the trial will be reported via Advantage eClinical® on a pregnancy report form. With the subject's permission, all protocol-required venous blood samples will be obtained, and the subject will continue to be followed for safety until Day 30 ± 4 days (Final Visit). Efforts will be made to follow all pregnancies reported during the trial to pregnancy outcome, as described in the protocol-specific MOP (e.g., delivery, spontaneous abortion, or therapeutic abortion), pending the subject's permission.

A female subject who participates in the trial and becomes pregnant will be asked to inform study personnel of a pregnancy occurring 30 days after Final Visit. For all reported pregnancies, subjects will be asked to provide pregnancy outcome upon delivery or pregnancy termination to the CTU.

Serious adverse outcomes of pregnancy affecting the mother and/or fetus or neonate (e.g., spontaneous abortion, congenital anomaly(ies) in fetus or child, late fetal death, or reports of adverse drug reactions in a newborn/neonate that is fatal, life-threatening, resulting in persistent

or significant disability/incapacity or resulting in or prolonging hospitalization) will be documented in a SAE form and reported to the CROMS PVG within 24 h of clinical site awareness of the events.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

Treatment-emergent SAEs and AEs will be followed until resolution or until considered stable in the clinical judgment of the investigator. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition in the clinical judgment of the study investigator, with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded in the subject's source document and eCRF.

9.5 Halting Rules

9.5.1 Study Halting Criteria

Systemic or Lab Criteria:

- One subject has an SAE that is determined to be related to the study product. OR
- Two or more subjects experience Grade 2 or higher related TEAE (laboratory or systemic) that is coded in the same HLGT per MedDRA classification, and if AE is not resolved within 48 hours through Day 4. Resolution is defined as return to a baseline status. Note: If the event starts on Day 4, the review period will be extended to Day 5.

  Note: VS abnormalities will be considered part of a systemic disorder or an organ-specific condition, as described in Appendix B: Adverse Events Toxicity Grading Criteria, in order to be included among the Study Halting Criteria.

Ototoxicity Criteria:

- Two or more subjects experience Grade 2 or higher ototoxic change of pure-tone audiometry through Day 30 ± 4 days that is confirmed by repeat testing, generally within 24 h, and assessed as related to IMP.
- A single subject experiences significant ototoxic change in DEOAEs, characterized by decrease in reproducibility to lower than 70% at three consecutive frequencies where responses were previously obtained, through Day 30 (± 4 days) and is confirmed by repeat testing, generally within 24 h:

If the predefined criteria for the halting rules are met, the SMC will review the study data and provide guidance on how to proceed.

A halted study will resume upon recommendation by the Sponsor (DMID).

9.6 Safety Oversight

9.6.1 Safety Monitoring Committee (SMC)

Safety oversight will be conducted by a SMC, which is an independent group of experts that monitors subject safety and advises DMID. SMC members will be separate and independent of study personnel participating in the trial and will not have scientific, financial, or other conflicts of interest related to the trial. The SMC will consist of a minimum of three members with appropriate expertise to contribute to the interpretation of the data from the trial.

The SMC will meet as follows:

- Organizational meeting (before study initiation).
- *Ad hoc* meeting.
    - When study halting criteria are met.
    - At the request of DMID to review a potential safety concern identified by either the Site PI or DMID MM.
- Scheduled meeting.
    - The SMC will review cumulative, interim safety data when available after completion of all subjects in cohorts T1 to T4.
    - The SMC will review final safety data after completion of all subjects in all cohorts, including T5, when available after database lock.

Procedures for SMC reviews/meetings will be defined in the SMC charter. The SMC will review safety data and provide recommendations to DMID, NIAID on resumption or stopping of a temporary halted trial, and safety monitoring in future clinical trials with apramycin.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring will be conducted to ensure that human subject protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet DMID, GCP/ICH, and regulatory guidelines, when appropriate. Site visits may be conducted by an authorized representative of DMID or other regulatory agencies to inspect study data, subjects' medical records, and eCRFs in accordance with ICH guidelines, GCP, and the respective local and national government regulations and guidelines.

The investigator will permit authorized representatives of DMID and the respective local and national health authorities to inspect facilities and records relevant to the trial, if needed.

A separate monitoring plan developed by DMID will describe protocol-specific items to be monitored.

Site visits will be made at standard intervals defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but not be limited to, review of regulatory files, accountability records, subjects' source documents, eCRFs, ICFs, clinical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to the CTU, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with the Site PI to discuss any problems and actions to be taken and document visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

The objectives of the study are to obtain lung and plasma PK and safety data for apramycin. There are no formal hypotheses being tested in this Phase 1 trial.

11.2 Sample Size Considerations

Enrollment of 20 subjects assigned into 5 cohorts, T1-T5 (T5 will undergo bronchoscopy with BAL after completion of cohorts at the 24 h nominal timepoint, with the optimum timepoint to be selected after completion of T1-T4), with 4 subjects per cohort is based on clinical experience and judgment and should provide adequate clinical information to meet the objectives of the study. Power calculations have not been performed.

11.3 Safety Review

An SMC will be appointed to oversee the safe conduct of the trial. A scheduled SMC meeting will be held after all subjects in timepoint cohorts T1 to T4 complete the trial to review safety data. If criteria for halting the trial (as listed in Section 9.5.1) are met, an *ad hoc* SMC meeting will be held to review all available safety data and to make recommendations about the dosing of all further subjects in the trial. A statistician may present data in a closed session of an SMC meeting. If group T5 will enroll, a final SMC meeting will be held to review all data and advise on safety monitoring in future clinical trials of apramycin.

11.4 Final Analysis Plan

The ICH Guidance E9 (Statistical Principles for Clinical Trials) will be followed for all statistical content. Summaries of frequencies and percentages will be presented for categorical data, and summary statistics such as minimum, median, mean, SD, and maximum values will be presented for continuous data. Details of plasma and BAL SUP (containing ELF) and BAL AM apramycin PK, lung PK/plasma apramycin exposure ratios, safety, ECG interval measurements (QT, PR, QRS, and HR), and ECG morphology data analyses and presentations will be described in the Statistical Analysis Plan (SAP) and accompanying Tables, Listings, and Figures (TLF) templates. The SAP for PK and safety data will be prepared and finalized by the SDCC before analysis of the PK data after completion of cohorts T1-T4. A final analysis containing safety data and PK data from cohorts T1-T4 and cohort T5, if enrolled, will be performed by the SDCC after final data lock and included in the clinical study report (CSR). Any change from originally planned statistical analyses will be reported in the CSR.

11.4.1 Analysis Populations

The safety population will include all subjects who received any amount of the IMP. The per protocol safety population subset will include all subjects who received a complete dose of study drug (apramycin) and analyzed as treated.

