Title: Phase 1 Feasibility Trial of Inhaled Tobramycin in Preterm Infants with Bronchopulmonary Dysplasia

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Sponsor: Erik Allen Jensen, MD

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Title:	**Phase 1 Feasibility Trial of Inhaled Tobramycin in Preterm Infants with Bronchopulmonary Dysplasia**
Short Title	Phase 1 Trial of Inhaled Tobramycin in BPD
Drug or Device Name(s):	Tobramycin solution (300mg/5mL) for inhalation USP
FDA IND	145836
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ABBREVIATIONS AND DEFINITIONS OF TERMS

a-TEOAE	Automated transient evoked otoacoustic emissions
AAP	American Academy of Pediatrics
ABR	Auditory brainstem response
AE	Adverse event
AKI	Acute kidney injury
BAEP	Brainstem auditory evoked potential
BAL	Bronchoalveolar lavage
BPD	Bronchopulmonary dysplasia
CCP	Center for Clinical Pharmacology
CF	Cystic fibrosis
CFU	Colony forming units
CHOP	Children's Hospital of Philadelphia
CI	Confidence interval
DSMB	Data safety monitoring board
ET	Endotracheal tube
FDA	Federal Drug Administration
FiO_2	Fraction of inspired oxygen
GNR	Gram-negative rod
HPLC-MS/MS	High-performance liquid chromatography tandem mass spectrometry
IDS	Investigational drug services
IND	Investigational new drug
IV	Intravenous
JCIH	Joint Committee on Infant Hearing
KDIGO	Kidney disease improving global outcomes
MAP	Mean airway pressure
MIC	Minimum inhibitory concentration
MTD	Maximum tolerated dose
NeoCLD	Newborn and Infant chronic lung disease (program at CHOP)
N/IICU	Newborn and infant intensive care unit
OAE	Otoacoustic emissions
OI	Oxygenation index
PI	Primary investigator
PK	Pharmacokinetics
PMA	Postmenstrual age
RSS	Respiratory severity score
SAE	Serious adverse event
SGOT/AST	Aspartate aminotransferase
SGPT/ALT	Alanine aminotransferase
SpO_2	Pulse oxygen saturation
TSI	Tobramycin solution for inhalation
USP	United States pharmacopeia
VROA	Visually reinforced orientation audiometry

ABSTRACT

Context:
Bronchopulmonary dysplasia (BPD) is among the most common and consequential chronic complications associated with preterm birth. Among infants with BPD who continue to require invasive mechanical ventilation near term corrected gestation, airway colonization with a pathogenic Gram-negative rod (GNR) bacteria is an independent risk factor for prolonged need for supplemental oxygen and home oxygen therapy. Inhaled tobramycin is an effective therapy for treating airway infections secondary to GNR bacteria and has shown clinical benefit when used in several pediatric and adult chronic respiratory conditions. The safety and efficacy of inhaled tobramycin in preterm infants with BPD and GNR bacteria present in the airways is unknown.

Objectives:
The objective of this open-label, phase 1, inter-patient dose escalation feasibility trial is to identify a safe, well tolerated dose of inhaled tobramycin for use in very preterm infants with BPD receiving invasive mechanical ventilation who have evidence of a pathogenic GNR detected by tracheal aspirate culture.

Study Design:
Prospective open-label, phase 1, inter-patient 3+3 dose escalation feasibility trial. An untreated, observational cohort of eligible infants will also be enrolled.

Setting/Participants:
This trial will take place in the newborn and infant intensive care unit (N/IICU) at the Children's Hospital of Philadelphia. This will be the only site. Up to 103 infants will be enrolled in this study to produce up to 24 infants with evaluable trial data and up to 28 participants in the observational cohort. The trial will use the following inclusion/exclusion criteria:

Inclusion Criteria:

1. Male or female infants born <32 weeks' gestation
2. Diagnosed with BPD (use of supplemental oxygen or respiratory support at 36 weeks postmenstrual age[1])
3. Postmenstrual age ≥36 weeks at study enrollment
4. Treatment with invasive mechanical ventilation at enrollment without planned tracheal extubation within 7 days after enrollment
5. Tracheal aspirate culture positive for a pathogenic GNR bacteria (see list of eligible organisms in section 3.4.3) within 7 days of beginning the study phase – this testing will be performed during the screening phase and must be met to participate in the phase 1 trial or observational cohort
6. Parental/guardian permission (informed consent).

Exclusion Criteria:

1. Serum creatinine >0.4mg/dL within 14 days prior to enrollment
2. Congenital or acquired disease of the kidney or renal collecting system that adversely affects renal function
3. Congenital or acquired hepatobiliary disease that adversely affects liver function
4. Treatment with a systemic antibiotic within 7 days prior to enrollment

[1] Postmenstrual age (PMA) is calculated as the gestational age at birth plus the chronological age

5. Treatment with a nephrotoxic medication (see list in appendix A), excluding diuretics, within 48 hours prior to enrollment
6. Treatment with a continuous infusion of a neuromuscular blocker (see list in Appendix B) within 48 hours prior to enrollment
7. Known intolerance to aminoglycoside antibiotics
8. Current treatment with high frequency or other oscillating mechanical ventilation
9. Presence of a cancer diagnosis
10. Maternal family history of early onset hearing loss defined as the need for an assistive hearing device prescribed before 30 years of age
11. Persistent endotracheal tube leak >20%.
12. Any prior use of an investigational drug as part of an FDA approved IND protocol.
13. A subject who, in the judgement of the Investigator, is not an appropriate candidate for this research study.

Study Interventions and Measures:
The study will investigate tobramycin solution for inhalation (300mg/5mL). Up to 4 different dosages will be investigated: 78mg, 150mg, 216mg, and 300mg, all administered every 12 hours for up to 14 days.

The primary study outcome is a composite safety endpoint defined by the presence of any of the following events occurring at any time point during the 14-day trial:

1) Trough serum tobramycin level (measured 11 hours after the administered dose) ≥1mcg/mL
2) Increase in serum creatinine level by ≥0.3mg/dL above pre-trial baseline
3) Increase in serum creatinine level >1.5-fold above pre-trial baseline
4) Urine output <0.5mL/kg/hr for 12 consecutive hours
5) Any serious adverse event possibly attributable to the study drug

Occurrence of the primary outcome in ≥2 infants at a single dosage level will define the "maximum tolerated dose" and result in cessation of the trial.

Additional exploratory secondary safety endpoints are:

1) New onset or worsened coughing associated with a change in respiratory status (SpO_2 <80% for >10 seconds; need for increase in FiO_2 by >20%)
2) Obstruction of the endotracheal tube requiring tube replacement
3) Unplanned tracheal extubation
4) Desaturation (SpO_2 <80% for >10 seconds) during administration of inhaled tobramycin
5) Rates of pre-discharge failed audiology examinations
6) Rates of new intra-patient microbial resistance to tobramycin

The exploratory secondary efficacy endpoints are:

1) Tracheal aspirate tobramycin level
2) Change in tracheal aspirate pathogenic bacterial colony forming unit (CFU) counts measured by high resolution, quantitative culture
3) Change in the following measures of administered respiratory support: fraction of inspired oxygen (FiO_2), ventilator mean airway pressure (MAP), and respiratory severity score (MAP x FiO_2)

4) Change in the following measures of hypoxemia (SpO_2<80%): Intermittent hypoxemia (events lasting 10s-3min), prolong hypoxemia (events lasting >1min), and daily proportion of time in hypoxemia
5) Change in tracheal aspirate cytokine levels, neutrophil to total WBC ratio, and patterns in the airway microbiome
6) Change in dynamic lung compliance, airway resistance, peak expiratory flow, and carbon dioxide (CO_2) elimination

PROTOCOL SYNOPSIS

Study Title	Phase 1 Feasibility Trial of Inhaled Tobramycin in Infants with Bronchopulmonary Dysplasia
Funder	Children's Hospital of Philadelphia
Clinical Phase	Phase I
Study Rationale	Bronchopulmonary dysplasia (BPD) is among the most common and consequential chronic complications of preterm birth. Colonization or infection of the airway by a pathogenic Gram-negative rod (GNR) bacteria in preterm infants with BPD receiving invasive mechanical ventilation is an independent risk factor for prolonged use of supplemental oxygen and home oxygen therapy. Respiratory infection with *Pseudomonas aeruginosa* (the most common GNR detected in airways of infants with BPD) is a known risk factor for poor pulmonary function and adverse clinical outcomes in several chronic respiratory conditions. Tobramycin is an aminoglycoside antibiotic that has been shown to eradicate bacterial airway colonization with *P. aeruginosa* and other pathogenic GNR bacteria in older children and adults when delivered to the lungs via nebulization. The safety and efficacy of inhaled tobramycin in preterm infants with BPD is unknown and there are no robust data that inform the optimal dosing strategy for use in this population.
Study Objective(s)	**Primary** • To determine the maximum, safely tolerated dose of tobramycin solution for inhalation (selected between 78mg, 150mg, 216mg, and 300mg administered every 12 hours) in very preterm infants with BPD who are actively receiving invasive mechanical ventilation and have evidence of a pathogenic GNR bacteria (listed in section 3.4.3) detected by tracheal aspirate culture. **Secondary** • To evaluate the association between the dose of inhaled tobramycin, plasma tobramycin pharmacokinetics, and peak tracheal aspirate tobramycin levels. • To compare the change (pre vs. post study) in tracheal aspirate pathogenic bacterial colony forming unit (CFU) counts measured by high resolution culture, (1) between participants in the phase 1 trial and untreated infants in the observational cohort, and (2) between trial participants stratified by the administered dose of inhaled tobramycin. • To compare the change in measures of administered respiratory support (FiO_2, ventilator mean airway pressure, and respiratory severity score) during the 14-day study period, (1) between participants in the observational cohort and phase 1 trial, and (2) between trial participants stratified by the administered dose of inhaled tobramycin.

	• To evaluate changes (pre vs. post treatment) in airway cytokine levels, neutrophil/WBC ratio, and patterns in the airway microbiome. • To compare changes in the following measures of hypoxemia (SpO_2<80%): Intermittent hypoxemia (events lasting 10s-3min), prolong hypoxemia (events lasting >1min), and daily proportion of time in hypoxemia during the 14-day study period, (1) between participants in the observational cohort and phase 1 trial, and (2) between trial participants stratified by the administered dose of inhaled tobramycin. • To evaluate longitudinal change in dynamic lung compliance, airway resistance, peak expiratory flow, and CO_2 elimination during the 14-day study period, (1) between participants in the observational cohort and phase 1 trial, and (2) between trial participants stratified by the administered dose of inhaled tobramycin.
Study Drug	Tobramycin solution (300mg/5mL) for inhalation USP
Study Design	Open-label, inter-patient 3+3 dose escalation trial. An untreated observational cohort of eligible infants will also be enrolled and outcomes compared to trial participants.
Subject Population key criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Male or female infants born <32 weeks' gestation 2. Diagnosed with BPD (use of supplemental oxygen or respiratory support at 36 weeks postmenstrual age) 3. Postmenstrual age ≥36 weeks at study enrollment 4. Treatment with invasive mechanical ventilation at enrollment without planned tracheal extubation within 7 days after enrollment 5. Tracheal aspirate culture positive for a pathogenic GNR bacteria (eligible organisms listed in section 3.4.3) within 7 days of beginning the study phase – this testing will be performed during the screening phase and must be met to participate in the phase 1 trial or observational cohort 6. Parental/guardian permission (informed consent). **Exclusion Criteria** 1. Serum creatinine >0.4mg/dL within 14 days prior to enrollment 2. Congenital or acquired disease of the kidney or renal collecting system that adversely affects renal function 3. Congenital or acquired hepatobiliary disease that adversely affects liver function 4. Treatment with a systemic antibiotic within 7 days prior to enrollment 5. Treatment with a potentially nephrotoxic medication (see list in Appendix A), excluding diuretics, within 48 hours prior to enrollment

	6. Treatment with a continuous infusion of a neuromuscular blocker (see list in Appendix B) within 48 hours prior to enrollment 7. Known intolerance to aminoglycoside antibiotics 8. Current treatment with high frequency or other oscillating mechanical ventilation 9. Presence of a cancer diagnosis 10. Maternal family history of early onset hearing loss defined as the need for an assistive hearing device prescribed before 30 years of age 11. Persistent endotracheal tube leak >20%. 12. Any investigational drug use (as part of an FDA approved IND protocol) prior to enrollment. 13. A subject who, in the judgement of the Investigator, is not an appropriate candidate for this research study.
Number of Subjects	Up to 103 infants will be enrolled in this study to produce up to 24 infants with evaluable trial data and up to 28 participants in the observational cohort. The Children's Hospital of Philadelphia will be the only study site.
Study Duration	Each subject's participation will last from enrollment until hospital discharge. The phase 1 trial will last up to 14 days. The entire study is expected to last up to 3 years.
Study Phases	
Screening Phase	Potential subjects will be screened using daily census logs and the protocol inclusion and exclusion criteria. Infants who meet study eligibility criteria will be enrolled if a parent or guardian consents to once-weekly collections of a screening tracheal aspirate culture. If a screening culture is positive for a pathogenic GNR, the infant will be eligible to participate in the phase 1 trial or observational (non-treated) cohort, but can be enrolled in only one.
Study Phase	
Phase 1 Trial	The phase 1 trial will last up to 14 days, with each infant receiving a specified dose of tobramycin solution for inhalation every 12 hours, administered via vibrating mesh nebulizer. The drug dose (78mg, 150mg, 216mg, or 300mg) will be determined as per the inter-patient dose escalation 3+3 design protocol. If the 300mg dose level is completed without evidence of safety concerns (i.e., stopping rules are not met), up to 3 additional infants will be enrolled to receive 300mg to provide a more robust assessment of drug dose tolerability and PK. During the trial, each infant will undergo continuous pulse oximetry, and blood and tracheal aspirate sampling and respiratory mechanics measurements at pre-specified time points to assess dose safety and potential efficacy. Clinical data will also be recorded daily throughout the trial.
Observational (non-treated) Cohort	Infants enrolled in the untreated observational cohort will undergo continuous pulse oximetry for 14 days after enrollment. Respiratory

	mechanics measurements will be performed at set timepoints. Clinical data will be collected daily from the medical record.
Follow-Up Phase	All infants enrolled in the phase 1 trial and observational cohorts will be followed until hospital discharge. Pre-defined clinical data will be abstracted periodically from the medical record. For infants enrolled in the phase 1 trial, tracheal aspirate samples for high resolution culture, 16s sequencing, and cytokine level measurement will be collected ≤48 hours and 14 days after receiving the final dose of inhaled tobramycin. Infants enrolled in the observational cohort will undergo tracheal aspirate collection for culture ≤48 hours after completing the 14-day study phase. Infants in both cohorts will undergo respiratory mechanics measurements ≤48 hours after completing the study phase.
Safety Evaluations	The primary study endpoint of the phase 1 trial is a safety outcome defined as the occurrence of any of the following: • Trough serum tobramycin level (measured 11 hours after the last administered dose) ≥1µg/mL • Increase in serum creatinine level by ≥0.3mg/dL above the pre-trial level • Increase in serum creatinine >1.5-fold above the pre-trial level • Urine output <0.5mL/kg/hr for 12 hours • Any serious adverse event possibly attributable to the study drug Additional exploratory secondary safety endpoints are: • New onset or worsened coughing associated with a change in respiratory status (SpO_2 <80% for >10 seconds; need for increase in FiO_2 by >20%) • Obstruction of the endotracheal tube requiring tube replacement • Unplanned tracheal extubation • Desaturation (SpO_2 <80% for >10 seconds) during administration of inhaled tobramycin • Rates of pre-discharge failed audiology examinations • Rates of new intra-patient microbial resistance to tobramycin
Efficacy Evaluations	The exploratory secondary efficacy endpoints are: • Tracheal aspirate tobramycin levels • Change in tracheal aspirate pathogenic bacterial colony forming unit (CFU) counts measured by high resolution, quantitative culture • Change in the following measures of administered respiratory support: fraction of inspired oxygen (FiO_2), ventilator mean airway pressure (MAP), respiratory severity score (FiO_2 x MAP) • Change in the following measures of hypoxemia (SpO_2<80%): Intermittent hypoxemia (events lasting 10s-3min), prolonged hypoxemia (events lasting >1min), and daily proportion of time in hypoxemia • Change in tracheal aspirate cytokine levels, neutrophil/WBC ratio, and patterns in the airway microbiome • Change in dynamic lung compliance and airway resistance

Pharmacokinetic Evaluations	Blood and tracheal aspirate samples for pharmacokinetic (PK) analyses will be collected. Patient specific PK parameters will be calculated using log-linear regression based on plasma peak and trough tobramycin concentrations. Population PK analyses will be conducted using nonlinear mixed effects modeling. Mean population PK parameters (clearance, volume of distribution) will be estimated using the first-order conditional estimation with interaction method. A 2-compartment model with first order absorption will be utilized based on prior knowledge of inhaled tobramycin pharmacokinetics.
Statistical and Analytic Plan	All subjects entered into the phase 1 trial will be included in the primary safety analysis. The primary study outcome, listed above under “Safety Evaluations,” will be evaluated as a dichotomous outcome based on the presence of any of the listed outcomes. A 3+3 design will be followed to determine enrollment and trial progression based on the results of each infant’s safety analysis.
DATA AND SAFETY MONITORING PLAN	A 4-member independent data safety monitoring board (DSMB) consisting of 2 neonatologists, 1 statistician, and 1 audiologist will monitor trial data, safety, progress throughout the trial.

TABLE 1: SCHEDULE OF STUDY PROCEDURES

Study Phase	**Screening**	**Phase 1 Trial**															**Follow-up**	
Study Days		**0[a]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15[b]**	**>15**
Informed consent	X																	
Review inclusion & exclusion criteria	X																	
Pre-trial medical record review	X																	
Prior & concomitant medications	X																	
Dispense study drug			X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Review current medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Review all laboratory, imaging data		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Continuous pulse oximetry		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Pulmonary mechanics measurements		X							X								X	
Tracheal aspirate	X	X				X			5X			X				X	2X	2X[c]
Blood draw for laboratory testing - Serum tobramycin trough - Comprehensive metabolic panel[d]			X			X			X			X				X		
Blood peak/trough for PK									2X									
Collect blood sample during clinical draws for PK analyses[e]			X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Assessment of respiratory support		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Adverse event assessment (daily)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Adverse event assessment (weekly)																		X
Review clinical audiology testing results	X																	X
Discharge medical record review																		X
		Observational (non-treated) cohort																
Tracheal aspirate	X																X	
Continuous pulse oximetry		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Assessment of respiratory support		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Pulmonary mechanics measurements			X						X								X	
Review clinical audiology testing results	X																	X
Discharge medical record review																		X

[a] "Day 0" procedures to be completed ≤7 days prior to trial Day 1

[b] "Day 15" procedures to be completed ≤48 hours after the final administered dose of inhaled tobramycin (phase 1 trial) or study day 14 (observational cohort)

[c] Tracheal aspirate sample collected 14 days after the final administered dose of inhaled tobramycin or on the day of planned tracheal extubation, if earlier

[d] Serum magnesium and phosphorus levels will be checked with the comprehensive metabolic panel on trial days 7 and 14.

[e] Up to 6 blood samples (200μL per sample) for pharmacokinetic analyses (PK) will be collected per infant concurrent with clinically ordered blood draws.

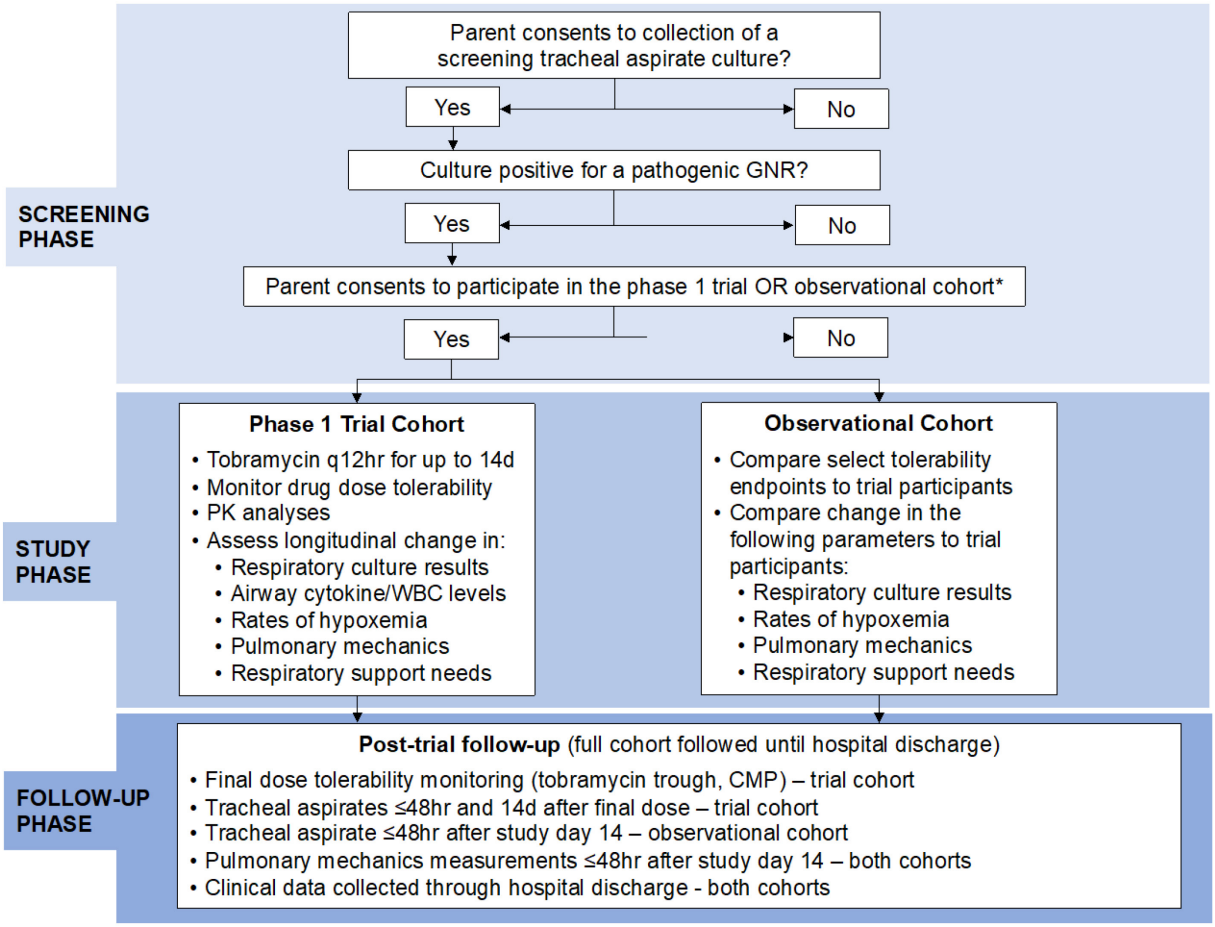


Figure 1. Study enrollment and phases flow diagram.

** Details of both the phase 1 trial and observational (non-treated) cohort will be discussed with eligible families during the consent conversations. Eligible infants may participate in either cohort but can be enrolled in only one cohort.*

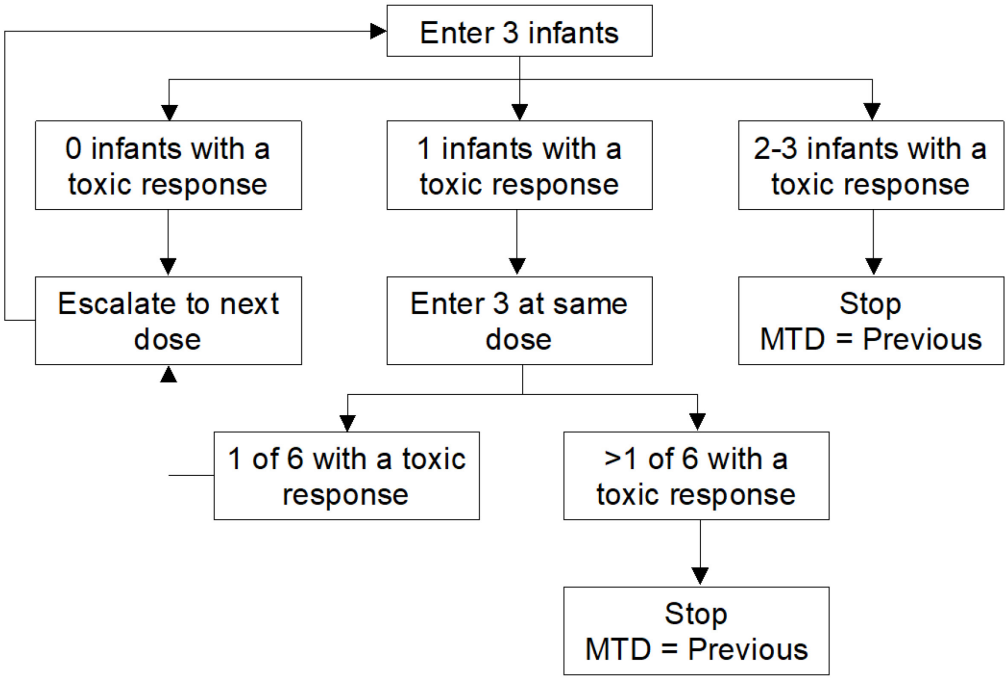


Figure 2. 3+3 dose escalation design schematic. *MTD: maximum tolerated dose*

Up to 4 different dosages of tobramycin solution for inhalation will be investigated using the above 3+3 trial design (78mg, 150mg, 216mg, and 300mg; all administered every 12 hours for up to 14 days). If the 300mg dose level is completed in 3 participants and none show evidence of a toxic response, up to 3 additional infants will be enrolled to receive to receive 300mg. The trial will be stopped if 2 of the additional infants receiving 300mg develop a toxic response and the 216mg dose will be used as the maximum tolerated dose in subsequent trials.

A toxic response is defined as the occurrence of any of the following safety endpoints during the 14-day phase 1 trial (see individual subject and full study stopping rules in section 5.2.4 and 5.2.5 for more details):

- Trough serum tobramycin level (measured 11 hours after the last administered dose) ≥1µg/mL
- Increase in serum creatinine level by ≥0.3mg/dL above the pre-trial level[1-3]
- Increase in serum creatinine >1.5-fold above the pre-trial level[1-3]
- Urine output <0.5mL/kg/hr for 12 hours[1-3]
- Any serious adverse event possibly attributable to the study drug[b]

[b] The likelihood an adverse event is attributable to a study intervention will be defined as outlined in section 8.3.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

The objective of this phase 1, 3+3 dose escalation clinical trial is to identify a well-tolerated dose of tobramycin solution for inhalation for use in very preterm infants with BPD who are actively receiving invasive mechanical ventilation and have evidence of a pathogenic Gram-negative rode (GNR) bacteria detected by tracheal aspirate culture.

1.2 Burden of bronchopulmonary dysplasia (BPD)

BPD is the most common and consequential complication of prematurity. BPD affects approximately 50% of extremely preterm infants, is a strong risk factor for life-long cardiopulmonary and neurodevelopmental impairment, and is a leading pediatric cause of disability-adjusted life years lost.[4-7] Disease acuity in BPD, defined by the respiratory support administered at 36 weeks postmenstrual age (PMA), predicts the degree of childhood impairment. Very preterm infants treated with invasive mechanical ventilation at 36 weeks PMA (grade 3 BPD) are 5 times more likely to undergo tracheostomy than very preterm infants with less severe disease.[8] Over 60% of very preterm infants with grade 3 BPD demonstrate severe cognitive impairment at 2 years and 1 in 5 die before their second birthday.[8] Finally, the risk of late death or severe neurodevelopmental impairment increases the longer an infant is exposed to invasive mechanical ventilation after 36 weeks PMA.[9] These data suggest that interventions that promote lung disease recovery and shorten the duration of invasive ventilation may produce long-term benefit among infants with the most severe forms of BPD.

1.3 Pathogenic bacterial colonization of the airway and BPD

Colonization of the airways in very preterm infants with pathogenic bacterial organisms and imbalance in the microbial communities in the airway (dysbiosis) have been implicated as inflammatory triggers and important risk factors in the development and exacerbation of BPD.[10-12] Airway colonization by a pathogenic GNR organism is associated with a 2-fold increase in the rates of supplemental oxygen use at 36 weeks PMA and discharge to home.[11] In an analysis of 121 very preterm infants receiving mechanical ventilation at 36 weeks PMA in the newborn and infant intensive care unit (N/IICU) at the Children's Hospital of Philadelphia (CHOP), airway colonization with a GNR bacteria was associated with a 6-fold increase in the odds of death or need for home supplemental oxygen therapy (adjusted odds ratio 6.2, 95% CI 1.8-21).[10] *Pseudomonas aeruginosa* was the most commonly cultured GNR (30% of study infants) and was independently associated with increased risk for death or prolonged oxygen therapy (adjusted odds ratio 4.9, 95% CI 1.1-22).[10] In contrast, airway colonization with a Gram-positive organism was not a significant risk factor for respiratory morbidity.[10]

1.4 Difficulty treating airway infections with systemically administered antibiotics

Bacterial lower respiratory tract infections and aberrant colonization of the airways with pathogenic bacterial organisms present a significant therapeutic challenge. Systemically administered antimicrobials have limited penetration into the lung parenchyma and the airway lining epithelial fluid.[13] The utility of systemic antibiotics to treat respiratory infections may be further impaired in BPD, owing to the frequent presence of pulmonary hypertension and hypoxemia.[14] These physiologic perturbations can lead to pulmonary vasoconstriction and shunting of blood flow away from respiratory sites targeted for antimicrobial therapy.[14]

In contrast to systemically administered agents, inhaled antibiotics may maximize intra-pulmonary drug concentrations, optimize pharmacokinetic indices, and substantially reduce

systemic exposure and toxicity. Inhaled tobramycin has been shown to effectively decrease pathogenic bacterial density in the airways, reduce pulmonary exacerbation frequency, and improve the quality of life and lung function in patients with cystic fibrosis (CF) and non-CF bronchiectasis whose respiratory tract is colonized with *P. aeruginosa*.[15,16] Owing to the favorable risk:benefit ratio, inhaled antibiotics are also gaining popularity for other disease states such ventilator associated tracheobronchitis and pneumonia.[17-20]

1.5 Name and Description of Investigational Product

This study will utilize tobramycin solution for inhalation USP (United States Pharmacopeia). Tobramycin solution for inhalation USP is a sterile, clear, slightly yellow, non-pyrogenic, aqueous solution with the pH and salinity adjusted specifically for administration by nebulization. The chemical formula for tobramycin, USP is $C_{18}H_{37}N_5O_9$ and the molecular weight is 467.52. Tobramycin, USP is *O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1→4)-*O*-[2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl-(1→6)]-2-deoxy-L-streptamine. The structural formula for tobramycin, USP is shown in **Figure 3**. Each 5mL ampule contains 300mg of tobramycin, USP and 11.25mg sodium chloride in sterile water. Sulfuric acid and sodium hydroxide are added to adjust the pH to 6. Nitrogen is used for sparging. All ingredients meet USP requirements. The formulation contains no preservatives.

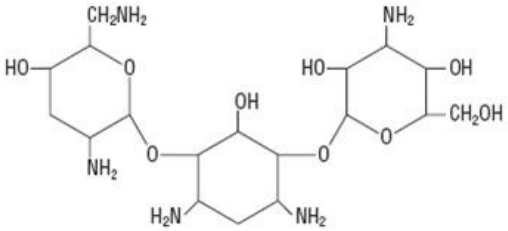


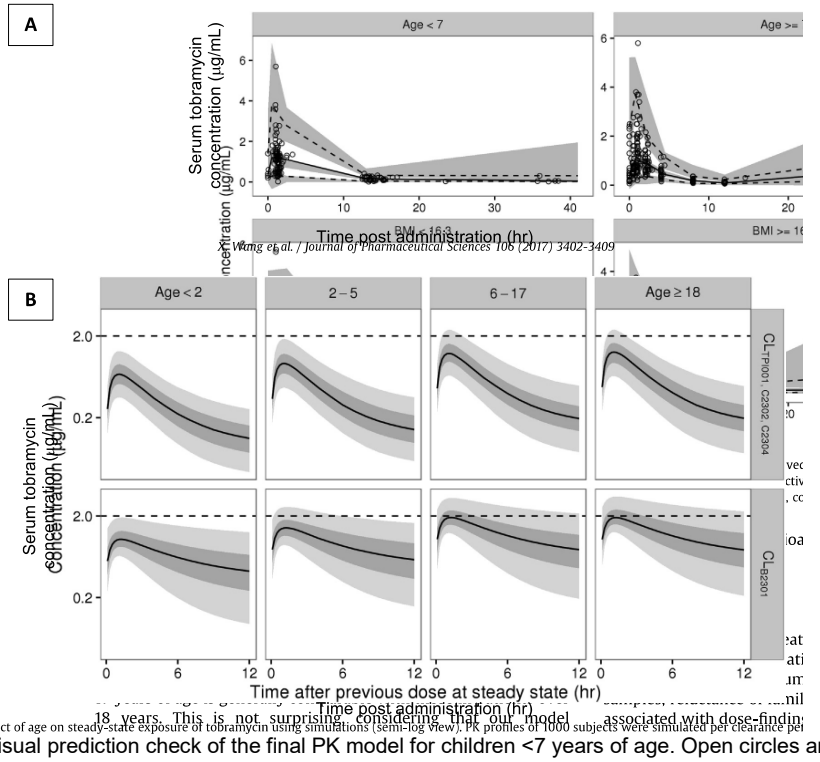
Figure 3. Structural formula for Tobramycin, USP

Tobramycin is a member of the aminoglycoside class of antibiotics. This class exert their antimicrobial activity by binding to the 30s subunit of the bacterial ribosome and inhibiting protein synthesis, thus inhibiting growth and division of the bacteria. Aminoglycosides, including tobramycin are not absorbed enterally, therefore must be administered locally such as through nebulization or intravenous instillation.

1.6 Findings from Clinical Studies

1.6.1 Human pharmacokinetics

Wang et al. studied the pharmacokinetics (PK) of tobramycin solution for inhalation in patients aged between 6 months and 44 years using data from 4 clinical trials investigating 300mg of tobramycin administered via nebulized aerosolization.[21] The final PK model used a 2-compartment, first-order absorption model.[21] Serum tobramycin levels reached near undetectable trough levels 10-12 hours after administration in children <7 years of age (**Figure 4A**) and peak serum tobramycin levels were below 1-2μg/mL in all children <2years of age (**Figure 4B**).[21] Relative bioavailability in patients between 6 months and 7 years of age increased with age (i.e. younger children demonstrated lower serum concentration levels).[21] Importantly, the investigators noted that systemic exposure is not predictive of clinical efficacy due to direct dosing at the infection site (lung).[21] They concluded that *P. aeruginosa* eradication rates and safety profiles in patients aged <7 years were similar to older patients and that no dose adjustment is needed in young children.[21]



Wang et al. / Journal of Pharmaceutical Sciences 106 (2017) 3402-3409

Figure 4. **(A)** Visual prediction check of the final PK model for children <7 years of age. Open circles are observed concentrations. Central solid line is the median of observed concentrations, dashed lines are the 2.5% and 97.5% quantile of observed concentrations. The central shaded area represents the 95% confidence interval (CI) of the median of the simulated profiles and the upper and lower 95% CI for the 2.5% and 97.5% quartiles, respectively. **(B)** Effect of age on steady-state exposure of tobramycin using simulations (semi-log view). PK profiles of 1000 subjects were simulated per clearance per age group. Central solid lines represent the median concentration; shaded areas frame the 50% (25th-75th percentile) and 90% (5th-95th percentile) of the subjects. Horizontal dashed lines represent the safe serum trough threshold utilized by the study authors. Top panel shows simulations using data form 3 trials[22-24] and the bottom simulation data from an additional trial[25].