The lung PK population will consist of all the subjects who received a complete dose of apramycin, underwent BAL at the assigned sampling timepoint with BAL return volume adequate for analysis and have at least one quantifiable drug concentrations separately for BAL SUP (containing ELF) and BAL AM.

The plasma PK analysis population will consist of all subjects who received a complete dose of apramycin and have at least one quantifiable post-dosing plasma drug concentration measured.

The PK analysis subset will be based on the lung and plasma PK population, which includes all subjects who completed the lung PK and plasma PK part of the trial without any protocol violations that would likely affect the PK results and who have evaluable plasma PK and BAL PK concentration data for apramycin from which at least a subset of the designated PK parameters can be determined. If any subjects are found to be noncompliant with respect to dosing or have incomplete data, a decision to include them in the analysis will be made on a case-by-case basis.

11.4.2 Demographics and Baseline Characteristics

Subject demographics and baseline characteristics will be summarized. The number of subjects who enroll in the trial, and the number and percentage of subjects who complete each assessment, will be presented. The percentage of subjects who withdraw from the trial or discontinue the study drug, and reasons for withdrawal or discontinuation, will be summarized.

11.4.3 Safety Analysis

11.4.3.1 Adverse and Serious Adverse Events

TEAEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities® (MedDRA). All AEs occurring after study drug dosing will be summarized using frequency counts and percentages. The following summaries will be presented for treatment-emergent AEs and SAEs:

- Overall (i.e., regardless of severity or relationship to treatment).
- By severity grade (mild, moderate, or severe).
- By relationship to study drug.

- By MedDRA level hierarchy (system organ class [SOC], higher level group term [HLGT] and preferred term [PT]).

Unless otherwise specified, at each level of subject summarization in reporting the incidence of TEAEs, a subject will be counted once if the subject reported one or more TEAEs. If more than one occurrence of a TEAE is reported, the TEAE of the worst severity or the worst-case relationship assessment will be summarized.

11.4.3.2 Additional Safety Analyses

Descriptive summary statistics (mean, SD, median, minimum, and maximum) for clinical laboratory data, 12-lead standard ECG parameters, and VS at admission and each applicable post-dosing visit, including changes from baseline values, will be calculated. Baseline values will be the last values recorded before administration of study drug. For change-from-baseline summaries, subjects with an undefined change from baseline, due to missing data, will be excluded. Clinical significance of abnormalities will be indicated in the listings.

11.4.4 PK Analysis

PK parameters will be estimated for total plasma apramycin, and for ELF and AM total apramycin by noncompartmental analysis (NCA) methods using Phoenix® WinNonlin® version 8.0 or higher.

11.4.4.1 Plasma PK parameters:

When evaluable, estimated plasma PK parameters will include:

- $AUC_{(0-\infty)}$: Area under the concentration time-curve from time zero to infinity.
- $AUC_{(0-t)}$: Area under the concentration time-curve from time zero to time t.
- $AUC_{(0-24)}$: Area under the concentration time-curve from time zero to 24 h.
- C_{max}: Maximum observed concentration.
- T_{max}: Time of maximum observed concentration.
- K_e: Elimination rate constant.
- $t_{1/2}$: Terminal elimination half-life.
- CL_T: Total clearance.
- Vd: Volume of distribution.

Other PK parameters may be calculated, as appropriate.

The free portion (fu, % unbound) of plasma apramycin will be calculated from the ratio of free to total apramycin concentration in plasma PK samples obtained at 0.5 h (± 5 min, immediately after the end of infusion) and 36 h (± 1 h) after the start of infusion.

11.4.4.2 Bronchoalveolar ELF and AM PK parameters:

When evaluable, estimated PK parameters from BAL ELF and BAL AM will include:

- $C_{max:}$ Maximum observed concentration.
- $AUC_{(0\text{-}24):}$ Area under the concentration time-curve from time zero to 24 h.
- $AUC_{(0\text{-}t):}$ Area under the concentration time-curve from time zero to the last concentration. above the lower limit of quantitation.
- $AUC_{(0\text{-}\infty):}$ Area under the concentration time-curve from time zero to infinity.
- T_{max}: Time of maximum observed concentration.
- t½: Terminal elimination half-life.

The concentration of apramycin in ELF will be calculated using the urea method (Tenero 2013, Gottfried 2017, Rizk 2018). The formulae for calculating ELF concentration will be provided in the SAP.

11.4.4.3 Ratio of exposure parameters of Lung PK to Plasma PK:

When evaluable, the following exposure ratios of total apramycin will be calculated:

- ratio of C_{max} in ELF over C_{max} in plasma.
- ratio of C_{max} in AM over C_{max} in plasma.
- ratio of $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$ in ELF over $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$ in plasma.
- ratio of $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$ in AM over $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$ in plasma.

The results of PK parameters for apramycin in plasma, ELF, AM, and the exposure (C_{max} and AUC) ratios of lung/plasma (total and free) will be listed by subject, and summarized with descriptive statistics including n, mean, SD, coefficient of variation, median, minimum, maximum, geometric mean and geometric SD at each timepoint.

Graphical presentations of concentration vs. time profiles will be provided for apramycin and will include individual subject and mean concentration profiles. Semi-log concentration profiles will be provided for individual subjects. The ratios of lung PK exposure / plasma PK exposure vs time profiles for total plasma apramycin will be presented graphically for the ELF and AM components of BAL samples.

11.4.5 Missing Values and Outliers

All attempts will be made to collect all data per protocol. No imputation will be performed for missing values. Outliers will not be excluded from the primary analyses. Outliers identified during the PK analysis will be discussed in the analysis report.

11.4.6 Analysis of ECG Parameters

11.4.6.1 ECG Analysis and Interpretation

For replicate ECGs recorded on Day -1 and Day 1, the ΔΔQTcF, calculated as difference between mean change-from-baseline QTcF values at each timepoint on Day 1 after treatment with apramycin relative to time-matched ECG recording before dosing in Day -1, will be evaluated to quantify the QTcF prolongation. For single ECG recordings after dosing, ΔQTcF, will be calculated as difference between mean change-from- baseline QTcF values recorded at the pre-dose timepoints on Day -1 and Day 1.

QTcF corrections will be calculated using Fridericia's formula:

- $QTcF=QT/RR^{0.33}$

Other endpoints to be evaluated include HR (bpm), PR (msec), QRS (msec), and cardiac abnormalities (morphologies, rhythm, and conduction).