In a separate study, Rosenfeld et al. found that peak serum concentrations occurred approximately 1 hour (range 0.5-2hr) after administration of 300mg of tobramycin solution for inhalation to children aged 0.6-5.8 years (**Figure 5**).[26] The average peak serum concentration of 0.6μg/mL (range: <0.2 to 1.2μg/mL) was within the acceptable, safe trough range for intravenous tobramycin. Serum tobramycin levels reached negligible values in most study patients within 4 hours after administration (**Figure 5**).[26]

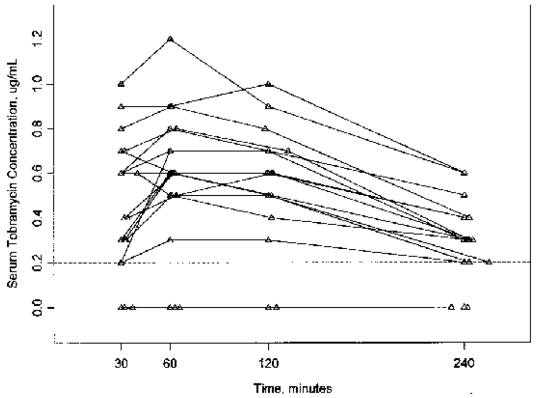


Figure 5. Serum tobramycin concentration – time profiles in 19 children (age range 0.6-5.8yr) treated with 300mg of inhaled tobramycin. Time shown along the x-axis indicates minutes since administration of the inhaled dose. In 3 patients, all levels were below the detectable limit of 0.2μg/mL (dotted line).[26]

1.6.1.1 Clinical Studies in Older Children (>6 years) and Adults

Tobramycin solution for inhalation is approved by the US Food and Drug Administration for the treatment of respiratory tract infections by *P. aeruginosa* in patients with CF who are at least 6 years of age. The CF Foundation "strongly recommends" the use of inhaled tobramycin for the treatment of "initial or new growth of *P. aeruginosa* from an airway culture."[16] They rate the certainty of benefit with this therapy as "high," and estimate the net benefit as "substantial."[16] An expert panel from the European CF Society also recommends the use of inhaled tobramycin for the treatment of newly acquired *P. aeruginosa* infection.[27] Multiple controlled trials (**Table 2**) conducted in older children and adults support the safety and efficacy of inhaled tobramycin in this population. Several of these trials, combined with data from younger children (**Table 3,** section 1.6.1.2) were included in a recent Cochrane review. This meta-analysis supports the use of inhaled tobramycin in children with CF who have culture proven respiratory tract infections with *P. aeruginosa*.[28] Inhaled tobramycin has also demonstrated efficacy for the treatment of respiratory tract infections due to *P. aeruginosa* and other GNR bacteria in non-CF bronchiectasis (**Table 2**), chronic obstructive pulmonary disease (COPD), and ventilator-associated infections.[18,29-31]

Table 2. Published randomized trials evaluating tobramycin solution for inhalation (TSI) in older children (≥6 years) or adult patients with *P. aeruginosa* present in the sputum.

Author / Year	Dose / Frequency	Disease	Participants' ages	Study duration	Trial size	Key findings
Ramsey 1999[32]	300mg BID	CF	≥ 6 years	24 weeks (cycled 28 days on, 28 days off)	N=520	• Improved pulmonary function • Decreased hospitalizations
Murphy 2004[33]	300mg BID	CF	6-15 years	56 weeks (cycled 28 days on, 28 days off)	N=184	• Decreased hospitalization rates
MacLuskey 1989[34]	80mg TID	CF	7-24 years	32 months	N=27	• Stability in pulmonary function, controls showed decline
Smith 1989[35]	600mg TID	CF	Mean age 17.3 ± 6.1 years	12 weeks	N=22	• Improved symptoms, decrease in bacterial density
Ramsey 1993[36]	600mg TID	CF	Mean age 17.1 ± 1.3 years	12 weeks (cycled 28 days on, 28 days off)	N=71	• Improved pulmonary function
Scheinberg 2005[37]	300mg BID	non-CF bronchiectasis	≥ 18 years	12 weeks (cycled 14 days on, 14 days off)	N=41	• Improvements in respiratory symptoms
Drobnic 2005[38]	300mg BID	non-CF bronchiectasis	38-75 years	6 months	N=30	• Decrease in the number of hospital admissions • Shorter duration of hospitalizations
Barker 2000[39]	300mg BID	non-CF bronchiectasis	Mean age 67 ± 13 years	28 days	N=74	• Decreased sputum bacterial load • Improvement in respiratory symptoms • No new bacterial resistance

1.6.1.2 Clinical Studies in Infants and Young Children (≤ 6 years)

Several clinical trials have demonstrated the safe use of inhaled tobramycin in infants (<1 year of age) and young children (**Table 3**). The Australian Cystic Fibrosis Bronchoalveolar Lavage (BAL) study enrolled 170 infants (mean age 3.6 ± 1.6 months at enrollment).[40,41] In

Table 3. Published randomized trials of tobramycin solution for inhalation (TSI) in young children with *P. aeruginosa* present in the sputum.

Author / Year	Dose / Frequency	Disease	Eligible participants' ages	Study duration	Trial size	Key findings
Ratjen 2019[24]	300mg BID	CF	3 months to >7 years	28 days with optional cross-over for additional 28 days	N=51	• Effective eradication of early P. aeruginosa infection • Favorable safety profile
Ratjen 2010[25]	300mg BID	CF	≥6 months (42% of cohort aged <6 years)	28 days vs. 56 days	N=88	• >90% of participants free of P. aeruginosa after 1 month of treatment • Favorable safety profile
Gibson 2003[42]	300mg BID	CF	6 months to 6 years (29% of the cohort aged <3 years)	28 days	N=21	• Reduced density of P. aeruginosa in the lower airway with TSI • Reassuring mean peak (1.0 ± 0.4 mcg/mL) and trough (0.4 ± 0.5 mcg/mL) serum tobramycin levels • No change in renal or auditory function with TSI
Treggiari 2011[43]	300mg BID	CF	1-12 years (30% of the cohort aged 1-3 years)	28 days +/- oral ciprofloxacin. Treatment provided either quarterly or only with positive sputum culture over 18 months period	N=304	• No benefit with addition of oral ciprofloxacin • Minimal emergence of antibiotic resistant strains • No difference in renal, liver, or audiometric function in between trial arms
Taccetti 2012[44]	300mg BID	CF	>1 year (35% in the TSI arm aged ≤5 years)	28 days TSI + oral ciprofloxacin vs. inhaled colistin + oral ciprofloxacin	N=223	• TSI with similar efficacy as colistin • 65% free of P. aeruginosa 6 months after TSI + ciprofloxacin therapy • No serious adverse events
Wiesemann 1998[45]	80mg BID	CF	>4 years	12 months	N=22	• Shorter time to conversion to P. aeruginosa negative respiratory culture
Proesmans 2013[46]	300mg BID	CF	0-18 years (7% <2 years of age)	28 days	N=58	• TSI vs. inhaled Na-colistimethate resulted in similar eradication of P. aeruginosa (80-90%)

total, 46 of the study infants were treated with 2 weeks of systemic antibiotic therapy followed by inhaled tobramycin (300mg twice daily) for 2 months.[40,41] No adverse effects secondary to inhaled tobramycin were reported in the trial.[41] A recent retrospective cohort study reported the use of inhaled tobramycin in 32 infants (<1 year of age) with CF.[47] The mean age at initiation was 4 months, with 72% aged <6 months at first treatment.[47] The median dose of inhaled tobramycin was 80mg (IQR 80-120); 4/32 (22%) were treated for 14 days and the remainder (78%) were treated for 28 days.[47] Most (83%) were free of *P. aeruginosa* by the end of the treatment course and (53%) remained free 6 months after treatment.[47] Overall, inhaled tobramycin was well tolerated.[47] Only 1 patient required therapy discontinuation owing to cough.[47]

The limited available data from infants without CF support the safe use of inhaled tobramycin when administered to infants who: (1) do not have pre-existing renal disease,

and (2) are not receiving concurrent nephrotoxic intravenous antibiotics. A single center retrospective cohort study from the University of Oklahoma identified 40 pediatric patients without CF who received inhaled tobramycin for pneumonia or tracheitis during invasive mechanical ventilation.[48] The median age at treatment was approximately 1 year with an interquartile range extending as low as 6 months.[48] Most of the described patients received 300mg twice daily with a low interquartile range of 60mg twice daily.[48] Although the authors do not report respiratory outcomes, they indicate inhaled tobramycin continues to be one therapy used for the treatment of respiratory infections in young children receiving invasive respiratory support at their institution. The authors report that two infants developed acute kidney injury while receiving inhaled tobramycin (one after 5 days of therapy, the other after 17 days of therapy).[48] Both received 300mg twice daily during the treatment course.[48] One received intravenous gentamicin within 48 hours of initiating tobramycin (last gentamicin trough 1.3mcg/mL) and the second had recently undergone surgical palliation for complex congenital heart disease.[48] Both infants had detectable serum tobramycin trough levels (1.9 and 3.2 mcg/mL).[48] Two additional case reports describe the use of inhaled tobramycin (300mg twice daily) in preterm infants without CF (32 weeks' gestation, 2.5 months of age treatment; 34 weeks' gestation, 1 month of age at treatment).[49,50] Although both infants developed detectable tobramycin levels above the goal trough range, they also both received concurrent IV doses of nephrotoxic antibiotics (including IV tobramycin in one case) and were diagnosed with pre-existing renal disease.[49,50] Finally, analysis of data from the Pediatric Health Information System (PHIS) database suggests that inhaled tobramycin in used some infants with severe BPD. A recent study found that 3% (93/3252) of very preterm infants with severe BPD (gestational age <32 weeks; <1 year of age at treatment) in the dataset were prescribed inhaled tobramycin between 2007-2016. Taken together, these data indicate that: (1) inhaled tobramycin is used in clinical practice to treat infants (some as young as 3 months of age) with and without CF – including those with severe BPD, and (2) administration of inhaled tobramycin at doses ≤300mg, coupled with close monitoring of serum levels, is likely to be safe in preterm infants diagnosed with severe BPD who are ≥36 weeks PMA at the time of treatment.

1.6.2 Potential toxicity with inhaled tobramycin

1.6.2.1 Nephrotoxicity

Nephrotoxicity was not seen during clinical trials of tobramycin solution for inhalation in children or adults. However, nephrotoxicity has been associated with aminoglycosides as a class. Aminoglycosides in systemic circulation are eliminated via glomerular filtration and can induce toxicity to the proximal tubule. The acute tubular necrosis that can occur is most commonly mild and reversible (owing to the ability of the proximal tubules to regenerate).[51] The considerably reduced serum exposure to tobramycin with inhalational administration substantially lowers the risk of acute kidney injury (AKI).[52] At present, evidence suggesting a possible link between inhaled tobramycin and AKI in infants and young children is limited to case reports.[48,53] In nearly all of these cases, there was evidence of renal dysfunction prior to the initiation of inhaled tobramycin and/or concomitant treatment with additional nephrotoxic agents.[48,53]

1.6.2.2 Ototoxicity

Ototoxicity, as measured by complaints of hearing loss or by audiometric evaluations, did not occur in any of the clinical trials evaluating tobramycin solution for inhalation in children or adults.[54] Moreover, there is no convincing evidence that inhaled tobramycin administered without concurrent systemic aminoglycoside therapy results in ototoxicity in pediatric

patients. A multicenter European trial comparing a 28-day and a 56-day course of inhaled tobramycin (300mg twice daily) in children as young as 6 months of age with CF did not find a single case of hearing loss during extended follow-up.[25] Similarly, a 2018 Cochrane review evaluating long-term use of inhaled tobramycin in CF patients did not identify a single case of hearing loss among 258 study participants.[15] In a multicenter Australian study evaluating audiometric function before and after treatment with inhaled tobramycin (treatment duration ranged 59-119 days) in 142 children <6 years of age, only 1 child receiving both inhaled tobramycin and intermittent intravenous tobramycin developed mild, unilateral sensorineural hearing loss.[55] This child inadvertently received a 300mg (21.3mg/kg) IV tobramycin dose, instead of a nebulized dose, in addition to the concurrently prescribed IV tobramycin dose.[55]

In addition to the lack of data linking inhaled tobramycin to ototoxicity in young children, the overwhelming abundance of data indicate that systemically administered aminoglycosides do not cause hearing loss in infants when used as directed. A single center study of 2347 infants found no increased risk of an abnormal audiology evaluation among infants who received gentamicin compared to those who did not.[56] Two studies, including one evaluating 84,808 hospitalized infants, found no relationship between the presence of sensorineural hearing loss and the cumulative aminoglycoside dose, the average daily dose, or the duration of administration.[57,58]

One small observational study (n=81) reported an increased risk of sensorineural hearing loss in infants who received systemic aminoglycoside therapy during concurrent treatment with a neuromuscular blocking agent.[59] Whether this finding represents a true toxicity or is confounded by unmeasured illness severity is unknown. Notably, this study found no association between the dosage or duration of systemic aminoglycoside therapy and the risk for sensorineural hearing when the antibiotic was used in infants who were not co-treated with a paralytic agent.[59] Out of an abundance of caution, this phase 1 trial will exclude infants treated with a neuromuscular blocker within 48 hours of enrollment.

Genetic risk for aminoglycoside induced ototoxicity: Homoplasmic A1555G and C1494T mutations in the 12s mitochondrial rRNA gene may predispose to aminoglycoside-induced hearing loss.[60,61] These mutations are present in approximately 0.2% of infants.[62] Although clinical testing for these maternally-inherited mutations is not yet performed in an anticipatory manner, avoiding use of aminoglycosides in patients with a maternal family history of early onset sensorineural hearing loss may be beneficial.

Vestibular toxicity following treatment with aminoglycoside antibiotics: Vestibular toxicity, leading to symptoms such as dizziness, imbalance, and nausea, is a rare complication (~2%) of systemically administered aminoglycosides reported in adults.[63] To our knowledge, there are only 3 single-patient case reports, all describing medically complex adults, reporting vestibular dysfunction after inhaled tobramycin use.[63-66] There are no reports of vestibular toxicity in children receiving inhaled tobramycin. There are no diagnostic tests capable of accurately quantifying vestibular function in hospitalized infants. As per standard of care for very preterm infants with BPD in the CHOP N/IICU, all infants enrolled in the phase 1 trial will be referred to a neonatal follow-up clinic and undergo standardized cognitive and motor function testing through the first 2 years of life.

1.6.2.3 Additional adverse reactions

Data from randomized, placebo-controlled trials of inhaled tobramycin in children and adults with CF indicate inhaled tobramycin is generally well tolerated. In 2 studies inclusive of 258 patients who received inhaled tobramycin (300mg twice daily with alternating periods of 28 days on and off treatment for 24 weeks), voice alteration (13% vs. 7% in the placebo group) and tinnitus without loss of hearing (3% vs 0% in the placebo group) were the only adverse experiences reported by significantly more patients treated with inhaled tobramycin than placebo.[54] Changes in voice/cry are not expected in this study of infants receiving invasive mechanical ventilation. Additional reported adverse events that may be related to inhaled tobramycin use include cough, increased sputum production, bronchospasm, and hemoptysis. Allergic reactions and anaphylaxis are possible adverse reactions of any drug.

1.7 Rationale for drug dosages and duration under investigation

The recommended dose of inhaled tobramycin in children with CF who are ≥6 years of age is 300mg administered every 12 hours for at least 28 days. Randomized trials and observational studies have evaluated the safety and efficacy of this regimen in children as young as 6 months of age with CF and not found evidence of toxicity.[25,26,55,67] At present, there are no universally established dosing recommendations for inhaled tobramycin in children without CF. Published reports in young children without CF describe safe administration with doses ranging from 80-300mg every 12 hours.[48,68,69] A consensus summary from the Society of Infectious Disease Pharmacists recommends a dose of 80mg every 12 hours for acute exacerbations of non-CF bronchiectasis.[17]

This trial will investigate up to 4 different doses of inhaled tobramycin: 78mg, 150mg, 216mg, and 300mg – each administered every 12 hours. A dose of 80mg has been shown to be safe and effective in children <6 years of age.[45-47] This trial will investigate an initial dose of 78mg rather than 80mg (a 2.5% decrease in dose) to enable accurate dilution from a 300mg/5mL drug vial using a 3mL syringe (see section 7.1.3 for further details on drug dosing and aliquoting).

The FDA-approved dose of *intravenous* (IV) tobramycin for infants is 7.5mg/kg per day. This IV dose far exceeds the amount of drug expected to reach the systemic circulation with nebulized therapy (**Table 4**). During nebulization, less than 15% of the administered drug reaches the airways. This equates to ≤12mg of tobramycin for each 78mg dose. The available data indicate that <5% of inhaled tobramycin that reaches the airways is absorbed into systemic circulation, resulting in serum peak levels that are approximately 95% lower with inhaled versus intravenous administration. Based on these data, we anticipate that a starting dose of 78mg of inhaled tobramycin administered every 12 hours will not generate detectable serum tobramycin trough levels or result in toxicity. This study will investigate doses as high as 300mg twice daily if the 3+3 dose escalation design does not raise concern for potential toxicity at lower doses. This maximum possible dose was chosen as the available PK data indicate there is no need to decrease the dose of inhaled tobramycin below the recommended dose of 300mg when treating children <6 years of age (see section 1.6.1).[21]

Although the current recommended duration of therapy for the eradication of a *P. aeruginosa* lung infection in young children with CF is a 28-day treatment course, studies have demonstrated clinical benefit after 14 days of therapy.[32,36,47] These data suggest that longer treatment courses may not be necessary. Therefore, a 14-day course is expected to

generate sufficient data to evaluate dose safety and tolerability and provide preliminary efficacy data to inform the design of possible future phase 2 and phase 3 trials.