All statistical analyses and reporting of ECG data will be conducted on the mean of the replicate intervals within a timepoint on Day -1 and Day 1. Descriptive statistics including two-sided 90% CI will be generated for observed, change-from-baseline for all ECGs recorded in the study, and time-matched change-from-baseline ECG intervals for replicate ECGs recorded on Day -1 and Day 1.

11.4.6.2 Categorical Analyses

QTcF intervals will be categorized and tabulated for each treatment. The following criteria will be used to specify noteworthy QTcF intervals:

- QTcF >450 msec
- QTcF >480 msec
- QTcF >500 msec
- QTcF increase ≥30 msec from baseline
- QTcF increase ≥60 msec from baseline

Categorical analysis of PR, QRS, and RR intervals will also be performed. The following criteria will be used to specify noteworthy non-QTc intervals (PR, QRS, and RR intervals) and HR:

- PR change-from-baseline >25% resulting in PR >200 msec
- QRS change-from-baseline >25% resulting in QRS >120 msec
- HR change-from-baseline >25% decrease resulting in HR <50 bpm

- HR change-from-baseline >25% increase resulting in HR >100 bpm

The number and percent of subjects in each category will be calculated. Comparisons across timepoints may be conducted if sufficient counts are observed.

11.4.6.3 Cardiac Abnormalities:

The morphological waveform analysis of the ECG data at each timepoint will be utilized for the summary of new onset cardiac abnormalities for treatment. Results will be summarized in frequency tables with counts and percentages for both number of subjects and number of timepoints.

11.4.7 Analysis of Audiology Data

Descriptive summary statistics (mean, SD, median, minimum, and maximum) of ear audiometric and otoacoustic variables at baseline and each applicable post-dosing visit, including changes from baseline values, will be calculated. Baseline values will be the last values recorded before administration of study drug. For change-from-baseline summaries, subjects with an undefined change from baseline, due to missing data, will be excluded. Clinical significance of abnormalities will be indicated in the listings.

All events occurring after study drug dosing will be summarized using frequency counts and percentages. The following summaries will be presented for each event:

- Overall (i.e., regardless of severity or relationship to treatment).
- By severity grade (mild, moderate, or severe).

Detailed analysis plans will be documented in the SAP.

Results of the safety and PK evaluations will be reported in the CSR.

11.4.8 Interim Reports

Plasma and BAL (ELF and AM) apramycin concentration and PK data from cohorts T1 to T4 will be analyzed and summarized.

Interim safety data will be presented to the SMC.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The CTU will maintain appropriate medical and/or research records for the trial, in compliance with ICH E6 GCP, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a DMID-sponsored, DMID-affiliated, or manufacturer-sponsored trial, the CTU will permit authorized representatives of DMID, to include The Emmes Company, LLC (the SDCC), DynPort Vaccine Company, LLC (DVC), and regulatory agencies to review (and, when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Forms for use as source documents will be derived from eCRFs and will be provided by the SDCC and CTU. Additional source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, ECG print-outs, and subject files and records kept at the pharmacy, laboratories, and medico-technical departments involved in the trial.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the CTU is responsible for conducting routine quality assurance and quality control activities to internally monitor study progress and protocol compliance. The Site PI will provide direct access to the CTU, source data/documents, and reports for monitoring and auditing by DMID, and inspection by local and regulatory authorities. The Site PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the CTU for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The PI will ensure that the trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25692 (1997), if applicable. The PI's institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection for federally funded research.

14.2 Institutional Review Board

The CTU will provide for the review and approval of this protocol and associated ICFs by an appropriate IRB/EC listed on the FWA. Any amendments to the protocol or consent materials will also be approved before they are used, unless change is for the safety of the subject. Only those IRB members who are independent of the investigators and DMID will provide an opinion on study-related matters. Verification of IRB approval of the protocol and the written ICF will be transmitted by the investigator or designee before shipment of the study drug. No deviations from or changes to the protocol will be initiated without prior approval of an appropriate amendment unless change is for the safety of the subject.

14.3 Informed Consent Process

The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will adhere to the ICH Harmonized Tripartite Guideline for GCP. Informed consent will be obtained before any protocol-specified procedures or interventions are carried out, and in accordance with 21 CFR 50.25 and 45 CFR 46. Information will be presented both orally and in written form.

An investigator or designee will describe the protocol to potential subjects face-to-face. The ICF may be read to the subjects, but, in any event, the investigator shall give the subjects ample opportunity to inquire about details of the trial and ask any questions before signing the ICF.

Study staff will inform subjects that the trial involves research, and explain the purpose of the trial, those aspects of the trial that are experimental, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, the procedures of the research study, including all invasive procedures, and the probability for assignment to

treatment cohorts. Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. They will also be informed of alternative procedures that may be available, and the important potential benefits and risks of these available alternative procedures. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact (e.g., the Investigator) for answers to any questions relating to the research project. Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. Subjects will be informed that participation is voluntary and that they are free to withdraw from the trial for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Neither the investigator, nor the trial staff, will coerce or unduly influence a subject to participate or continue to participate in the trial. The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. Subjects will be informed that the monitor(s), auditor(s), IRB, DMID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the subject's confidentiality, to the extent permitted by applicable laws and regulations, and that, by signing a written ICF, the subject is authorizing such access. Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential.

ICFs will be in a language fully comprehensible to the prospective subjects. Informed consent shall be documented using a written consent form approved by the IRB and signed and dated by the subject and the person who conducted the informed consent discussion. The signature confirms that the consent is based on information that has been provided and all questions have been answered to the prospective subject's satisfaction. Each subject's signed ICF will be kept on file by the investigator for possible inspection by regulatory authorities and/or DMID and regulatory compliance persons. The subject will receive a copy of the signed and dated written ICF and any other written information provided to the subjects and will receive copies of any signed and dated ICF updates and any amendments to the written information provided to subjects.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

Children aged < 18 years will be excluded from participation because insufficient data are available in adults to judge potential risk in children, and as a Phase 1 trial, there is no known benefit.

Neither women nor minorities will be routinely excluded from participation in the trial. Subjects will be recruited without regard to gender or race. It is expected that race and gender distributions in the trial will approximate the proportion to their numbers within the community.

Women of childbearing potential will be included but will be repeatedly counseled to use effective measures (Section 5.1) to avoid becoming pregnant from the time of screening until Day 30 (± 4 days) (last visit) after the dose of study drug is received because the effects of the study drug on the unborn fetus are not known.

14.5 Subject Confidentiality

Subject confidentiality is held strictly in trust by the participating investigators, their staff, and DMID and its agents. This confidentiality is extended to cover testing of biological samples, and also clinical information related to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the trial or data will be released to any unauthorized third party without prior written approval from DMID. This information and data will not be used by the Site PI or other study personnel for any purpose other than conducting the trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the Site PI or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of the trial; (3) information which is necessary to disclose in order to provide appropriate medical care to a subject; or (4) study results which may be published as described in Section 16.

The study monitors or other authorized representatives of DMID may inspect all documents and records required to be maintained by the Site Investigator, including, but not limited to, medical records (office, clinic or hospital) and pharmacy records for the subjects in the trial. The CTU will permit access to such records.

14.6 Certificate of Confidentiality

To protect privacy, we have received a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the subject, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the Federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported, including child and elder abuse, sexual abuse, or wanting to harm themselves or others.

The release of individual private information or specimens for other research will only occur if consent was obtained from the individual to whom the information, document, or biospecimen pertains, or for the purposes of other research that is in compliance with applicable Federal regulations governing the protection of human subjects in research.

14.7 Study Discontinuation

DMID has the right to terminate the trial or the CTU's participation at any time. Reasons for terminating the trial may include, but are not limited to:

- Incidence or severity of TEAEs indicates a potential health hazard.
- Data recording is inaccurate or incomplete.
- Investigator does not adhere to the protocol or applicable regulatory guidelines in conducting the trial.

If the trial is discontinued, subjects who have signed the ICF and received the study drug will continue to be followed for safety for the duration of the trial. No further study treatments will be administered to other subjects.

14.8 Future Use of Stored Specimens

Blood (plasma) and BAL fluid samples (ELF and AM) will be collected as outlined in the protocol. Subjects will be given a choice during the informed consent process to have their residual linked samples stored indefinitely for future research, have their residual samples de-linked from any subject information and stored indefinitely, or have their residual linked samples destroyed at completion of the study. The use of long-term stored linked samples will be

conducted under the restrictions regarding confidentiality, as outlined in the preceding paragraphs.

Only coded specimens will be sent to the sponsor with the code identifiers maintained by the principal investigator and The Emmes Company, LLC, the NIAID SDCC. Any future research studies will utilize only the residual long-term stored specimens from subjects consenting to future use, which will be stored at the DMID CAR. Residual, coded samples from the plasma PK and BAL (ELF and AM) PK assays may also be used for future exploratory research.

Residual clinical samples will be available upon the completion of the study; however, future use clinical samples may be requested from DMID and shipped from the DMID CMS at any time.

The samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on the samples. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality.

There are no benefits to subjects in the collection, storage and subsequent use of their specimens for future research. Reports about future research done with subjects' samples will NOT be kept in their health records.

Subjects may be given the option to decide if they want their samples to be used for future research or have their samples destroyed at the end of this trial. The subject's decision can be changed at any time by notifying the study doctors or nurses in writing. However, if the subject originally consents to future use and subsequently changes his/her decision, any data from a previously collected sample may still be used for this research.

15 DATA HANDLING AND RECORD KEEPING

The Site PI is responsible to ensure the accuracy, completeness, legibility, and timeliness of reported data. All data collection forms will be completed legibly to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data collection forms will be provided by the SDCC and the CTU will use them to develop the CTU's source documents to record and maintain data for each subject enrolled in the trial. Data reported in the eCRF derived from source documents will be consistent with the source documents or the discrepancies will be explained.

DMID and/or its designee will provide guidance to investigators and other study personnel on making corrections to the data collection forms, source documents and eCRFs.

15.1 Data Management Responsibilities

All source documents and laboratory reports will be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. TEAEs will be graded, assessed for severity and causality, and reviewed by the Site PI or designee.

Data collection is the responsibility of the clinical trial staff at the CTU under the supervision of the Site PI. During the trial, the investigator will maintain complete and accurate documentation for the trial.

The Emmes Company, LLC will serve as the SDCC for the trial, and will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including, but not limited to treatment-emergent AE/SAEs, ConMeds, MH, PE, clinical laboratory data, and ECG data [ECG intervals and interpretations]) will be entered into a 21 CFR 11-compliant Internet Data Entry System provided by the SDCC. The data system includes password protection and internal quality checks (e.g., automatic range checks) to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents by CTU study personnel.

15.3 Types of Data

Data for the trial will include clinical and laboratory safety assessments, plasma and urine renal toxicity markers, 12-lead standard ECG interval measurements and interpretations, audiology assessments and interpretations, bronchoscopy procedure reports including pre- and post-

bronchoscopy records, plasma PK and lung PK (ELF and AM) bioanalytical assay results and corrected ELF and AM concentration data, and concentration of urea in plasma, ELF and AM at each specified timepoint.

15.4 Timing/Reports

A final CSR will be prepared after all safety, 12-lead standard ECG, and plasma and lung PK data are available. See Section 9.6.1 and Section 11.4.8 for additional reporting requirements.

15.5 Study Records Retention

Study files and ICFs will be maintained for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of DMID, if applicable. It is DMID's responsibility to inform the investigator when these documents no longer need to be retained.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the investigator, or the CTU staff. Corrective actions for protocol deviations are to be developed by the CTU and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the Site PI/study staff to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations will be promptly reported to DMID via SDCC's Advantage eClinical®.

All protocol deviations, as defined above, will be addressed in subject source documents. A completed copy of the DMID Protocol Deviation Form will be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations will be sent to the local IRB/EC per their guidelines. The Site PI/study staff is responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

Following completion of the study, the lead Principal Investigator is expected to publish the results of this research in a scientific journal. All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting. As part of the result posting, a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov. For this trial the responsible party is DMID/NIAID/NIH, which will register the trial and post results.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