Table 4. Anticipated maximum peak serum tobramycin concentrations and daily IV dose equivalence for the drug dosage under investigation in this phase 1 trial

Inhaled tobramycin dose	Anticipated maximum peak serum concentration (per dose)[a]	Approximate IV dose equivalents based on anticipated systemic exposure[b]
78mg	0.31 μg/mL	0.24 mg/kg/day
150mg	0.6 μg/mL	0.46 mg/kg/day
216mg	0.86 μg/mL	0.66 mg/kg/day
300mg	1.2 μg/mL	0.92 mg/kg/day

[a] Calculated using data from Rosenfeld et al. indicating maximum peak serum tobramycin levels were ≤1.2μg/mL following treatment with 300mg of inhaled tobramycin in young children.[26]
[b] Intravenous (IV) dose equivalents calculated based on the anticipated maximum peak serum concentrations shown in the table and observed peak serum concentrations of 26.0 ± 2.1μg/mL after administration of a 10mg/kg intravenous dose of tobramycin in pediatric patients.[70]

1.8 Compliance Statement

This study will be conducted in full accordance of all applicable CHOP Research Policies and Procedures and all applicable federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, 312, and the Good Clinical Practice (GCP): Consolidated Guideline approved by the International Conference on Harmonization (ICH). Adverse event reporting will be targeted, so that potential adverse events arising from the use of inhaled tobramycin (for example, inhalational side-effects as well as renal adverse events) will be reported, while other adverse effects attributed to the underlying disease or clinical care will not be reported. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain informed consent, and will report unanticipated problems involving risks to subjects or others in accordance with the CHOP IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

The objective of this trial is to evaluate the safety and tolerability of tobramycin solution for inhalation in very preterm infants with BPD who have evidence of a pathogenic GNR detected by tracheal aspirate culture.

2.1 Primary Objective

The primary objective of this study is to determine the maximum well-tolerated dose of tobramycin solution for inhalation (selected between 78mg, 150mg, 216mg, and 300mg administered twice daily) administered to very preterm infants with BPD who are actively receiving invasive mechanical ventilation and have evidence of a pathogenic GNR detected by tracheal aspirate culture.

2.2 Secondary Objectives

The secondary, exploratory objectives are to:

- Evaluate the association between the dose of inhaled tobramycin, plasma tobramycin pharmacokinetics, and peak tracheal aspirate tobramycin levels.

- Compare the change (pre vs. post study) in tracheal aspirate pathogenic bacterial colony forming unit (CFU) counts measured by high resolution culture, (1) between participants in the phase 1 trial and untreated infants in the observational cohort, and (2) between trial participants stratified by the administered dose of inhaled tobramycin.
- Compare the longitudinal change in measures of administered respiratory support (FiO_2, ventilator mean airway pressure, and respiratory severity score) during the 14-day study period, (1) between participants in the phase 1 trial and observational cohort and (2) between trial participants stratified by the administered dose of inhaled tobramycin.
- Compare changes (pre vs. post treatment) in tracheal aspirate cytokine levels, neutrophil/WBC ratio, and patterns in the airway microbiome, stratified by the administered dose of inhaled tobramycin.
- Change in the following measures of hypoxemia (SpO_2<80%): Intermittent hypoxemia (events lasting 10s-3min), prolonged hypoxemia (events lasting >1min), and daily proportion of time in hypoxemia
- Compare the longitudinal change in dynamic lung compliance, airway resistance, peak expiratory flow, and CO_2 elimination during the 14-day study period, (1) between participants in the observational cohort and phase 1 trial and (2) between trial participants stratified by the administered dose of inhaled tobramycin.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This study is an open-label, phase 1, inter-patient 3+3 dose escalation feasibility trial. A prospective, observational (non-treated) cohort of eligible infants will also be enrolled and outcomes compared to the trial participants. The study phases are depicted in **Figure 6** and described in the sections that follow.

3.1.1 Screening Phase

Potential subjects will be screened using daily census logs and the protocol inclusion and exclusion criteria. Male and female infants who meet the criteria will be eligible to undergo a once-weekly screening tracheal aspirate culture while receiving invasive mechanical ventilation. Infants with a screening culture that is positive for a pathogenic GNR bacteria will become eligible to enroll in the phase 1 trial of inhaled tobramycin or the untreated, observational cohort. Infants must begin the phase 1 trial or observational (non-treated) cohort 14-day study period within 7 days of collection of the screening tracheal aspirate sample that is positive for a pathogenic GNR (**Figure 6**). Infants whose screening culture(s) is/are negative for a pathogenic GNR bacteria will continue to be screened weekly for as long as they meet study eligibility criteria. This plan for serial screening is consistent with the regular surveillance used to monitor for Pseudomonas infection in children with CF and reflects the increasing risk of developing a GNR respiratory infection with longer duration of invasive mechanical ventilation.[10,71,72]

3.1.2 Phase 1 Trial

The phase 1 trial will last up to 14 days, with each infant receiving an inhalational dose of tobramycin solution administered every 12 hours via vibrating mesh nebulizer. The dose (78mg, 150mg, 216mg, or 300mg) will be determined as per the 3+3 design protocol (**Figure 2**). During the investigational drug trial, each infant will undergo blood and tracheal

aspirate sampling and respiratory mechanics measurements at pre-specified time points to assess dosage safety and potential efficacy. Continuous pulse oximetry will be performed beginning 24-48hr prior to initiating the study drug and continued until completion of the 14-day trial. Review of clinical data and assessment for adverse events will be conducted daily throughout the 14-day trial.

3.1.3 Observational (non-treated) Cohort

Infants enrolled in the observational (non-treated) cohort will undergo 14 days of clinical data collection. During the study phase, respiratory mechanics measurements will be performed on study days 1 and 7. Respiratory mechanics measurements will also be performed ≤48 hours after the final study day as indicated in section 3.1.4. Pulse oximetry will be performed throughout the 14-day study period.

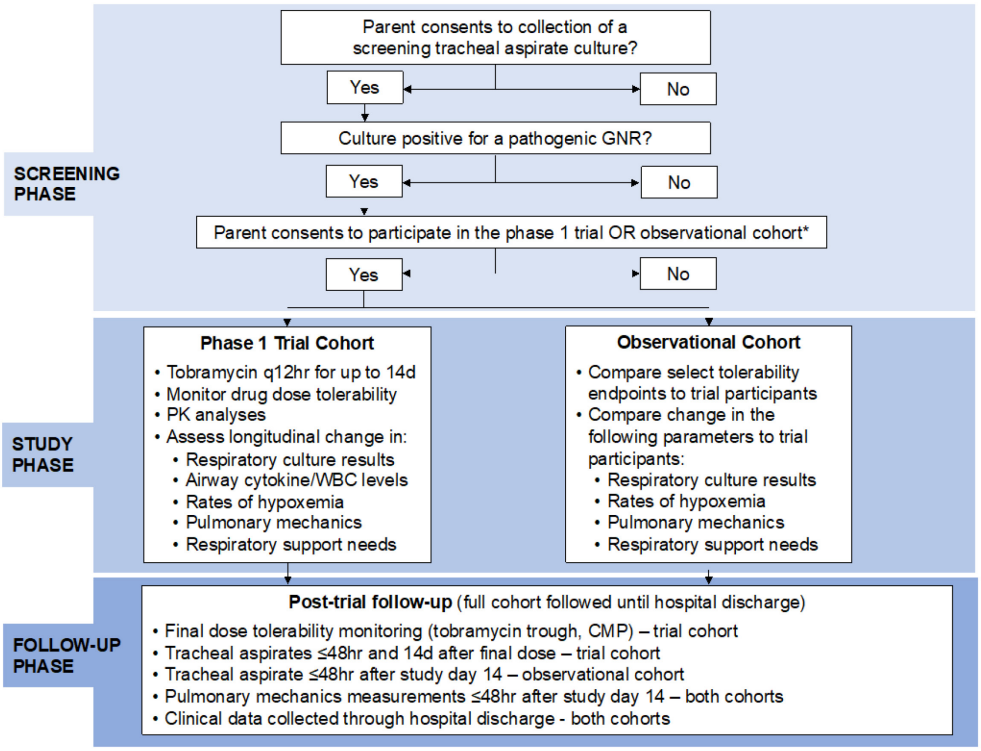


Figure 6. Diagram of study phases. *GNR, Gram-negative rod. * Details of both the phase 1 trial and observational (non-treated) cohort will be discussed with eligible families during the consent conversations. Eligible infants may participate in either cohort but can be enrolled in only one cohort. Infants will enter into one of the two study cohorts within 7 days from the collection of the screening tracheal aspirate.*

3.1.4 Follow-up Phase

All enrolled study infants will be followed until hospital discharge with pre-defined clinical data abstracted periodically from the medical record. For infants enrolled in the phase 1 trial, tracheal aspirate samples for microbiology testing and cytokine level measurements will be collected ≤48 hours and 14 days (or on the day of planned tracheal extubation, if earlier) after receiving the final dose of inhaled tobramycin. For infants enrolled in the observational cohort, a tracheal aspirate for high resolution culture will be collected ≤48 hours after study day 14. Infants enrolled in both cohorts will undergo the third and final respiratory mechanics measurements ≤48 hours after the final study day.

3.2 Allocation to Treatment and Masking

Infants who satisfy the inclusion and exclusion criteria and a parent or guardian consents for the infant to participate in the phase 1 trial will be allocated to receive up to 14 days of tobramycin solution for inhalation at the dosage determined by the 3+3 trial design. Drug dosage will not be masked (blinded).

3.3 Study Duration, Enrollment, and Number of Sites

3.3.1 Duration of Study Participation

The study duration for each subject will last from enrollment until hospital discharge. For infants who participate in the phase 1 trial of inhaled tobramycin, the drug trial will last up to 14 days. The last tracheal aspirates obtained for research purposes will be collected up to 14 days after completing the final administered dose of inhaled tobramycin. For infants who participate in the observational (non-treated) cohort, clinical data will be collected daily for 14 days beginning after enrollment. Respiratory mechanics data will be recorded at study entry (day 0 for the trial participants, day 1 for the observational cohort), study day 7, and ≤48 hours after the final study day.

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

Study site: Neonatal/infant intensive care unit at the Children's Hospital of Philadelphia.

Up to 103 infants will be enrolled in the study to produce up to 52 evaluable subjects (phase 1 trial + observational cohort). These numbers are estimated according to the enrollment flow diagram shown in **Figure 7**. Up to 26 infants are expected to receive the study drug to produce up to 24 infants with evaluable trial data. These numbers for the phase 1 trial account for the 24 infants needed to enroll up to 6 infants per each of the 4 evaluated doses (assuming 1 infant with a toxic response per dose level) plus withdrawal of up to 2 infants (~10%) prior to receiving *14 drug doses* for reasons other than a toxic response. We will target a minimum of 12 infants in the observational cohort. Enrollment in the observational (non-treated) cohort will be capped at 28 infants.

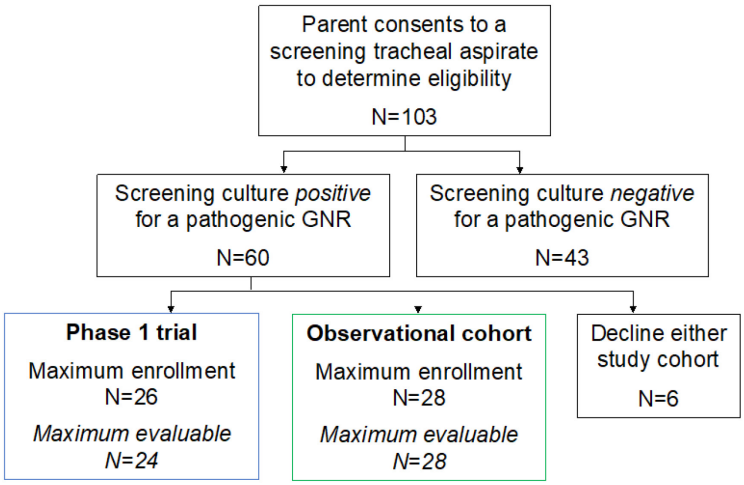


Figure 7. Flow diagram of estimated maximum enrollment numbers

3.4 Study Population

3.4.1 Inclusion Criteria

1. Male or female infants born <32 weeks' gestation

2. Diagnosed with BPD (use of supplemental oxygen or respiratory support at 36 weeks postmenstrual age)
3. Postmenstrual age ≥36 weeks at study enrollment
4. Treatment with invasive mechanical ventilation at enrollment without planned tracheal extubation within 7 days after enrollment
5. Tracheal aspirate culture positive for a pathogenic GNR bacteria (see list of eligible organisms in section 3.4.3) within 7 days of beginning the study phase – this testing will be performed during the screening phase and must be met to participate in the phase 1 trial or observational cohort
6. Parental/guardian permission (informed consent).

3.4.2 Exclusion Criteria

1. Serum creatinine >0.4mg/dL within 14 days of enrollment
2. Congenital or acquired disease of the kidney or renal collecting system that adversely affects renal function
3. Congenital or acquired hepatobiliary disease that adversely affects liver function
4. Treatment with a systemic antibiotic within 7 days prior to enrollment
5. Treatment with a potentially nephrotoxic medication (see list in Appendix A), excluding diuretics, within 48 hours prior to enrollment
6. Treatment with a continuous infusion of a neuromuscular blocker (see list in Appendix B) within 48 hours prior to enrollment
7. Known intolerance to aminoglycoside antibiotics
8. Current treatment with high frequency or other oscillating mechanical ventilation
9. Presence of a cancer diagnosis
10. Maternal family history of early onset hearing loss defined as the need for an assistive hearing device prescribed before 30 years of age
11. Endotracheal tube leak >20%.
12. Any investigational drug use (as part of an FDA approved IND protocol) prior to enrollment.
13. A subject who, in the judgement of the Investigator, is not an appropriate candidate for this research study.

Subjects that do not meet all of the above criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

3.4.3 Definition of Pathogenic GNR Airway Bacteria

One or more of the following pathogenic GNR organisms must be cultured from a tracheal aspirate sample within 7 days prior to enrollment to meet trial eligibility:

- Pseudomonas aeruginosa
- Klebsiella species
- Enterobacter species
- Stenotrophomonas maltophilia
- Escherichia coli
- Acinetobacter baumannii
- Serratia marcescens

4 STUDY PROCEDURES

Below is a summary of the study procedures that will occur in each study phase. A list of main study procedures can also be found in **Table 1** (page xiv).

4.1 Screening Phase

Census documents in the CHOP N/IICU will be reviewed bi-weekly during the study period to identify potentially eligible subjects. For subjects likely to be eligible, medical records will be reviewed using the inclusion and exclusion criteria. Study participation and enrollment criteria will first be discussed with the subject's attending physician. After obtaining approval of the attending physician, the parent(s) or guardian(s) will be approached, and the details of the study will be discussed. First, consent will be obtained to collect a screening tracheal aspirate culture up to once weekly for as long as eligibility is maintained and a pathogenic Gram-negative rod organism is not identified. The parent or guardian will be informed that the results of the tracheal aspirate culture(s) will be used to determine eligibility for the phase 1 trial of inhaled tobramycin and the observational cohort. Details of the two study cohorts will be discussed during the initial consent conversations. However, a second consent discussion for participation in the inhaled tobramycin trial or observational (non-treated) cohort will take place for those in whom the tracheal aspirate culture is positive for a pathogenic GNR. After learning of the study goals, procedures, risks and benefits, the parent(s) of eligible infants will be invited to participate in the phase 1 trial or observational cohort and if interested, informed consent will be obtained. Informed consent will be obtained prior to any study related procedures being performed. All screening culture results will be disclosed to families of enrolled infants. *See section 9.6 for a description of the informed consent procedure.*

4.2 Observational (non-treated) cohort

The medical record of each infant enrolled in the observational cohort will be reviewed regularly until hospital discharge to collect pre-specified clinical data. Each infant in the observational cohort will also undergo respiratory mechanics measurements on study days 1, 7 and ≤48 hours after completing the 14-day study period (see section 4.3.3). A tracheal aspirate for culture will also be collected ≤48 hours after completing the 14-day study period (see section 4.3.2). Oxygen saturation data (SpO_2) will be recorded using continuous pulse oximetry. To collect this data, a research pulse oximeter will be placed in addition to the pulse oximeter used for clinical monitoring. This second device will enable reliable data collection, storage, and analysis. Nursing and medical staff will be instructed to use the clinical oximeter for all clinical monitoring.

4.3 Phase 1 Trial of Tobramycin Solution for Inhalation

The phase 1 trial of inhaled tobramycin will utilize a 3+3 inter-patient dose escalation design (**Figure 8**). The safety and tolerability of up to 4 different doses of tobramycin will be evaluated: 78mg, 150mg, 216mg, and 300mg. Each dose will be administered every 12 hours for up to 14 days via vibrating mesh nebulizer. If the 300mg dose level is completed in 3 participants and none show evidence of a toxic response, up to 3 additional infants will be enrolled to receive to receive 300mg. The trial will be stopped if 2 of the additional infants receiving 300mg develop a toxic response and the 216mg dose will be used as the maximum tolerated dose in subsequent trials. The study procedures utilized to monitor drug safety and efficacy are listed in **Table 5**.

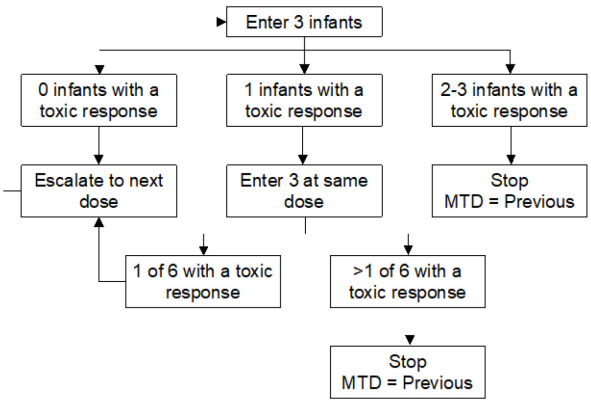


Figure 8. 3+3 dose escalation design schematic. *MTD: maximum tolerated dose*

4.3.1 Blood draws

All blood samples required as part of this research protocol will be collected by heel stick, venipuncture, or from an indwelling catheter (e.g., arterial line) by nursing staff or CHOP phlebotomy, in adherence with standardized, written hospital guidelines. Samples will then be transferred to the CHOP clinical or research laboratories for analysis. Required blood sample volumes per laboratory test are: serum tobramycin level (0.5mL), comprehensive metabolic panel (1mL), PK blood samples (200µL). In addition to the pre-specified study blood draws, 200µL of blood will be collected for PK analyses on up to 6 different occasions during the phase 1 trial at the times of clinically ordered blood draws. In total, the amount of blood drawn for this study will not exceed 9.1mL over the 14-day study period. This amount of blood extraction is not expected to incur risk to the study patients. As an additional step to protect the safety of enrolled subjects, study team members, in consultation with nursing staff and the medical team, will ensure blood volume extraction (for clinical + research purposes) does not exceed the maximum allowable weight-based limits specified by local and NIH guidelines (no more than 5 mL/kg drawn in a single day).