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18 APPENDICES

Appendix A: Schedule of Study Procedures and Evaluations

Table 1: Schedule of Events

Procedures and Evaluations	Out-patient	In-patient	In-patient	Treatment Period (In-patient)												Follow-up (out-patient)		Early Termination	Unscheduled
	Day -28 to -3	Day -2	Day -1	Day 1								Day 2		Day 3		Day 14 (± 3 days)	Day 30 (±4 days)		
	Screening [1]	Check-in	Baseline	Pre-dose	0 h (dose)	0.5 h (±5 min)	1 h (±5 min)	2 h (±5 min)	4 h (±10 min)	8 h (±15 min)	16 h (±15 min)	24 h (±1 h)	36 h (±1 h)	48 h (±1 h)	60 h (±1 h)				
Informed consent	X																		
Demographics	X																		
Inclusion and exclusion criteria	X [2]																		
Review of Inclusion and exclusion criteria [3]		X [3]	X [3]	X [3]															
Medical history (including family history of auditory loss, renal disorders and sudden death)	X																		
Medical History update [4]		X [4]	X [4]	X [4]															
Prior medication	X	X	X	X															
Concomitant medication					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Complete Physical examination (PE)	X	X													X	X		X	
Abbreviated PE			X																
Symptom-directed (Focused) PE				X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Vital signs (BP, HR, RR, T) [5]	X	X	X	X		X	X		X		X	X	X	X	X	X		X	X
Height, Weight, BMI calculation	X																		
Weight		X														X		X	
Spirometry (FEV1 and FEV1/FVC)	X																		
12-lead ECG (single recording) [6]	X	X										X	X	X	X	X		X	X
12-lead ECG (triplicate recording)[7]			X [7]	X [7]			X [7]		X [7]		X [7]								
Viral Serology [8]	X																		
Alcohol breathalyzer test	X	X																	
Urine drug toxicity and cotinine tests	X	X																	
Clinical laboratory tests [9]	X	X		X								X		X		X		X	X
Calculation of glomerular filtration rate (CKD-EPI) [10]	X	X		X								X		X		X		X	
Urinalysis [11]	X	X		X								X		X		X		X	X
Serum β-HCG pregnancy test (females only)	X																		
Serum FSH (post-menopausal females only)	X																		
Urine pregnancy test (females only)		X																	
Blood sample for exploratory biomarkers [12]		X		X										X		X	X	X	
Urine sample for exploratory biomarkers [13]		X		X										X		X	X	X	
Ear otoscopy, Valsalva test, tympanometry, stapedial reflexes[14]	X																		
Ear otoscopy[14]		X												X		X	X	X	
Ear audiometry and otoacoustic tests [14]	X	X												X		X	X	X	
Admission [15]		X																	
Enrollment and Assignment to T cohorts [16]			X																
Discharge [17]															X				
IV administration of apramycin [18]					X-------[21]														

Check of infusion site					X	X	X				X	X	X	X	X	X		X	
Treatment- emergent adverse events (TEAE) and serious AEs (SAEs) [19]					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood (plasma) sampling for apramycin PK [20]				X		X	X	X	X	X	X	X	X	X	X			X	
Protein Binding Test [21]						X							X						
Administration of sedative or anesthetic medication [22]						X		X	X	X		X							
Bronchoscopy /BAL (for ELF and AM PK) [23]						X		X	X	X		X							
Oxygenation support (TBC)					X	X	X	X	X	X	X	X							
Drinking schedule [24]					X	X	X	X	X	X	X	X	X	X				X	
Counseling [25]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	

1. Screening including Ear -Nose-Throat (ENT)/Audiology assessments are planned to be completed in a single Screening Visit. If schedule cannot be accommodated, ENT/Audiology assessments will be completed on a second visit within the screening period.
2. Inclusion and exclusion criteria regarding ENT/Audiology assessments to be reviewed by audiologist.
3. Review of inclusion and exclusion criteria on Day -2 to determine eligibility for admission; on Day -1, before 12-lead ECG, to determine eligibility for enrollment; and on Day 1 pre-dose to confirm the subject remains eligible for dosing.
4. Medical history update to include pre-dose events.
5. Vital signs include systolic and diastolic BP, heart rate, respiratory rate and oral temperature.
6. 12-lead ECG single recording with 10 sec rhythm strip.
7. 12-lead ECG: triplicate safety ECGs with 10 sec rhythm strips on Day -1 at the same timepoints as the projected timepoints for ECGs to be recorded on Day 1, and on Day 1 within 30 min of dosing, and at 1h (± 5 min), 4h (± 10 min) and 16 h (± 15 min) after starting the apramycin dose. Record ECG before VS and PK draws and, at 4 h post-dosing, before administration of sedation or anesthetic for BAL in cohort T3.
8. Hepatitis B surface antigen (HBsAg), hepatitis virus C antibody (HCV) and human immunodeficiency virus (HIV) antibody.
9. Clinical laboratory tests under fasting conditions: hematology, coagulation, and clinical chemistry panels and thyroid stimulating hormone (TSH) & free T3/free T4. (See Section 8.2.1)
10. The CKD-EPI equation, expressed as a single equation, is GFR = 141 × min(Scr/κ, 1)α × max(Scr/κ, 1)-1.209 × 0.993Age × 1.018 [if female] _ 1.159 [if black], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κor 1, and max indicates the maximum of Scr/κ or 1 [Levey 2009].(Formula will be used and result will be included in the lab report)
11. Urinalysis (UA) by dipstick. If abnormal for blood, protein and glucose, perform microscopic UA. The results of microscopic UA supersede those of UA by dipstick. UA may be postponed in a menstruating female. Abnormal UA in a menstruating female is not graded as a TEAE; the UA should be completed after the end of menstruation.
12. Cystatin C in serum.
13. Kidney-injury molecule 1 (KIM-1) in urine.
14. Ear otoscopy, Valsalva test, tympanometry, stapedial reflexes, and pure-tone audiometry with standard frequencies and distortion product otoacoustic emissions (DPOAEs) at screening. Ear otoscopy, and pure-tone audiometry including high frequencies and DPOAEs at check-in (Day -2 [or Day -4 or Day -3 if needed]) and at Days 3 (+2 days), 14 (± 3 days), and 30 (± 4 days), or ET.
15. Subjects will be admitted to the ward on Day -2 after confirmation of eligibility.
16. Assignment to Lung PK timepoint cohorts (T1 – T5) and Enrollment to be performed before 12-lead ECG on Day -1. – Subjects with ECGs on Day-1 and pre-dose on Day 1 with clinically significant abnormalities according to the PI should not be eligible for receiving study drug and should be withdrawn and replaced.
17. Subjects may be discharged from the CTU after Day 3 collection of the 60-h plasma PK sample.
18. Duration of apramycin infusion is 30 ± 5 min.
19. Adverse events from the start of dosing to the end of the trial are considered treatment-emergent adverse events (TEAE) or serious AEs (SAE).
20. Blood samples for plasma PK are taken pre-dose, and at 30 (± 5) minutes (end of infusion) and 1 h (±5 min), 2 h (±5 min), 4 h (±10 min), 8 h (±15 min), 16 h (±15 min), 24 h (±1 h), 36 h (±1 h), 48 h (±1 h) and 60 h (±1 h) after start of the infusion - Blood samples for plasma PK are taken at the nominal timepoints. If collection of BAL is delayed, plasma PK will be collected at the nominal timepoint and again within 5 min after collection of the last BAL sample. In that case, total apramycin concentration will be measured in both samples, but only the drug concentration in the delayed plasma PK sample will be used to calculate the Lung PK/Plasma PK ratio.
21. A protein binding test for the measurement of free apramycin will be performed using aliquots of plasma PK samples collected at 0.5 h (± 5 min) and 36 h (± 1 h) after start of infusion.
22. Type of sedation or light anesthesia medication for bronchoscopy/BAL procedure and local anesthetic TBD by the anesthesia and bronchoscopy protocols.
23. Each subject is assigned to a single bronchoscopy/BAL procedure. Lung PK samples (for measurement of total apramycin concentration) after initiation of infusion and corresponding cohorts (T) are: (Cohort T1), 30 min (±5 min); (Cohort T2), 2 h (±5 min); (Cohort T3), 4 h (±10 min); (Cohort T4), 8h (± 15 min); and (Cohort T5), 24 h (± 1 h). Lung PK timepoints are aligned with plasma PK samples collected for the measurement of total apramycin concentration at 30 min (±5 min), 2 h (±5 min), 4 h (±10 min), 8 h (±15 min) and 24 h (±1 h) after start of apramycin infusion, If BAL is delayed, plasma sample will be collected at the nominal timepoint and again within 5 minutes after the last BAL sample collection. Urea will be measured at the same timepoints in plasma and BAL (ELF) samples.
24. Subjects to be kept hydrated water starting 30 min before until 24 h after start of infusion (document time and volume of water intake).
25. Provide counseling at the end of each encounter with the subject. Counsel on use of contraception (males and females), avoidance of pregnancy (women of childbearing potential), avoidance of sperm donation, and avoidance of prohibited medications, illicit drugs, alcohol, nicotine products, vigorous exercise, and exposure to loud noises.