Table 5. Study procedures utilized to monitor drug safety, tolerability, and efficacy for participants in the phase 1 trial.

		Study Day						**Follow-up**	
Procedure	**0[a]**	**1**	**4**	**7**	**10**	**14**	**All (1-14)**	**15[b]**	**28[C]**
Tracheal aspirate	**X**		**X – after 7th dose**	**X – after 14th dose**				**X**	**X**
Test:									
Quantitative culture	X							X	X
Cell count differential	X							X	X
Cytokine levels	X			X				X	X
16s sequencing	X			X				X	X
Tobramycin level[d]			X	5X	X	X			
Blood draw		**X – after 2nd dose**	**X – after 7th dose**	**X – after 14th dose**	**X – after 20th dose**	**X – after 28th dose**			
Test:									
Tobramycin trough[e]		X	X	X	X	X			
Comprehensive metabolic panel[f]		X	X	X	X	X			
Tobramycin peak[g]				X					
Random tobramycin levels[h]							X		
Continuous pulse oximetry	X						X		
Pulmonary mechanics measurements	X			X				X	
Daily medical record review							X		
Weekly medical record review[i]								X	X

[a] "Day 0" procedures to be completed ≤7 days prior to administration of the first study drug dose. The quantitative tracheal aspirate culture and cell count differential obtained during the screening phase will be used for the laboratory values. Prior to initiating study drug therapy, one additional tracheal aspirate will be collected for cytokine level measurements and 16s sequencing.

[b] "Day 15" procedures to be completed ≤48 hours after administration of the last study drug dose

[c] Follow-up tracheal aspirate for quantitative culture will be collected 14 days after the last administered dose of inhaled tobramycin or on the day of planned tracheal extubation if earlier

[d] Tracheal aspirate for tobramycin level measurements obtained on trial day 7 will be collected approximately 1hr, 3hr, 6hr, 9hr, and 11hr after drug administration. Samples collected on trial days 4, 10, and 14 will be collected approximately 11hr after administration of doses number 8, 20 and 28, respectively.

[e] Serum and blood tobramycin trough levels will be drawn concurrently, 11 hours after administration of the previous dose

[f] Blood draws for comprehensive metabolic panels should be bundled with draws for tobramycin trough levels or other clinically ordered blood draws. Comprehensive panel to include a magnesium and phosphorus level on day 7 and 14.

[g] Serum and blood tobramycin peak levels will be drawn concurrently, 1 hour after completion of the previous dose

[h] Up to 6 blood tobramycin levels per infant will be drawn concurrent with clinically ordered blood draws.

[i] Weekly medical record review to assess for adverse events will take place until hospital discharge

4.3.2 Tracheal aspirate collection

Following bedside review of the collection procedures, nursing or respiratory staff will collect all tracheal aspirates in a standardized manner under the direct (in-person) guidance of a study team member. Our research team has extensive experience coordinating endotracheal sample collection for microbiome and cytokine analyses. In most cases, the timing of tracheal aspirate collection for research can be planned by the investigative and nursing teams to coincide with routine, clinical suctioning of the endotracheal (ET) tube. However, if the collection of a tracheal aspirate sample must occur at a set time (e.g., timed after an inhaled tobramycin dose) and routine clinical suctioning will not occur at that time, a research only collection will be performed. Clinical and research suctioning of the endotracheal tube will be performed as described in the paragraph below. In the CHOP N/IICU, infants with BPD who are receiving invasive mechanical ventilation undergo routine endotracheal suctioning several times per day. Most undergo clinical suctioning at least every 3 hours, and often more frequently (as much as every 20-30 minutes) owing to the high secretion burden in infants with GNR bacteria in the airways.

Tracheal aspirate samples will be collected by advancing a suction catheter just proximal to the end of the ET tube (length of ET tube is indicated at the bedside for all intubated infants). Tracheal secretions will be aspirated into a sterile Lukens trap. After tracheal secretion collection and removal of the suction catheter from the ET tube, the suction catheter will be cleared by aspiration of 1mL of sterile 0.9% saline (note: saline should not be instilled directly into the ET tube). This is the standard clinical procedure used to collect airway samples in the CHOP N/IICU. A minimum of 0.5mL of tracheal fluid will be collected – resulting in a total of approximately 1.5mL of fluid in the Lukens trap after flushing the suction catheter with 1mL of saline. In our experience, this procedure provides sufficient sample recovery for the specified analyses (see sections 5.1.2 and 5.1.3) and is consistent with tracheal aspirate volumes collected in prior studies.[73-75] If less than 0.5mL of tracheal aspirate is recovered after a single suctioning, tracheal suctioning will be repeated at least 1 hour later, at the time of the next clinical suctioning. Of note, saline lavage is not performed prior to ET tube suctioning as this does not result in greater recovery of mucous material in infants and may adversely affect clinical stability.[76] A sterile (new) suction catheter will be used for all microbiological sampling as per unit protocol. Tracheal aspirate samples for tobramycin assays may be collected with a new suction catheter or the existing in-line suction catheter.

4.3.3 Respiratory mechanics measurements

Respiratory mechanics data will be recorded at 3 timepoints: study entry (day 0 for participants in the phase 1 trial, day 1 for participants in the observational cohort), on study day 7, and ≤48 hours after completion of the 14-day study period. Respiratory mechanics will be measured using a Philips Respironics NM3 respiratory profile monitor placed in-line with the ventilator circuitry. Each recording will last for up to 4 hours. The Respironics sensor will be placed between the Neoflow sensor, if present, or directly to the endotracheal tube if not, and the “y” connector of the ventilator tubing. This device is FDA approved for use in infants (510 (k) K091459). The Respironics NM3 continuously monitors several mechanical and physiological respiratory parameters including minute ventilation, dynamic lung compliance, airway resistance, peak expiratory flow, and carbon dioxide elimination. These data will be downloaded to a dedicated, password protected study computer following each recording session. Respiratory mechanics measurements will be timed to correspond with quiet sleep periods.

The Respironics NM3 monitor sensor adds a negligible amount of dead space to the ventilator circuitry (0.8mL, <3% of inspired tidal volume for eligible study participants) and has been successfully used without adverse effects in infant studies,[77,78] including those conducted in the CHOP N/IICU (IRB 12-008686). The same procedure for collecting respiratory mechanics data will be used for infants enrolled in the phase 1 trial and those enrolled in the observational cohort.

4.3.4 Continuous pulse oximetry

Continuous peripheral oxygen saturation (SpO_2) measurements will be obtained beginning 24-48hr prior to initiating the study drug and continued throughout the 14-day study period using a Masimo Rad-G pulse oximeter. A 2-second averaging time and 2-second sample rate will be used for all oximeter recordings. These parameters provide a more reliable measurement of the number and duration of hypoxemic events compared to the oximeters used for clinical care, which employ longer averaging times and less frequent sampling. The study pulse oximetry probe may be placed on any extremity in accordance with unit policy. The study pulse oximetry probe and connected pulse oximeter will be used in addition to the probe and oximeter used for routine clinical care. The study pulse oximeter will be used for research purposes only. The study pulse oximeter will not have programmed alarms, however saturation data will be displayed to ensure the oximetry probe and oximeter are functioning properly. Raw data will be extracted from the pulse oximeter every 72 hours and stored on a single password protected CHOP workstation for cleaning and analysis. The oximeter data will not be provided to the clinical team.

4.4 Follow-up Phase

The medical records of each enrolled subject will be periodically reviewed until hospital discharge for the following information:

- Occurrence of adverse events that may be related to the investigational drug
- Weekly abstraction of respiratory support and microbiological data (including all culture results and use of any antimicrobials)
- Weekly abstraction of respiratory medication administration (drug name and class)
- Discharge review of respiratory support data, audiology screening results, medications prescribed for home use, and discharge disposition

For the infants enrolled in the phase 1 trial, tracheal aspirate samples will be collected at 2 separate time points during the follow-up phase (as shown in **Table 5**). The first time point will be within 48 hours of completing the last dose of inhaled tobramycin. The second time point will be 14 days after the last administered dose of inhaled tobramycin or on the day of tracheal extubation, if earlier. Two tracheal aspirate samples will be collected at each timepoint to obtain enough fluid from the respiratory tract to complete the described laboratory testing (one sample will be analyzed in the clinical laboratory– see section 5.1.3.3; and the second is used for research laboratory analyses – see section 5.1.3.1). For infants enrolled in the observational (non-treated) cohort, a single tracheal aspirate for high resolution culture will be obtained within 48 hours after study day 14, or at the time of extubation, if earlier. The procedures described in section 4.2.2 will be used to obtain these samples. The final respiratory mechanics measurements in both study cohorts be obtained ≤48 hours after completing the 14-day study period.

4.5 Concomitant Medications

All medications administered within 7 days prior to enrollment through the end of the phase 1 trial will be recorded for infants who participate in the drug trial. All antibiotics administered to infants enrolled in the observational cohort will be recorded. The dates of administration and drug dosages will be recorded.

4.6 Rescue Medication Administration

Subjects may receive rescue medications for adverse reactions to the study medication at the discretion of the treating medical team. Data on the administered medications and dosages will be recorded.

4.7 Subject Completion/Withdrawal

Subjects will be withdrawn from the phase 1 trial for any of the following reasons:

- Occurrence of any of the primary safety endpoints listed in section 5.2.1
- Initiation of an intravenous antibiotic, continuous infusion of a neuromuscular blocking agent, or a new cancer diagnosis
- Tracheal extubation
    - Infants who have not yet completed the phase 1 trial and are re-intubated ≤48 hours after extubation may restart the trial procedures at the same stage as when the extubation occurred.
    - Infants will not resume the investigational drug trial if 2 instances (combined) of endotracheal tube obstruction or unplanned extubation occur during the 14-day phase 1 trial.

Infants enrolled in the observational (non-treated) cohort will discontinue respiratory mechanics measurements if invasive mechanical ventilation is halted during the 14-day study period and not resumed within 48 hours. Clinical data collection will continue as appropriate.

Withdrawal from the study is also permitted at any time by parent or physician request or at the discretion of the investigator to protect the subject for reasons of safety (i.e., presence of adverse events) or for administrative purposes. Early withdrawal will not result in any prejudice to the subject's care. It will be documented whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents and on the case report forms and reported to the IRB and DSMB as described in section 8.

4.7.1 Early Termination Procedures

Subjects who are removed from the phase 1 trial early will undergo measurement of at least 1 serum tobramycin trough, a comprehensive metabolic panel, and collection of 2 tracheal aspirate samples after administration of the final tobramycin dose – unless these procedures are refused by the parent or physician. Laboratory testing may be repeated at the discretion of the research and medical teams for safety purposes. Review of the medical record will also continue as appropriate unless refused by the parent or guardian.

4.7.2 Addition of Trial Participants after Early Termination

Additional research subjects will be added to the phase 1 trial as per the 3+3 design (**Figure 8**). In addition, subjects who are removed early from the trial for reasons other than

a toxic response or safety concern related to the investigational drug will be replaced 1:1 if fewer than 14 *doses* of inhaled tobramycin were administered. Up to 2 subjects total and no more than 1 per dose level, who receive ≥14 *doses* of inhaled tobramycin, are removed early from the trial for reasons other than a toxic response or safety concern related to the investigational drug, and do not meet criteria for any of the primary tolerability endpoints will inform progression through the 3+3 trial design *without requiring participant replacement*. This is supported by the available data which show that elevated serum tobramycin trough levels in infants and young children are typically observed within the first 7 days of inhalational administration during every 12-hour dosing.[48-50] PK analyses in young children demonstrate similar serum tobramycin levels are obtained after 28 days and after 56 days of inhaled tobramycin use suggesting negligible serum drug accumulation or impaired renal function when initial levels are reassuring.[21]

At least 3 infants must receive the full 14-day treatment course (28 doses) of the dose that is ultimately defined as the maximum tolerated dose at the end of this trial. Participants will be added as needed to fulfill this requirement. If the 300mg dose level is completed in 3 participants and none show evidence of a toxic response, up to 3 additional infants will be enrolled to receive to receive 300mg. This will provide a more robust assessment of drug tolerability and PK. The trial will be stopped if 2 of the additional infants receiving 300mg (2 out of 5 or 2 out of 6) develop a toxic response. In that event, the 216mg dose will be considered the maximum tolerated identified in this study.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Medical Record Review

5.1.1.1 Screening Phase

The following data will be abstracted from the medical record during the screening phase:

- Date of birth
- Gestational age
- Birth weight
- Sex
- Race/ethnicity
- Prenatal exposure to corticosteroids
- Postmenstrual age at study enrollment
- Weight, length, and head circumference at enrollment
- Respiratory support (including settings and FiO_2) at enrollment
- Currently prescribed medications and those administered with 7 days prior to enrollment (name, dose, route, and interval).
- Prior microbiological data from birth through enrollment, including culture results and antibiotic exposures (drug, duration, dates of exposure)
- Results of any prior audiological examination or testing
- Diagnoses present in the active problem list

5.1.1.2 Phase 1 Trial

The medical record will be reviewed as follows throughout the phase 1 trial:

- Daily review of the medical record and discussion with the medical team, nursing, and respiratory staff to assess for adverse events

- Daily review of weight, vital signs (heart rate, blood pressure, oxygen saturation), and fluid balance (including urine output) recorded by nursing staff in the electronic medical record (EPIC) flow sheet
- Daily respiratory support data (mode of ventilation, settings, FiO_2, and calculated respiratory severity score: mean airway pressure x FiO_2) recorded in EPIC
- Daily review of administered medications (name, dose, route, and interval)
- Daily review of any clinically ordered laboratory tests and radiographic imaging.

5.1.1.3 Observational (non-treated) cohort

The medical records of infants who are enrolled in the observational cohort will be reviewed daily for 14 days beginning after enrollment. Respiratory support data (mode of ventilation, settings, FiO_2, and calculated respiratory severity score: mean airway pressure x FiO_2) will be abstracted from EPIC.

5.1.1.4 Follow-up Phase

The medical record will be reviewed from the end of the 14-day phase 1 trial/observational period until hospital discharge as follows:

- Weekly review of the medial record to assess for serious adverse events (only infants enrolled in the phase 1 trial)
- Results of all audiology evaluations conducted prior to hospital discharge
- Date, age, and postmenstrual age at hospital discharge
- Medications and respiratory support prescribed for home use

5.1.2 Continuous pulse oximetry

A research pulse oximeter (see section 4.3.4) will be used throughout the 14-day study period to measures reliable SpO_2 levels. Oximeter monitoring will begin 24-48 hours prior beginning the phase 1 drug trial to establish baseline values.

5.1.3 Clinical Data Warehouse

Routine physiologic monitoring data (heart rate, oxygen saturation, blood pressure) will be obtained from the clinically prescribed bedside multiparameter monitor on phase 1 trial and observational cohort study participants. This data will be downloaded from CHOP data warehouse and analyzed by the study team. These data will be stored on the CHOP research server.

5.1.4 Clinical Laboratory Evaluations

5.1.4.1 Screening phase

Tracheal aspirate sampling will be performed for the following laboratory evaluations during the screening phase to establish eligibility:

- Tracheal aspirate cell count with differential
- Tracheal aspirate quantitative culture with antimicrobial sensitivities

Of note, this sampling will be performed even in infants with a recent clinically obtained tracheal aspirate culture as quantitative cultures are not part of routine clinical practice.

5.1.4.2 Phase 1 Trial

Blood sampling will be performed for the following laboratory evaluations:

- Serum tobramycin level monitoring (note: this sampling differs from blood tobramycin levels used for the PK analyses described in section 5.1.4.2)
- Serum electrolytes
- Liver function assessment
- Renal function assessment

Tracheal aspirate sampling will be performed for the following laboratory evaluations:

- Tracheal aspirate cell count with differential
- Tracheal aspirate quantitative culture with antimicrobial sensitivities

The laboratory tests listed in sections 5.1.3.1 and 5.1.3.2 (and shown in **Table 6**) will be performed in the clinical laboratory at CHOP. All laboratory tests performed in the CHOP clinical lab meet the regulatory requirements of the State Health Department, CLIA 88, FDA, AABB, CAP and JCAHO. The timing of collection of these tests is outlined in **Table 5** and in sections 4.2 and 4.3

Table 6: Clinical Laboratory Tests

Category	Tests
Drug monitoring	Serum tobramycin
Metabolic panel	Serum sodium, potassium, chloride, bicarbonate, glucose, calcium, total protein, albumin. Magnesium and phosphorus will be collected on day 7, 14
Liver function tests	SGOT/AST, SGPT/ALT, total Bilirubin, alkaline phosphatase
Renal function tests	BUN, creatinine
Microbiology	Quantitative culture of tracheal aspirate fluid with antimicrobial sensitivities
Body fluid cell count	Cell count and differential of tracheal aspirate fluid

5.1.4.3 Follow-up Phase

For the phase 1 trial participants, tracheal aspirate sampling will be performed for the following laboratory evaluations at two timepoints during the follow-up phase (≤48 hours after receiving the final dose of inhaled tobramycin and 14 days after receiving the final dose of inhaled tobramycin or on the day of planned tracheal extubation, if earlier):

- Tracheal aspirate cell count with differential
- Tracheal aspirate quantitative culture with antimicrobial sensitivities

For observational cohort participants, tracheal aspirate sampling for the above laboratory tests will be performed once during the follow-up phase, ≤48 hours after day 14 of the observation period.

5.1.5 Research Laboratory Evaluations

5.1.5.1 Tracheal Aspirates

The following laboratory analyses will be performed using tracheal aspirate samples collected during the study treatment phase:

- Tobramycin level
- Cytokine level analysis (including: IL 1β, 6, 8, 17A/F, 18, 22, 23, TNF-α, MIF, IFN-γ)
- 16s microbial sequencing

Additional tracheal aspirate samples will be collected during the follow-up phase for the following laboratory analyses:

- Cytokine level analysis (including: IL 1β, 6, 8, 17A/F, 18, 22, 23, TNF-α, MIF, IFN-γ)

- 16s microbial sequencing

Cytokine level analyses will be performed in the CHOP Translational Core Laboratory. Owing to inevitable variation in dilution during the collection process, cytokine level will be normalized to total protein levels in the tracheal aspirate fluid and compared to typical ranges found in published studies to ensure validity.[73,79] Tracheal aspirate tobramycin level analyses will be performed in the laboratories of University of Florida College of Pharmacy. Whether tracheal aspirate drug levels require correction for dilution is uncertain due to a lack of available evidence. We will explore differences in the study results with and without urea correction for dilution and compare the results to laboratory controls. The 16s microbial sequencing will be performed in the laboratories of the CHOP/Penn Microbiome Program. All laboratory procedures will be conducted using validated assays. The results of these laboratory tests will be reported as part of the data collection process for the research study, but will not appear in the medical record nor be used to make clinical decisions.

5.1.5.2 Blood Sampling for Pharmacokinetic Analyses

Blood (0.2mL) will be collected for pharmacokinetic analyses at 2 set time points on day 7 plus up to 6 random collections coincident with clinically obtained draws. The lower limit of quantification for serum tobramycin concentrations in the CHOP clinical laboratory is 0.6 µg/mL. Based on this quantification limit, we anticipate that the majority of subjects will have clinically undetectable trough and/or peak levels, limiting the ability to characterize serum pharmacokinetics. Measurement of blood plasma tobramycin levels will be performed at the University of Florida College of Pharmacy using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). This will enable quantification of drug levels down to a level of 50-100 ng/mL and facilitate estimation of patient-specific pharmacokinetics. Individually identifiable will be removed from samples and the samples will be labeled with unique identifiers. The CHOP study team will be able to link the unique identifiers to individually identifiable information, but the individually identifiable information will not be available to outside researchers.

5.1.6 Respiratory mechanics measurements

A Philips Respironics NM3 respiratory profile monitor will be used to collect respiratory mechanics data. Measurements will be performed at 3 time points: at study entry (study day 0 for infants enrolled in the phase 1 trial, study day 1 for infants enrolled in the observational cohort), study day 7, and ≤48 hours after completing the 14-day study period. Each recording period will last 4 hours and coincide with a period of quiet sleep. The NM3 monitor is FDA approved for use in infants (510 (k) K091459). The Respironics NM3 monitor will be used to collect data on the following endpoints: dynamic compliance (mL/cm H_2O), peak expiratory flow (L/min), airway resistance (cm H_2O/L/sec), and carbon dioxide elimination (mL/min). These data will be downloaded to a dedicated, password protected study computer following each recording session. For this analysis, only results obtained from mandatory, ventilator driven non-pressure support breaths will be used.

5.2 Safety Evaluations

5.2.1 Primary composite safety endpoint

The primary objective of this trial is to assess the safety and tolerability of up to 4 different dose levels of tobramycin solution for inhalation in preterm infants receiving invasive mechanical ventilation with a tracheal aspirate culture positive for a pathogenic GNR. Following the 3+3 trial design (**Figure 8**), tolerability of each dose will be defined as no

more than 1 in 6 infants per dose level developing any of the following safety endpoints during the 14-day trial:

- *Serum tobramycin trough (measured 11 hours after the prior dose) ≥1μg/mL*
  Serum tobramycin trough levels will be performed as per the schedule outlined in section 4.2. Quantification of serum tobramycin levels will be performed in the CHOP Clinical Laboratory as described in section 5.1.3. Current standards (including pharmacy guidelines at CHOP for conventional dosing strategies) and manufacturer labelling for intravenous tobramycin recommend maintaining serum tobramycin trough levels <2μg/mL.[80] Out of an abundance of caution, any measured trough value ≥1μg/mL will define a toxic response.
- *Increase in serum creatinine level by ≥0.3mg/dL above pre-trial level*
- *Increase in serum creatinine >1.5-fold above the pre-trial level*
- *Urine output <0.5mL/kg/hr for 12 hours*

  These 3 measures of reduced kidney function indicate stage 1 (the least severe stage) of acute kidney injury (AKI) as defined by the Kidney Disease Improving Global Outcomes (KDIGO) practice guidelines.[1-3] Serum creatinine levels will be measured as per the schedule outlined in section 4.2. Quantification of serum creatinine levels will be performed by the CHOP Clinical Laboratory as described in section 5.1.2.

  Nursing staff monitor urine output of all infants in the CHOP N/IICU throughout each day by weighing every soiled diaper. Typically, this is performed every 3 hours, coinciding with nursing care times. As per routine clinical care, “combo” diapers containing both urine and stool will be utilized when calculating total urine output. Data on urine output is recorded in the electronic medical record (EPIC) and will be reviewed by the study and clinical teams prior to the administration of each investigational drug dose.

- *Any serious adverse event possibly attributable to the study drug.*

  The occurrence of adverse events identified by clinical monitoring or laboratory testing (in addition to those described above) will be monitored throughout the trial and reported as described in section 8. A serious adverse event will constitute a toxic response.

5.2.2 Secondary exploratory safety endpoints

Additional exploratory safety endpoints that will be recorded are:

- *New onset or worsened coughing associated with a concomitant drop in SpO_2 to <80% for >10 seconds, need for an increase in FiO_2 by an absolute change >20%, or need clinical recovery using positive pressure support delivered via manual bag ventilation*
- *Obstruction of the endotracheal tube requiring tube replacement*
- *Unplanned tracheal extubation*
- *Desaturation (SpO_2 <80% for >10 seconds) during administration of inhaled tobramycin*
- *Rates of pre-discharge failed audiology examinations*
- *New intra-patient antimicrobial resistance*

5.2.3 Limitations of Audiology Testing in Hospitalized Infants

Inhaled tobramycin therapy in infants with BPD is not expected to result in ototoxicity. Ototoxicity, as measured by complaints of hearing loss or by audiometric evaluations, did

not occur with tobramycin inhalation solution therapy during clinical studies.[54] Moreover, intravenous aminoglycoside use has not been shown to result in hearing loss in preterm infants when used as directed (see section 1.6.2.2 for a discussion of these studies).

Some studies evaluating inhaled tobramycin in older children and adults performed audiometric testing prior to and after completing a drug course and did not find significant evidence of persistent hearing loss.[55,81,82] However, such an approach is not appropriate for hospitalized preterm infants. Physiologic test measures which include Auditory Brainstem Response (ABR) or Otoacoustic Emissions (OAE), must be used to screen newborns and infants for hearing loss. Newborns with tracheostomy or ventilator dependency may be screened later due to their complex medical conditions. Patient awake state, movement and electrical interference from equipment can impact testing. In addition, chronic fluid or abnormal middle ear pathology, common in this population, may cause hearing loss that can be transient or chronic in nature.

Complex medical presentations common in this population and environmental influences may impact initial test results, supporting the need for continued audiologic follow-up. One study of 78 preterm infants with BPD found auditory brainstem response (ABR) abnormalities in 22% of participants.[83] All subjects who failed the ABR demonstrated normal hearing by visual reinforcement orientation audiometry (VROA) at 8-12 months.[83] Notably, 10 of 14 infants (71%) who failed the gold standard VROA at follow-up had a normal ABR at hospital discharge.[83] Another study of 216 hospitalized infants compared the diagnostic reliability of automated transient evoked otoacoustic emissions (a-TEOAE), automated auditory brainstem response (a-ABR), and conventional brainstem auditory evoked potential (BAEP/ABR).[84] These tests produced modest specificity (71-91%) and poor positive predictive value (9-25%) when compared to repeat testing BAEP/ABR conducted 2-3 months later.[84] Finally, BAEP/ABR can only detect hearing abnormalities occurring between 0.5-4hz.[85] Most ototoxic medications affect frequencies >4hz.[85]

The American Academy of Pediatrics (AAP) recommends that all infants at risk of hearing loss should undergo subsequent screening by 24 to 30 months of age. Importantly, recommendations for audiologic follow-up should be customized based on individual patient risk factors and likelihood of delayed-onset hearing loss. Accordingly, the Joint Committee on Infant Hearing (JCIH) recommend beginning in 2019 that that all infants with a risk indicator for hearing loss who pass the initial newborn hearing screen should be referred for an audiologic assessment by 9 months of age.[86]

Owing to challenges of completing audiologic testing in preterm infants who are receiving intensive care (e.g., ongoing invasive mechanical ventilation) and obstacles that contribute to obtaining these tests in this population (infants with severe BPD in the CHOP N/IICU require a median of 3 attempts at audiology screening before a test can be successfully performed – regardless of the eventual result), all audiologic screening in study participants will be performed prior to hospital discharge as per standard of care in the CHOP N/IICU. All study infants will undergo pre-discharge ABR and OAE testing as well as tympanometry as indicated by the audiologic evaluation. The results of these evaluations will be recorded as part of the study data. The rate of failed pre-discharge hearing tests in a recent cohort of infants who received care in the CHOP N/IICU and were similar to those eligible for this study was 36%.[87] Based on these data, a rate of pre-discharge hearing screening abnormalities ≥50% among the infants treated with inhaled tobramycin will trigger adverse event reporting to the IRB and FDA. In addition, the rates of failed pre-discharge audiometric evaluations will be compared between infants who participate in the

investigational drug trial and those who are enrolled in the study but do not participate in the drug trial (e.g., lack of GNR in the airway or parental refusal to participate in the phase 1 trial). A statistically significant difference in the rates of failed evaluations between these two groups will also trigger adverse event reporting to the IRB and FDA.

In the CHOP N/IICU, all infants with abnormal audiologic test results are referred for additional evaluations by Audiology and Otolaryngology. Consistent with the above recommendations from the JCIH[86], infants who attend the CHOP newborn follow-up program and pass their newborn hearing screening are also referred for repeat audiologic testing by at least 9-12 months of age or as soon as possible thereafter if discharge occurs at a later age. In addition, all infants seen in the CHOP newborn follow-up program undergo detailed neurologic evaluations including standardized motor and cognitive testing.

5.2.4 Individual Subject Stopping Rules

A study subject will be removed from the phase 1 trial and will not receive additional doses of the study drug if he/she develops a toxic response defined as any of the following:

- Trough serum tobramycin level (measured 11 hours after the last administered dose) ≥1µg/mL
- Increase in serum creatinine level by ≥0.3mg/dL above the pre-trial level[1-3]
- Increase in serum creatinine >1.5-fold above the pre-trial level[1-3]
- Urine output <0.5mL/kg/hr for 12 hours[1-3]
- Any other serious adverse event possibly attributable to the investigational drug

Subjects may also be withdrawn from the study for reasons that do not meet the above criteria as described in section 4.7.

If an infant commences the trial and the primary medical team subsequently chooses to prescribe a systemic antibiotic (e.g., for the positive tracheal aspirate or another suspected infection that was unknown/not present at the time of enrollment), the infant will stop the phase 1 trial early. No additional doses of inhaled tobramycin will be administered but final laboratory testing will be performed.

5.2.5 Study Stopping Rules

The study stopping rules will follow the 3+3 dose escalation design shown in **Figure 9**. The study will be stopped if more than 1 subject develops a drug-related toxic response (any of the safety endpoints listed under section 5.2.1) at a single dosage level. Further enrollment will be stopped, but data analysis for those enrolled will continue until completed.

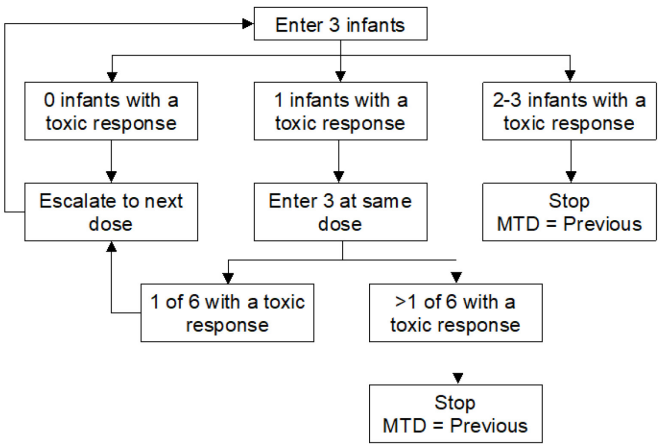


Figure 9. 3+3 dose escalation design schematic. *MTD: maximum tolerated dose*

5.3 Efficacy Evaluations

Measures of potential drug efficacy will be assessed as secondary, exploratory endpoints. These will include the following outcomes:

- *Change (pre vs post treatment) in tracheal aspirate pathogenic bacterial colony forming unit (CFU) counts measured by high resolution culture*

  Tracheal aspirates for high resolution culture will be obtained ≤7 days prior to initiating inhaled tobramycin (as part of the screening procedures), ≤48 hours after completing the final drug dose, and 14 days after completing the final drug dose (or on the day of planned tracheal extubation if earlier) (see schedule under section 4.3). For infants enrolled in the observational (non-treated) cohort, tracheal aspirate for culture will be obtained ≤7 days prior to initiating the 14-day observation period (as part of the screening procedures) and ≤48 hours after completing the observation period. These tests will be performed in the CHOP clinical laboratory as described in section 5.1.3.

  Reduction in number and eradication of pathogenic GNR bacteria from the airways is anticipated to result in clinical benefit. Evidence from preterm infants with BPD indicates the presence of these organisms in the airway is associated with worse clinical outcomes.[10,11] Moreover, data from children with CF demonstrate that early treatment of *P. aeruginosa* infection of the respiratory tract leads to improved clinical outcomes.[16,88,89] As such, change in quantitative tracheal aspirate culture results following treatment with inhaled tobramycin is anticipated to be a suitable surrogate marker to assess the likelihood of clinical benefit. For this phase 1 feasibility trial, we will evaluate the relative intra-patient change in bacterial CFU counts and assess the association between the longitudinal change in CFU count, airway cytokine levels, and the RSS.

- *Tracheal aspirate tobramycin levels*

  Tracheal aspirate samples used for the measurement of tobramycin levels will be collected at the following times: (1) a single tracheal aspirate will be collected on days 4, 10, and 14, 11-hours after completion of the 8th, 20th, and 28th doses respectively; and (2) 5 samples will be collected on trial day 7, approximately 1-hour, 3-hours, 6-hours, 9-hours, and 11-hours after completion of the 14th tobramycin dose (see section 4.3) Tracheal aspirate drug levels will be measured in the laboratory of the University of Florida College of Pharmacology (see section 5.1.3). Tracheal aspirate drug levels will be compared to published concentrations needed to overcome the minimum inhibitory concentration necessary to kill 90% of detected bacterial isolates (MIC_{90}).[90,91] Longitudinal change in tracheal aspirate levels will be modeled as part of the PK analyses. Correction for serum urea levels will be performed.

- *Longitudinal change in the following respiratory support measures during the 14-day study period:*

  - Fraction of inspired oxygen (FiO_2)
  - Ventilator mean airway pressure (MAP)
  - Respiratory severity score (MAP x FiO_2)

  FiO_2 and mean airway pressure (MAP) are established clinical measures of respiratory disease severity. They characterize the amount of respiratory support (supplemental oxygen and positive airway pressure) required to maintain cardiorespiratory stability in infants receiving invasive respiratory support. The respiratory severity score (RSS) is a

simplified severity score calculated as the MAP multiplied by the FiO_2. The RSS is used in place of the oxygenation index (OI) owing to the infrequent availability of data on the partial pressure of arterial oxygen (PaO_2) in hospitalized infants. The RSS correlates well with OI in infants receiving mechanical ventilation and is a useful predictor of poor respiratory outcomes in preterm infants.[92-94] FiO_2 and MAP values (measured by the mechanical ventilator and reported on the ventilator graphical interface), are recorded at least hourly in the respiratory flow sheet in the electronic medical record (EPIC). These data will be used to calculate daily, time-weighted values for FiO_2, MAP, and RSS. Longitudinal change in these respiratory support measures during the 14-day study period will be compared (1) between infants who participate in the phase 1 trial and those enrolled in the observational (non-treated) cohort, and (2) among participants in the phase 1 trial, stratified by the administered dose of inhaled tobramycin.

Owing to the expected heterogeneity in lung disease and respiratory support requirements of the enrolled infants, a protocolized ventilator management algorithm will not be utilized during this study. However, routine practice in our unit is to wean FiO_2 in infants with BPD to maintain SpO_2 ≥90%. Most infants with BPD who require invasive respiratory in our unit are supported using a time-cycled, pressure-limited, volume-guarantee mode of ventilation that delivers 8-10mL/kg of tidal volume at mandated rates of 15-25 breaths per minute. The Draeger V500 ventilator is used in all infants receiving acute care in the CHOP N/IICU who are prescribed intermittent mechanical ventilation. On this mode of support, the ventilator automatically adjusts the positive inspiratory pressure (PIP) – within set limits – to deliver the goal tidal volume. Change in the PIP along with any clinical adjustments in the positive end expiratory pressure (PEEP) will be the primary determinants of any observed changes in the delivered MAP. Only infants with an endotracheal tube leak ≤20% will be enrolled in this trial to avoid ventilator leak compensation and artificial inflation of the PIP levels.[95] Of note, the Draeger V500 ventilator calculates the endotracheal leak proportion during all modes of intermittent ventilation employed in our unit.

- *Change (pre vs post) in tracheal aspirate cytokine levels, neutrophil/WBC ratio, and patterns in the airway microbiome:*

  Tracheal aspirates collected ≤7 days prior to initiating inhaled tobramycin, ≤48 hours after completing the final drug dose, and 14 days after completing the final drug dose will be analyzed. Patterns in the airway microbiome will be assessed using 16s sequencing performed in the laboratories of the CHOP/Penn Microbiome Program. The following cytokine levels will be measured in tracheal aspirate samples: IL 1β, 6, 8, 17A/F, 18, 22, 23, TNF-α, MIF, IFN-γ. These analyses will be performed in CHOP Translational Core Laboratory. Tracheal aspirate cell counts will be obtained by the CHOP clinical laboratory. Changes in the observed patterns in the airway microbiome, neutrophil/WBC ratio, and tracheal aspirate cytokine levels (compared to the pre-treatment baseline) will be assessed, stratified by the treatment dosage level (see section 6.3 for further details of the analytical plan). For this phase 1 feasibility trial, we will evaluate the relative, intra-patient change in these measures as the association between these measures and clinical benefit in the study population is uncertain.