Appendix B: Adverse Events Toxicity Grading Criteria

ABBREVIATIONS: Abbreviations utilized in the Tables:

ADL = Activities of Daily Living

CTU = Clinical Trial Unit

ULN = Upper Limit of Normal

IV = Intravenous

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables, use the scale below to estimate grade of severity:

GRADE 1	**Mild**	Events require minimal or no treatment; do not interfere with the subject's daily activities.
GRADE 2	**Moderate**	Events result in a low level of inconvenience or concern with therapeutic measures; may cause some interference with functioning.
GRADE 3	**Severe**	Events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment; are usually incapacitating.

SERIOUS OR LIFE-THREATENING AEs

Clinical events considered to be serious or life-threatening include, but are not limited to seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, and severe depression.

COMMENTS REGARDING THE USE OF THESE TABLES

- Standardized and commonly used toxicity tables (Division of AIDS, National Cancer Institute [NCI] Common Toxicity Criteria [CTC], and World Health Organization [WHO]) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of subjects in DMID trials.
- For parameters not included in the following Toxicity Tables, the CTU will refer to the "Guide for Estimating Severity Grade" located above.
- Criteria are generally grouped by body system.

Some protocols may have additional protocol specific grading criteria, which will supersede the use of these tables for specified criteria.

Table 2: Toxicity Grading Tables – CLINICAL AEs

Clinical AEs	Reference range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
VITAL SIGNS [1]				
Fever - °C	36.1 - 37.2 [1]	37.3 - 38.4	38.5 - 38.9	>38.9
Fever - °F	97.0 - 99.0 [1]	99.1 - 101.1	101.2 - 102.0	>102.0
Tachycardia - bpm	50 - 100 [2,3,4,5]	101 - 115	116 - 130	>130 or ventricular dysrhythmias
Bradycardia – bpm		45 - 49	40 - 44	<40
Hypertension (systolic) - mmHg	130/89 [2,3,4,5]	131 - 150	151 - 160	>160
Hypertension (diastolic) - mmHg		90 - 95	96 - 100	>100
Hypotension (systolic) - mmHg		85 - 88	80 - 84	<80
Tachypnea – breaths per min	10 - 20 [2,3,4,5]	21 - 25	26 - 30	>30

Note 1: No recent hot or cold beverages or smoking. A protocol should select either ^{0}C or ^{0}F for inclusion

Note 2: Assume awake and in supine position for 5 min at rest. For TEAE, measurements at least 3 times with 2 concordant results (See Section 8.1.6)

Note 3: Abnormal HR and BP on first measurement may be repeated twice more with the subject resting between measurements for at least 5 min (See Section 8.1.6).

Note 4: Exceptions to screening BP and HR reference range are:

(a) Subjects with baseline HR ≥ 45 to 50 bpm may be accepted if they do not have symptomatic bradycardia, including syncope, heart disease, ECG abnormalities, or history of syncope or use of medication

(b) Subjects with baseline SBP up to 140 mmHg and DBP up to 90 mmHg may be accepted if they do not have MH of symptomatic hypertension, including paroxysmal hypertension, heart disease, renal disease, CNS disease, visual abnormalities or evidence of peripheral vascular disease, ECG abnormalities, use of medication, use of illicit drugs or FH of hypertension

Note 5: Isolated/individual abnormalities of VS would not be considered toward halting criteria. Abnormalities of VS will be described as “increased X” or “decreased X” (X = HR, BP, RR, temperature) if asymptomatic, transient and not associated with a systemic or organ-specific disorder and coded by MedDRA within the System Organ Class (SOC) “Investigations.” These abnormalities will be graded per criteria in this table, but not considered in determining whether study stopping criteria have been met. On the other hand, abnormalities of VS that are either secondary to systemic or organ-specific clinical syndrome or primary disorders will be coded in the appropriate SOC (e.g., “cardiac disorders”, “respiratory disorders”, “immunological disorders”, etc). These abnormalities will be considered in determining whether stopping criteria have been met.

Table 2: Toxicity Grading Tables – CLINICAL AEs

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
CARDIOVASCULAR			
Arrhythmia		Asymptomatic or transient signs; no medical intervention required.	Recurrent and/or persistent signs; symptomatic medical intervention required.
Hemorrhage	Estimated blood loss ≤100 mL.	Estimated blood loss >100mL; no transfusion required.	Blood transfusion required.
RESPIRATORY			
Cough	Transient cough no treatment required.	Persistent cough treatment required.	Interferes with daily activities.
Bronchospasm, Acute	Transient bronchospasm no treatment required; FEV1 71-80% of predicted peak flow.	Requires treatment; normalizes with bronchodilator; FEV1 60-70% of predicted peak flow.	No normalization with bronchodilator; FEV1 <60% of predicted peak flow.
Dyspnea	Does not interfere with usual and social activities.	Interferes with usual and social activities; no treatment.	Prevents usual and social activities OR requires treatment.
EAR AND LABYRINTH DISORDERS			
Hearing Impairment (Adult enrolled on a Monitoring Program (on a 1, 2, 4, 3, 6, and 8 kHz audiogram)	Threshold shift of 15 - 25 dB averaged at 2 contiguous test frequencies in at least one ear.	Threshold shift of >25 dB averaged at 2 contiguous test frequencies in at least one ear.	Threshold shift of >25 dB averaged at 3 contiguous test frequencies in at least one ear; therapeutic intervention indicated.
Tinnitus	Mild symptoms intervention not indicated.	Moderate symptoms; limiting instrumental ADL.	Severe symptoms; limiting self-care ADL.
Vertigo	Mild symptoms.	Moderate symptoms; limiting instrumental ADL.	Severe symptoms; limiting self-care ADL.
GASTROINTESTINAL			
Nausea	No interference with normal activity.	Some interference with normal activity.	Prevents daily activities.
Vomiting	No interference with activity OR 1-2 episodes in a 24-h period.	Some interference with activity OR >2 episodes in a 24-h period.	Prevents daily activity OR requires medical intervention.