- *Longitudinal change in the following measures of respiratory mechanics during the 14-day study period:*
  - Dynamic compliance (mL/cm H_2O)
  - Peak expiratory flow (L/min),

- Airway resistance (cm H_2O/L/sec)
- Carbon dioxide elimination (mL/min).

The Respironics NM3 respiratory parameter monitor will be used to record these data for 4 hours periods at study entry, on study day 7, and ≤48 hours after completing the 14-day study period. These data will provide important information on the potential effects of inhaled tobramycin on respiratory function.

- *Longitudinal change in the following measures of hypoxemia (SpO_2<80%):*
  - Intermittent hypoxemia (SpO_2<80% lasting for 10s-3min)
  - Prolonged hypoxemia (SpO_2<80% lasting for >1min)
  - Daily proportion of time with hypoxemia

  Daily frequency of intermittent and prolonged hypoxemic events are established, real-time indicators of cardiorespiratory stability in BPD.[96] Cumulative burden of hypoxemia predicts the severity of childhood neurodevelopmental delay.[97] These data will be recorded continuously throughout the study using a research pulse oximeter that will be placed in addition to the clinical multiparameter monitor. Nursing and medical staff will be instructed to use the clinically placed monitor for all care decisions.

5.4 Pharmacokinetic Evaluation

Each infant participating in the phase 1 trial will undergo blood sampling on day 7 to measure serum tobramycin peak and trough levels. Up to 6 random blood samples (200μL per sample) will also be collected concurrent with clinical blood draws. Based on prior studies, we anticipate that most trial participants, particularly those receiving the lowest dosages of inhaled tobramycin, will have serum trough levels below the lower limit of quantification in the clinical lab (0.6μg/mL).[21,48,90] Therefore, we will collect 200μL of blood for high resolution measurement of peak, trough and random plasma tobramycin levels as per section 6.3.4. Blood samples collected for PK analyses will be stored at -80c in vials labelled with a study ID number only. Sample batches will be shipped to and subsequently processed at the University of Florida College of Pharmacy. This laboratory has extensive experience with high-resolution drug level testing and has established assays for tobramycin.

6 STATISTICAL CONSIDERATIONS

6.1 Primary Endpoint

The primary study endpoint is a composite safety endpoint. The presence of any of the following events occurring at any timepoint during the 14-day phase 1 trial will define a toxic drug response:

- Trough serum tobramycin level (measured 11 hours after the last administered dose) ≥1μg/mL
- Increase in serum creatinine level by ≥0.3mg/dL above the pre-trial level
- Increase in serum creatinine >1.5-fold above the pre-trial level
- Urine output <0.5mL/kg/hr for 12 hours
- Any serious adverse event possibly attributable to the investigational drug

6.2 Secondary Endpoints

Additional exploratory secondary safety endpoints are:

- New onset or worsened coughing associated with a change in respiratory status (SpO_2 <80% for >10 seconds; need for increase in FiO_2 by >20%)

- Obstruction of the endotracheal tube requiring tube replacement
- Unplanned tracheal extubation
- Desaturation (SpO_2 <80% for >10 seconds) during administration of inhaled tobramycin
- Rates of pre-discharge failed audiology examinations
- Rates of new intra-patient microbial resistance to tobramycin
    - Bacterial antimicrobial sensitivities reported for the pre-treatment tracheal aspirate culture will be compared to the tracheal aspirate cultures collected during and post treatment. The presence of new-onset microbial resistance to tobramycin within a patient – a change from susceptible to resistant by the same organism – will be recorded

The exploratory secondary efficacy endpoints are:

- Tracheal aspirate tobramycin levels
- Change in tracheal aspirate pathogenic bacterial colony forming unit (CFU) counts measured by high resolution, quantitative culture
- Longitudinal change in the following measures of administered respiratory support: FiO_2, ventilator mean airway pressure (MAP), respiratory severity score (MAP x FiO_2)
- Change in the following measures of hypoxemia (SpO_2<80%): intermittent hypoxemia (events lasting 10s-3min), prolonged hypoxemia (events lasting >1min), and daily proportion of time with hypoxemia
- Change in tracheal aspirate cytokine levels, neutrophil/WBC ratio, and patterns in the airway microbiome
- Longitudinal change in dynamic compliance, peak expiratory flow, airway resistance, and carbon dioxide elimination

6.3 Statistical Methods

6.3.1 Baseline Data

Baseline and demographic characteristics will be summarized using standard descriptive statistics (e.g., means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

6.3.2 Safety Analysis

All subjects entered into the phase 1 investigational drug trial of inhaled tobramycin will be included in the safety analysis. The primary study outcome is a safety endpoint and will be evaluated as a dichotomous outcome based on the presence of any of the events listed in section 6.1. The 3+3 design flow diagram shown in **Figure 8** will be followed to determine enrollment and trial progression based on the results of each infant's safety analysis. In addition, the frequencies of AEs by type, body system, severity, and relationship to the study drug will be summarized. SAEs (if any) will be described in detail.

6.3.3 Exploratory Secondary Safety and Efficacy Analyses

The exploratory secondary safety and efficacy endpoints listed in section 6.2 will be analyzed as described in **Table 7** (next page). In brief, all study data will first be summarized using standard descriptive statistics as appropriate based on data type and distribution. Assessments of change over time for all longitudinal secondary outcome data will first be assessed graphically, with and without stratification by the administered tobramycin dose. Next, paired t-tests or Wilcoxon sign rank tests will be used to compare

pre-trial baseline values to those recorded at later time points. Non-paired tests will be used to compare data baseline and subsequent data points between the infants in the phase 1 trial and observational (non-treated) cohorts.

Table 7. Planned statistical techniques and data availability for the phase 1 trial outcomes

	Values available for:		
Study outcome	**Phase 1 trial cohort**	**Observational cohort**	**Statistical approach**
Secondary safety endpoints	Yes	Yes, for some endpoints	• Rates of the secondary tolerability endpoints will be summarized for each dose level. Descriptive, narrative details of these endpoints will also be provided as appropriate. • Data on the rates of endotracheal tube obstruction, unplanned tracheal extubation, and audiology testing results will be compared between the trial and observational cohorts. • Planned comparisons of audiology testing results are described in the Protection of Human Subjects document.
Secondary efficacy endpoints			
Negative TA culture for the target GNR	Yes	No	• Primary exploratory efficacy endpoint. • Rates will be summarized for the trial cohort as a whole and stratified by drug dose level.
Change in TA GNR pathogen CFU	Yes	No	• Absolute (pre vs post) and relative change ([post trial – pre trial] / pre trial) in the target organism CFU/mL on the $\log_{10}$ scale will be calculated for each phase 1 trial participant. Paired t-tests or Wilcoxon sign rank tests as appropriate will be used to compare pre- vs. post-trial absolute CFU/mL values for the whole cohort and stratified by drug dose level.
Tracheal aspirate tobramycin levels	Yes	No	• Values, stratified by drug dose, will be compared to established concentrations necessary to overcome the minimum inhibitory concentration needed to kill 90% of detected bacterial isolates (MIC_{90}).[90,91] Values will also be used in our PK analyses.
Tracheal aspirate cytokine levels	Yes	No	• Individual cytokine levels measured pre-trial, ≤48 hours after the final dose, and 14 days after the final drug dose will be summarized. Transformation (e.g. logarithmic) or categorization by centiles will be considered based on the data distribution. Correction for total protein levels in the tracheal aspirate will be employed to account for differences in dilution during sample collection. • The relative intra-patient change for the individual cytokine levels from pre-trial baseline to the 2 post-trial timepoints will be calculated and compared between dose levels. • The association between change in cytokine levels and tobramycin dose will be explored using bivariate and multivariate correlation analyses with Bonferroni correction for multiple testing.[100] • We will consider exploratory cluster and linear discriminate analyses to identify airway immunological profiles associated with differences in the response to inhaled tobramycin (e.g. change in CFU/mL).[101,102]
Respiratory support measures Hypoxemia (SpO_2 <80%)	Yes	Yes	• Continuously recorded FiO_2, MAP, and RSS values will be converted to time-weighted daily averages for each study day. • Continuously recorded SpO_2 data will be used to calculate daily rates of intermittent and prolonged hypoxemia and daily total hypoxemia exposure. • Assessment of longitudinal change using linear mixed effects models will be considered.
Pulmonary mechanics	Yes	Yes	• Infants in both study cohorts will undergo pulmonary mechanics measurements pre-trial, after 7d, and after 14d. • Intra-patient change, relative to pre-trial baseline, will be calculated for each participant and compared (1) between the two cohorts and (2) stratified by drug dose. • Assessment of longitudinal change using linear mixed effects models will be considered.

CFU, colony forming units; GNR, Gram-negative rod; TA, tracheal aspirate

We will *consider* constructing exploratory linear mixed effects models to evaluate the longitudinal change in repeated measures outcome data. Owing to the modest total study sample size and low numbers of infants treated with each tobramycin dose, these analyses will only be performed on an exploratory basis if graphical evaluations of the study data suggest strong evidence of a linear change over the course of the study period. These models will be considered: (1) for the phase 1 trial participants as a whole, (2) with stratification by the administered dose of inhaled tobramycin, and (3) between infants who participate in the phase 1 trial and those enrolled in the observational (non-treated) cohort (for the study outcome data collected in both cohorts). Treatment dose group and treatment week will be included in the models as independent variables (the latter is added to account for natural fluctuation in disease acuity over time). Random effects for intercept +/- slope will be considered based on their effect on model fit assessed using the Akaike information criterion. Backwards, stepwise elimination techniques to determine inclusion of possible confounding, independent covariates will be employed to account for measured differences between comparison groups. Adherence to accepted numbers of independent covariates based on the study sample size will be used to avoid model over-fitting.[98] If longitudinal models are performed, 95% confidence intervals for all estimates of effect will be reported and caution will be urged when interpreting these hypothesis generating results.

Patterns in the airway microbiome will be assessed by comparing (pre vs. post treatment) measures of alpha diversity (e.g. species counts, Shannon index) and beta-diversity (Weighted and unweighted UniFrac distances[99]). Principal coordinate analyses and evaluation of dominant taxa will also be used to compare the microbiome before versus after completing treatment and with stratification by the administered dose of inhaled tobramycin. Linear mixed effects models, appropriate for repeated measures data, will also be constructed to measure the association between the dose of inhaled tobramycin and measures of alpha diversity.

Lastly, hypothesis generating analyses will assess changes in bacterial CFU/mL (on the $\log_{10}$ scale) as a function of: (1) the GNR organism cultured from the tracheal aspirate, (2) pre-trial airway cytokine levels, (3) pre-trial RSS, and (4) pre-trial pulmonary mechanics measurements. These data will help inform the selection of pre-specified subgroups group analyses that will be performed in future randomized trials.

6.3.4 Pharmacokinetic analysis

Patient specific pharmacokinetic parameters will be calculated using log-linear regression as the equation: $K_{el} = (ln\ Cp_{max}/Cp_{min}) / t$, where K_{el} is the elimination rate constant from plasma, Cp_{max} and Cp_{min} and the peak and trough concentrations, respectively, and t is the time between samples. Based on this equation, the estimated concentration at the time of tracheal aspirate sampling can also be calculated. The correlation between tracheal and plasma tobramycin concentrations will then be determined.

Population pharmacokinetic (PK) analysis will be conducted using nonlinear mixed effects modeling with the program NONMEM (Icon Inc.). Dosing information and plasma tobramycin concentrations will be used to facilitate model development. Mean population PK parameters (clearance, volume of distribution) will be estimated using the first-order conditional estimation with interaction method. A 2-compartment model with first order absorption will be utilized based on prior knowledge of inhaled tobramycin.[21] Between subject variability and covariate effects (body weight, PMA, renal function, sex) will be

assessed. Owing to a lack of existing data on the likely plasma tobramycin levels after inhalational administration in the target population, it is uncertain whether the sampling strategy implemented in this trial will enable formal population PK analysis. Therefore, this analysis will be conducted strictly as an exploratory aim.

6.4 Sample Size and Power

The traditional 3+3 design is the prevailing method for conducting phase 1 drug trials.[103,104] The sample size for this design is dictated by the number of different dosages investigated in the trial and whether or not additional patients are added to individual dosage levels based on the occurrence of a toxic drug response. Up to 24 infants are expected to provide analyzable trial data as described in section 3.3.2. The purpose of the 3+3 trial design is to continue dose escalation (within set boundaries) until at least two patients among a cohort of three to six patients experience dose-limiting toxicities (i.e., ≥33% of patients with a dose-limiting toxicity at that dose level).[103,104] If the 300mg dose level is completed in 3 participants and none show evidence of a toxic response, up to 3 additional infants will be enrolled to receive to receive 300mg. The trial will be stopped if 2 of the additional infants receiving 300mg develop a toxic response and the 216mg dose will be used as the maximum tolerated dose in subsequent trials.

Enrollment in the observational cohort will run concurrent with enrollment in the phase 1 trial. A minimum of 12 infants and a maximum of 28 infants will be enrolled in the observational cohort. This variable enrollment number is selected to avoid delay in study closure if enrollment in the trial cohort out paces the observational cohort. Importantly, these numbers are anticipated to provide adequate statistical power to measure clinically meaningful differences in the frequency of intermittent hypoxemia, an important measure of cardiorespiratory stability.[96,97] Premature infants with BPD average 75 (SD $\pm$ 9) intermittent hypoxemic events (SpO_2 <80% for 10s-3min) per day during the first several months of life.[105,106] A sample size of 12 infants in the observational cohort and 12 in the trial cohort will provide >80% power (α=0.05) to detect a 15% difference in the mean daily frequency of intermittent hypoxemic events between the two groups. A sample size of 24 infants per group will provide >80% to detect an 11% difference in in the mean daily frequency of intermittent hypoxemic events.

6.5 Interim Analysis

The 3+3 study design requires analysis of each study participant's data in "real time" to determine trial progression. After each infant completes the trial, an interim analysis will be performed to determine whether to continue the trial at the present dosage and enrollment number, expand the number of infants enrolled at the present dosage, advance to the next dosage, or end the trial after reaching a maximum tolerated dosage (MTD) or maximum planned investigational dosage.

7 STUDY MEDICATION (TOBRAMYCIN INHALATIONAL SOLUTION, USP)

7.1 Description

Tobramycin inhalational solution, USP is a tobramycin solution for inhalation. It is a sterile, clear, slightly yellow, non-pyrogenic, aqueous solution with the pH and salinity adjusted specifically for administration by a compressed air driven reusable nebulizer. The structural formula is shown in protocol section 1.5. Each single-use 5mL ampule contains 300mg tobramycin, USP and 11.25mg sodium chloride in sterile water for injection. Sulfuric acid and sodium hydroxide are added to adjust the pH to 6.0. Nitrogen is used for sparging. All ingredients meet USP requirements. The formulation contains no preservatives.

7.1.1 Packaging

Tobramycin inhalation solution comes in a single dose, ready-to-use ampule containing 300mg tobramycin. Each foil pouch contains 4 ampules.

7.1.2 Labeling

The product label for tobramycin inhalational solution, USP (300mg/5mL) is included in Appendix C. As the use of tobramycin in this clinical trial is investigational, the FDA label requirements per regulations will be affixed to the investigational product.

7.1.3 Dosing

This phase 1, dose escalation trial will investigate up to 4 different dosages of tobramycin solution for inhalation: 78mg, 150mg, 216mg, and 300mg. Each dose will be administered every 12 hours. For administered doses less than 300mg, the investigational pharmacy at CHOP will draw-up the appropriate volume from the 300mg/5mL tobramycin inhalational solution, USP ampule (i.e., 78mg = 1.3mL; 150mg = 2.5mL; 216mg = 3.6mL) using a single 3mL syringe (which indicate 0.1mL increments) or 5mL syringe (which indicate 0.2mL increments) as appropriate. The measured drug dose will then be diluted with sterile normal saline to produce a solution for aerosolization totaling 5mL. This will ensure that the same volume of liquid is aerosolized at each dosage level. All reconstituted drug product will be stored for not more than 4 hours at room temperature or 24 hours under refrigeration prior to administration.

7.1.4 Drug delivery via vibrating mesh nebulizer

Each dose of tobramycin solution for inhalation will be administered using the Aerogen ® Nebulizer System (Aerogen solo nebulizing chamber and Pro-X Controller/power source). This system utilizes a vibrating mesh nebulizer and is the standard equipment used for the delivery of aerosolized medications in the CHOP N/IICU. The Aerogen ® Solo nebulizing chamber and Pro-X Controller/power source are suitable for use in infants receiving invasive mechanical ventilation and FDA approved for use in children >29 days of age (see FDA approval information and device manual included in Appendix D, E). All study participants will be greater than 29 days of age at study initiation owing to enrollment of infants who are <32 weeks gestational age and ≥36 weeks PMA (gestational age + chronological age). The Aerogen ® Nebulizer System does not contribute additional flow to the ventilator circuit.