Table 2: Toxicity Grading Tables – CLINICAL AEs

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Diarrhea	2-3 loose or watery stools in a 24-h period.	4-5 loose OR watery stools in a 24-h period.	6 or more loose or watery stools in a 24-h period OR requires IV hydration OR requires medical intervention.
Oral Discomfort / Dysphagia	Mild discomfort; no difficulty swallowing.	Some limits on eating /drinking.	Eating/talking very limited; unable to swallow solid foods.
LOCAL IV CATHETER REACTION			
IV site reaction	Not applicable.	Erythema with associated symptoms (e.g., edema, pain, induration, phlebitis).	Ulceration or necrosis; severe tissue damage; operative intervention indicated.
SYSTEMIC REACTIONS			
Anaphylaxis **	--	--	Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema or angioedema; hypotension.
***Definition*: A disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis, and loss of consciousness, and may lead to death.*			
Allergic Reaction	Pruritus without rash.	Localized urticaria OR requires oral therapy.	Generalized urticaria OR angioedema OR anaphylaxis OR requires epinephrine
Hypersensitivity (including drug fever)	Transient flushing or rash; temperature 38.0-38.4°C (100.4-101.1°F).	Rash; flushing; urticaria; dyspnea; temperature 38.5 - 38.9°C (101.2 – 102.0°F).	Symptomatic bronchospasm with or without urticaria; parenteral medication indicated; allergy-related edema or angioedema; hypotension; temperature >38.9°C (>102.0°F).
Headache	No interference with activity.	Repeated use of non-narcotic pain reliever for more than 24 h OR some interference with activity.	Significant; any use of narcotic pain reliever OR prevents daily activity OR requires triptans.
Fatigue	No interference with activity.	Some interference with activity.	Significant; prevents daily activity.

Table 2: Toxicity Grading Tables – CLINICAL AEs

Clinical AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Myalgia	No interference with activity.	Some interference with activity.	Significant; prevents daily activity.
SKIN			
Mucocutaneous	Erythema, pruritus.	Diffuse, maculo-papular rash, dry desquamation.	Vesiculation OR moist desquamation OR ulceration.
Pruritus	No or minimal interference with usual social and functional activities.	Greater than minimal interference with usual social and functional activities.	Inability to perform usual social and functional daily activities.
ALL OTHER CONDITIONS			
Illness or clinical AE (as defined according to applicable regulations)	Require minimal or no treatment; does not interfere with the subject's daily activities.	Result in a low level of inconvenience or concern with therapeutic measures; may cause some interference with functioning.	Interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment; are usually incapacitating.

Table 3: Toxicity Grading Tables – LABORATORY AEs

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Blood, serum, or plasma *			
HEMATOLOGY			
Hemoglobin decrease, female – g/dL	9.6 – 11.0	8.0 – 9.5	<8.0
Hemoglobin decrease, male – g/dL	11.0 – 12.9	8.5 – 10.9	<8.5
WBC increase – $x10^3$/ µL	>10.8 – 15.0	>15.0 – 20.0	>20.0
WBC decrease – $x10^3$/ µL	2.4 – <3.4	1.4 – <2.4	<1.4
Neutrophils decrease – $x10^3$/ µL	1.0 – <1.4	0.75 – <1.0	<0.75
Lymphocytes decrease – $x10^3$/ µL	<0.7 –0.4	<0.4 – 0.3	<0.30
Monocytes increase – $x10^3$/ µL	>0.9 – 2.0	>2.0 – 3.0	>3.0
Eosinophils increase – $x10^3$/ µL	>0.4 – 0.75	>0.75 – 1.0	>1.0
Basophils increase – $x10^3$/ µL	>0.2 – 0.5	>0.5 – 0.8	>0.8
Platelets decrease – $x10^3$/ µL	90 – <150	55 – <90	<55
COAGULATION			
PT INR ratio	>1.2 – 1.8	>1.8 – 2.1	>2.1
Prothrombin Time (PT) - seconds	>12.0 – 15.0	>15.0 – 18.6	>18.6
Activated Partial Thromboplastin Time (APTT) - seconds	>33.0 – 54.0	>54.0 – 75.0	>75.0
CHEMISTRY			
Sodium decrease – mmol/L	130 - 133	124 –129	<124
Sodium increase – mmol/L	145 - 150	151 – 156	>156
Potassium increase – mmol/L	5.3 – 6.0	6.1 – 6.5	>6.5
Potassium decrease – mmol/L	3.4– 3.0	2.5 – 2.9	<2.5
Total Carbon Dioxide (CO_2) increase – mmol/L	30 - 35	36 - 37	>37
Total Carbon Dioxide (CO_2) decrease – mmol/L	17 - 19	14 - 16	<14
Calcium decrease – mg/dL	7.8 – 8.6	7.0 – 7.7	<7.0
Calcium increase – mg/dL	10.3 – 11.4	11.5 – 12.5	>12.5
Magnesium decrease – mg/dL	1.2 – 1.5	1.1 – 0.9	<0.9

Table 3: Toxicity Grading Tables – LABORATORY AEs

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Blood urea nitrogen (BUN) increase, 18-39Y - mg/dL	21 - 58	59 – 120	>120
Blood urea nitrogen (BUN) increase, 40-59Y – mg/dL	25 - 58	59 – 120	>120
Glucose decrease, fasting – mg/dL	55 - 69	40 – 54	<40
Glucose increase, fasting – mg/dL	100 – 160	161 – 250	>250
Creatinine increase, male – mg/dL	1.28 – 1.90	1.91 – 3.80	>3.80
Creatinine increase, female – mg/dL	0.58 – 1.50	1.51 – 3.00	>3.00
Direct bilirubin	0.41 – 0.70	0.71 – 1.20	>1.20
Total bilirubin (serum) increase – mg/dL (with other LFTs in the normal range)	1.3 – 2.0	2.1 – 2.5	>2.5
Total bilirubin (serum) increase – mg/dL (accompanied by a >3 x ULN increase in ALT or AST)	1.3 – 1.7	1.8 – 2.4	>2.4
Total protein decrease – g/dL	5.1 – 5.9	4.6 – 5.0	<4.6
Albumin, decrease, Male, 18-30Y - g/dL	4.0 – 3.0	2.0– 2.9	<2.0
Albumin decrease, Female, 18-30Y – g/dL	3.8 – 3.0	2.0– 2.9	<2.0
Albumin decrease, Male, 31-50Y – g/dL	3.9 – 3.0	2.0– 2.9	<2.0
Albumin decrease, Female, 31-50Y – g/dL	3.7 – 3.0	2.0– 2.9	<2.0
AST increase – U/L	41 – 80	81 – 120	>120
ALT increase, male – U/L	45– 88	89 - 132	>132
ALT increase, female – U/L	33 – 64	65 – 96	>96
Alkaline phosphatase (AP) increase, males 18-20Y – U/L	126 – 250	251 – 375	>375
Alkaline phosphatase (AP) increase, females, 18-20Y – U/L	107 – 250	251 – 375	>375
Alkaline phosphatase (AP) increase, 21-150Y – U/L	122 – 250	251 – 375	>375

Table 3: Toxicity Grading Tables – LABORATORY AEs

Laboratory AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Urine			
URINALYSIS by Dipstick			
Protein	1+	2+	>2+
Blood (occult)	1+	2+	>2+
Glucose	1+	2+	>2+
URINE MICROSCOPY			
Red blood cells (RBC) per HPF	3-10	11-30	>30 and/or gross blood
White blood cells (WBC) per HPF	6-10	11-30	>30 and/or symptomatic urogenital infection
Bacteria (microscopic)	few	moderate	many

Note 1: With the exception of AST, ALT, AP, bilirubin, electrolytes, urine protein, BUN and creatinine, which should be within reference range, lab values of other analytes in the grade 1 range are acceptable for enrollment if (a) they are not considered to be clinically significant by the investigator and (b) there is no cluster of abnormal labs that combined are suggestive of an underlying disorder.

Note 2: Other Exceptions to screening laboratory tests' normal reference ranges are:

a. *Racially based low total WBC or neutrophil counts up to toxicity Grade 1 are allowed, but toxicity Grades 2 or 3 are exclusionary.*

b. *Labs performed as part of a panel but not listed above are to be recorded in the database. If abnormal, they are not exclusionary and are not to be graded per Toxicity table, however, the investigator would make a clinical decision about their clinical significance and, if clinically significant, they will be graded according to the criteria in Section 9.2.1 . (Examples include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), and nucleated red blood cell count (NRBC CT), which are included in a CBC with differential.)*

Note 3: If a subject was accepted into the trial with a laboratory value of an analyte that overlaps with values used for grading Grade 1 laboratory abnormalities, a TEAE will be reported if the on-study value of the same analyte increases to Grade 2 or higher.

Note 4: If the dipstick UA is abnormal, a microscopic UA will be performed, and the results will supersede the results of the dipstick UA.

Note 5: Menstruating females with a positive dipstick UA or microscopic UA may be retested following cessation of menses.

Note 6: Isolated laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to collection or laboratory error may be repeated once.

Table 4: Toxicity Grading Tables – ECG

ECG interval abnormality	Reference range	Grade 1	Grade 2	Grade 3
QTcF interval prolonged (msec): • Male • Female	• ≤ 450 msec • ≤ 470 msec	Asymptomatic, QTcF • 451 − 479 msec • 471 − 479 msec	Asymptomatic, QTcF 480-500 msec **OR** increase in interval 30-59msec above baseline	Asymptomatic, QTcF >500 msec OR increase in interval ≥60 msec above baseline
PR interval prolonged (msec)	≤210 msec	211-250 msec	>250 msec	Type II 2nd degree AV block **OR** ventricular pause >3.0 sec

Note 1: Events will be coded as treatment-emergent SAEs if there are life-threatening associated symptoms or signs (arrhythmia, CHF, hypotension, syncope, TdP, etc.).

Note 2: If a male subject was accepted into the trial with a QTcF value that overlaps with values used for grading Grade 1 QTcF prolongation, a TEAE will be reported if the QTcF value is higher than the baseline value.

Appendix C: Blood Volume Withdrawn During the Trial

Table 5: Laboratory Samples and Estimated Total Blood Volume (mL)

Study Periods	Out-patient	In-patient	In-patient	In-patient Dosing Period (Days 1 – 3)					Out-patient		ET
Study Visit	Screen	Check-in	Baseline	Dosing	Follow-up		Discharge		Follow-up	Final	
Study Day [a]	-28 to -3	-2	-1	1	2 (24 h)	2 (36 h)	3 (48 h)	3 (60 h)	14 (±3 days)	30 (±4 days)	
HEMATOLOGY [1]	4.0	4.0		4.0	4.0	4.0	4.0	4.0	4.0		4.0
CHEMISTRY and serum β-HCG and FSH [1]	8.5	8.5		8.5	8.5	8.5	8.5	8.5	8.5		8.5
COAGULATION [1]	2.7	2.7		2.7	2.7	2.7	2.7	2.7	2.7		2.7
TSH, Free T4, Free T3 [1]	3.5	3.5		3.5	3.5	3.5	3.5	3.5	3.5		3.5
Viral Serology (HIV, HBsAg, HCV) [2]	7.0										
Exploratory Marker (serum Cystatin) [3]		3.5		3.5			3.5		3.5	3.5	3.5
PK [4]				56.0	8.0	8.0	8.0	8.0			8.0
Total volume/visit	25.7	22.2	0	78.2	26.7	26.7	30.2	26.7	22.2	3.5	30.2
Cumulative total volume	25.7	47.9	47.9	126.1	152.8	179.5	209.7	236.4	258.6	262.1	

[a] Study Days shown correspond to days in each study period. For a view of the cumulative numbering of study days, please refer to Section 7.

[1] Clinical Safety blood tests (HEM, CHEM, COAG, TSH, fT4, fT3) are drawn at Screening Visit, on Day -2, and on Days 1, 2, 3 and 14 (± 3 days), or ET. Serum pregnancy test in all women is drawn at Screening. FSH is drawn only at Screening in post-menopausal women.

[2] Viral serology tests are drawn at Screening.

[3] Serum Cystatin test is drawn on Day -2, Day 3 (at 48 h) and Day 14 (± 3 days).

[4] PK plasma samples are drawn on Day 1 (30 min before dosing and at 0.5, 1, 2, 4, 8, 16 h after dosing); on Day 2 (24 and 36 h after dosing) and on Day 3 (48 and 60 h after dosing), or ET if occurs within 24 h of dosing.