Vibrating mesh nebulizers employ a central aperture plate that is perforated with precisely formed holes. A piezo ring vibrates the aperture plate which acts as a micropump drawing liquid through the holes to generate aerosolized particles with a mass median aerodynamic diameter (MMAD) of 1-5μm for a range of different medications.[107-110] Achieving particles of this size is essential as smaller particles are more prone to removal during exhalation and particles with an MMAD >5-10μm are susceptible to “rainout” in the nebulizer/ventilator circuitry and/or the large, conducting airways.[111-113]

Bench studies using a pediatric lung model in a mechanically ventilated patient with an endotracheal tube (5mm inner diameter) and a tracheostomy tube (4.4mm inner diameter) have assessed the proportion of aerosolized drug delivered distal to the breathing tube using the Aerogen ® Solo vibrating mesh nebulizer.[114,115] The maximum proportion of inhaled drug was achieved using a tracheostomy: 19.8% ± 3.0%, likely owing to its shorter length despite modestly small inner diameter.[115] Using an endotracheal tube, 13.6% ± 1.3%

of the drug was delivered when the bias flow was set to 2L/min and 10.6% ± 0.3% when the bias flow was set to 5L/min.[114] The ventilators used in the CHOP N/IICU to support infants with BPD use a bias flow of either 2.5L/min when set to the pediatric mode (most common for the target study population) or 4L/min when set to the neonatal mode. Extrapolating the published bench study data[114,115] to the mechanical ventilators employed in this phase 1 trial, without correction for additional loss owing to the smaller endotracheal tubes used in the study infants (3.5-4mm), we conservatively estimate that **11-13% of the aerosolized drug will reach the respiratory tract of enrolled study subjects.** Further loss of drug may occur prior to the alveolar/capillary interface owing to the narrow caliber of the airways in infants and tighter bronchial branching patterns in small children.[116-119] Despite this drug loss, the available data indicate the range of tobramycin dosages under investigation in this study will yield a dose that results in sufficient antimicrobial levels to significantly reduce the respiratory load of the targeted pathogenic organisms. In a study evaluating tobramycin levels in the epithelial lining fluid obtained by bronchoalveolar lavage 30-45 minutes after treatment with 300mg of inhaled tobramycin administered by jet nebulization to 12 young children with CF (mean age 3.3 years, range 0.9 to 5 years), the tobramycin concentration was within the target range in 11 or 12 patients, with a mean value of 90 μg/mL.[26] This level is above the minimum inhibitory concentration of 2-4μg/mL necessary to kill 90% of pseudomonas isolates (MIC_{90}) *in vitro* and the necessary sputum levels of approximately 50μg/mL needed to effectively eradicate *P. aeruginosa* in the airway.[90,91] Modern vibrating mesh nebulizers produce a 2-3 fold higher drug output than do jet nebulizers, suggesting similar respiratory drug levels may be reached at the lower drug doses under investigation in this study.[108,120,121]

7.1.5 Treatment Compliance and Adherence

The drug dosages will be administered, according to the research protocol, by the clinically assigned respiratory therapist and documented in the medical record by the bedside nursing staff. The study team will also document each dosage in separate study logs. Drug administration will be open label.

7.1.6 Drug Accountability

Adequate records of study drug receipt and disposition will be maintained. The CHOP Investigational Drug Service (IDS) Records of receipts, investigational drug orders, dispensing records, and disposition forms will be examined during the course of the study. The purpose of these records is to ensure regulatory authorities that the investigational new drug will not be distributed to any person who is not a study subject under the terms and conditions set forth in this protocol. The study medication will be prescribed by the Investigator or designee and may not be used for any purpose other than that described in this protocol.

The CHOP IDS will purchase supplies for the study and partially used and empty containers will be discarded at the time of dose preparation to agree with IDS operating procedures.

8 SAFETY MANAGEMENT

8.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study. These will constitute both the defined tolerability drug endpoints and additional events that are observed clinically or by diagnostic testing (imaging, laboratory analyses) throughout the trial and pose a risk to the study participant.

8.2 Adverse Event Reporting

The Primary Investigator will hold responsibility for recording and reporting unanticipated problems related to research that occur during and after study treatment. Unanticipated problems related to the research involving risks to subjects or others that occur during the course of this study (including SAEs) will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review. Adverse event reporting will be targeted, so that potential adverse events arising from the use of inhaled tobramycin (for example, inhalational side-effects as well as renal adverse events) will be reported, while other adverse effects attributed to the underlying disease or clinical care will not be reported. All episodes of noncompliance will be documented. All adverse events reported to the CHOP IRB will also be reported in a timely manner to the study DSMB.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example) or disease temporally associated with the use of a medicinal product, whether or not it is considered related to the medicinal product.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

The likelihood that the adverse event was related to the study drug or a study procedure will be classified using the following scheme:

- ***Unrelated:*** *There is no apparent relationship between the adverse event and a study intervention or procedure*
- ***Unlikely:*** *It is unlikely that there could be any causal relationship between the adverse event and a study intervention or procedure*
- ***Possibly:*** *It is a possibility that the adverse event is related to a study intervention or procedure*
- ***Probably:*** *The adverse event is probably related to a study intervention or procedure*
- ***Definitely:*** *The adverse event is definitely related to a study intervention or procedure*

The severity of an adverse event will be classified using the following scheme:

- ***Mild:*** Asymptomatic or mild symptoms; clinical or diagnostic observation only; no intervention indicated
- ***Moderate:*** *Minimal, local, or non-invasive intervention indicated*
- ***Severe:*** *Severe or medically significant, but not immediately life threatening; prolongation of hospitalization indicated/required; disabling.*
- ***Life-threatening:*** *Life-threatening consequences; urgent intervention indicated*

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- a life-threatening event (at risk of death at the time of the event),
- prolongation of existing hospitalization beyond what would be expected for a preterm infant who requires extended invasive mechanical ventilation near and beyond term corrected gestation, *or*
- a persistent or significant disability/incapacity.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event 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.

Of note, there is a distinction between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4.1 Relationship/Severity of SAE to Study Drug or Other Intervention

In accordance with CHOP IRB Guidelines, the relationship of each SAE to the study intervention and the severity of the SAE will be characterized as described in section 8.3.

8.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB and DSMB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly.

Written reports will be filed using the eIRB system and in accordance with the timeline summarized on page 36. External SAEs that are both unexpected and related to the study intervention will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

8.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant

new or reassessed information (e.g., concomitant medication, medical history) will be submitted to the IRB. The investigator will be responsible for ensuring that all SAEs are followed until either resolved or stable.

8.6 Investigator Reporting of a Serious Adverse Event to Sponsor

Reporting will be consistent with regulatory and protocol-specified requirements.

8.7 Sponsor Reporting of Serious Adverse Events to FDA

In the event that a serious adverse event attributed to the investigational drug or its delivery occurs, the reporting to the FDA will be by the study Sponsor according to FDA guidance.

8.8 Medical Emergencies

All infants enrolled in the study will be hospitalized in the CHOP N/IICU throughout the trial. All medical emergencies will be handled by the primary medical team responsible for the care of each study participant. The study team will be available for consultation if needed.

9 STUDY ADMINISTRATION

9.1 Treatment Assignment Methods

Participants in this open label 3+3 phase 1 inter-patient dose escalation trial will be assigned to the dosage of inhaled tobramycin based on the order in which they enter the study. No randomization or blinding will be employed in this phase 1 trial.

9.2 Data Collection and Management

For each subject, the hospital medical record will serve as the primary record (source documents). Case report forms will be generated for each subject and maintained in the research-focused electronic data capture system REDCap. On occasion, paper case report documents may be generated, but will be destroyed as soon as possible after that data has been entered into REDCap. See section 9.3 for a description of data confidentiality protection measures. All data stored to REDCap is routinely backed-up to ensure recoverability in the event of malfunction of the primary server.

9.3 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy. The Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. Research related records will be stored using the research-focused electronic data capture system REDCap. Study data may also be shared via secure email. Only the Investigator and other approved study personnel will have access to the password-protected study database or data shared via secure email. Some data may initially be recorded on paper. As soon as possible, that data will be transcribed to the REDCap database and the paper copies will be destroyed. If storage of paper documents for any period of time is needed, those documents will be kept in locked file cabinets within the locked offices of the department of neonatology. Data files will be coded whenever possible to ensure that exposure of patient identifiers is kept to the minimum degree necessary to carry out the research goals. REDCap includes an automated export mechanism to common statistical packages. The export mechanism offers robust de-identification and coding options including removal all identifiers. When we export data for analysis, we will not include any individually identifiable private information. All study data (including PHI) will be maintained for the longer of either (1) 6 years after publication of the study results or (2) 2 years after the last marketing approval for the investigational agent (study drug) or, if no

application is filed or approved, 2 years after the FDA is notified that the IND application is formally discontinued. Thereafter, the database with identifiers will be deleted from REDCap and only a de-identified version will be retained. All paper copies of case reports, if not destroyed during the course of the study, will be destroyed at this time.

No identifiable data will be used for future study without first obtaining IRB approval. The investigator will obtain a data use agreement between the provider (the PI) of the data and any recipient researchers (including others at CHOP) before sharing a limited dataset.

9.4 Regulatory and Ethical Considerations

9.4.1 Data and Safety Monitoring Plan

An independent 4-member Data Safety Monitoring Board (DSMB) will be convened to monitor for any safety events associated with this study. At the recommendation of the NHLBI safety review committee, the DSMB will consist of 2 neonatologists (1 will serve as chair), 1 audiologist, and 1 statistician. The DSMB may appoint additional members as they require.

Data collection and management with specific focus on ensuring subject confidentiality will be carried out as described in section 9. Adverse events will be monitored for and reported as described in section 8.

9.4.1.1 Responsibilities of the DSMB

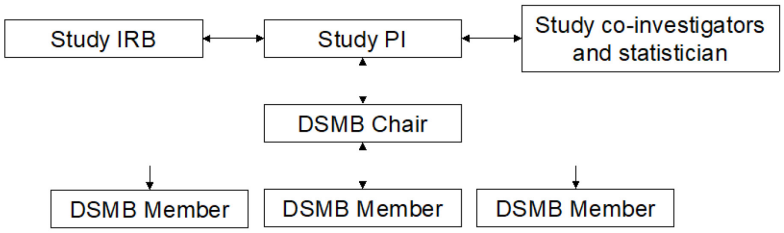
The DSMB is responsible for safeguarding the interests of study participants, assessing the safety and efficacy of study procedures, and for monitoring the overall conduct of the study. The DSMB will regularly review study safety data. As this is an open-label phase 1 dose escalation trial, all study data will be provided to the DSMB without masking of administered drug dose levels.

The DSMB is an independent group advisory to the investigators and affiliated institution, and is required to provide recommendations about starting, continuing, and stopping the study. The DSMB makes recommendations about:

- Participant safety and risk/benefit ratio of study procedures and interventions, including whether new data from other sources affects the study
- Initial approval of the protocol and subsequent amendments (with specific attention to study population, intervention, and study procedures)
- Initial approval of consent forms and subsequent amendments
- Study burden on participants
- Recruitment, and retention of participants
- Completeness, quality, and planned analysis of data
- Performance of individual centers and core labs
- Review of plans for incidental findings
- Ancillary study burden on participants and main study

9.4.1.2 Communication plan

The diagram shown below illustrates the flow of information between the DSMB and other entities in this study.



Communication with DSMB members will be primarily through the study PI. It is expected that study investigators will not communicate about the study with DSMB members outside of DSMB meetings. The investigator may contact the DSMB when needed for urgent concerns or clarifications of recommendations.

9.4.1.3 Scheduling, timing, and organization of DSMB meetings

The purpose of the first meeting is to review and discuss this charter, provide an overview of study activities, review and make recommendations about the protocol(s), informed consent materials, determine the frequency of interim analyses, finalize the DSMB data reporting template, and decide whether data will be unmasked to randomized group. Enrollment in a study cannot begin until the DSMB's and IRB's recommendations for approval.

- Meetings (in person or by teleconference) will be held **2 times a year**, with additional meetings scheduled as needed.

- Summaries of interim data analyses will be provided to the DSMB chair **prior to each drug dose escalation**. The DSMB chair may choose to convene a full DSMB meeting to discuss these findings and place the study on temporary hold as needed.

- The DSMB will conclude its operations when **all study procedures and planned primary data analyses are completed.**

The agenda for DSMB meetings and calls may be drafted by the PI and study biostatistician, and finalized after consultation with the DSMB Chair. The agenda and meeting materials will be distributed by 2 weeks before each meeting. The NHLBI Program Office may receive this material at the same time as DSMB members. When the agenda is distributed, DSMB members will be asked to report any new conflicts of interest since the last DSMB meeting. New conflicts will be reviewed by the Chair and study staff to determine if the conflict limits the ability of the DSMB member to participate in the discussion; according to the conflict of interest policy at the grantee institution.

The DSMB will review the following data: adverse events and other safety data, quality and completeness of study data, quality report(s) of any core(s), and enrollment data at each meeting to ensure proper trial conduct. The DSMB may also review formal interim analyses of drug tolerability endpoints prior to each planned drug dose escalation.
The expertise of the attending members should be appropriate for the agenda of the meeting. It's expected that all DSMB members will attend every meeting. The Chair may designate replacements for a meeting. For the purposes of voting on recommendations, a quorum is 3 members of the Board.

All standing Monitoring Board members are voting members. The Board may also decide in advance whether *ad hoc* members can vote.

Meetings are organized into open, closed, and executive sessions.

- **Open session** - information is presented to the DSMB by the study investigators, with time for discussion.

- **Closed session** - the DSMB, select study staff, and NHLBI program staff (at the Chair's discretion) discuss confidential data (any study data grouped by treatment arm), including information on drug dose safety.

  The principal investigator and staff involved in subject enrollment and treatment may not be present or review grouped data.

- **Executive session** (optional) -- DSMB members, and NHLBI program staff (at the Chair's discretion), may convene to discuss study issues independently.

- **Recommendations** (optional) **–** Meeting attendees may be reconvened to receive the DSMB's recommendations.

If the executive session occurs on a conference call, steps will be taken to ensure that only the appropriate participants are on the call, and to invite others to re-join the call only at the conclusion of the executive session.

9.4.1.4 Reports to the DSMB

For each meeting, the study biostatistician will prepare summary reports and tables to facilitate the oversight role of the DSMB. The DSMB will discuss at the first or subsequent meetings what data they wish to review and how it should be presented.

9.4.1.5 Statistical monitoring guidelines

At the first meeting, review of the protocol will include review of the statistical analysis plan. The DSMB will discuss the adequacy of that plan. The DSMB will discuss the statistical monitoring procedures they propose to follow to guide their recommendations about termination or continuation of the trial. These procedures could include guidelines for early termination for safety reasons.

9.4.1.6 DSMB recommendations

Voting on recommendations will follow Roberts' Rules of Order (**Robert's Rules of Order Newly Revised (11th Edition) RONR.**

Board Recommendations signed by the DSMB Chair, will be sent to the investigators within 7 calendar days after the meeting. Recommendations should include a statement as to whether the study, is approved to continue as planned, should continue with specified changes, or should be stopped. Requests for additional data from the investigators or study statistician should include an expected due date. In addition to recommendations memos issued to investigators (for review and IRB distribution), recommendations related to data analysis issues may be issued separately for statisticians.

Recommendations are distributed by the principal investigator to the NHLBI Program Office and the IRB.

9.4.1.7 Additional monitoring

Before the initiation of the clinical investigation, the sponsor-investigator will arrange a pretrial monitoring visit with the Office of Research Compliance (ORC) to confirm clinical trial readiness. After enrolling and starting administration of the investigational agent to the first subject, the sponsor-investigator will contact ORC to arrange a monitoring visit. Thereafter, ORC will monitor the study at least annually. Monitoring activities will be guided by ICH E6 section 5.18. Interim monitoring activities will include 100% review of regulatory files as well as a percentage of enrolled subject records, source documents, applicable informed consent forms, and case report forms. The percentage of subject data review may be amended throughout the course of the study based on an ongoing risk assessment. A tapered approach to monitoring may be employed, if conduct and documentation of the study reaches a level of reliability that would permit valid conclusions based upon a sampling of data.

9.4.2 Risk Assessment

The risks of participation in the phase 1 trial are greater than minimal. The primary medical risk to subjects relates to the administration of inhaled tobramycin. The anticipated potential risks associated with this medication are described in section 1.6.2. There may also be other unforeseen risks in this patient population. Robust monitoring of adverse events, including serial measurement of serum tobramycin trough levels, will be utilized to mitigate risk to the study participants. Any infant with a serum tobramycin trough level equal to or above the currently accepted standard of 2μg/mL, or any evidence of significant, new onset end-organ injury as measured by laboratory testing or clinical examination will be removed from the trial and no further doses will be administered.

Blood draws for research purposes may also pose a risk to the study participants. Infants with BPD receiving care in the CHOP N/IICU typically undergo multiple blood draws per week. Study team members will make every effort to coordinate with the medical team such that study blood collections might occur concurrent with other clinically indicated blood draws. All blood draws will be performed by nursing or phlebotomy staff according to unit guidelines, either via heel stick, venipuncture, or indwelling catheter. Phlebotomy may cause some pain, bleeding, or bruising. Rarely, taking blood may cause infection. To ensure collection of the necessary volume of blood for this research (up to 1.7mL per draw) does not incur risk to the patient, study team members, in consultation with nursing staff, will ensure that volume of blood extraction (for clinical + research purposes) does not exceed the maximum allowable weight-based limits specified by local and NIH guidelines (no more than 5 mL/kg drawn in a single day). In the event that excess blood draw is possible, the research team will coordinate with the medical team to prioritize laboratory testing to ensure patient safety.

Suctioning of the endotracheal tube is not anticipated to incur additional risk to the study participants as this is a routine clinical procedure for infants receiving invasive mechanical ventilation in the CHOP N/IICU. Infants receiving invasive mechanical ventilation undergo ET tube suctioning as part of routine clinical care several times throughout the day. Whenever possible, study staff will coordinate with nursing and respiratory therapy to collect ET aspirate samples at the time of planned clinical suctioning. ET suctioning

involves interruption of mechanical ventilation for ≤5 seconds, equivalent to the interval between 1-2 breaths for many infants.

Placement of the in-line sensor for the Philips Respironics NM3 respiratory parameter monitor requires brief (≤5 seconds) interruption of mechanical ventilation. Mechanical ventilation is briefly interrupted in infants with BPD on a daily basis as part of routine clinical care for reasons such as suctioning of the endotracheal tube, repositioning of the infant, and replacement or maintenance of the ventilator equipment. These brief interruptions are well tolerated in most infants.

Pulse oximetry is part of routine clinical care. For this study, an additional research monitor will be used. The adhesive used can on rare occasions result in minor skin irritation.

The other risk to participation in this study is breach of subject confidentiality. The study will employ extensive data security measures (see sections 9.2 and 9.3) to protect participant privacy.

9.4.3 Potential Benefits of Trial Participation

Colonization/infection of the lungs with pathogenic GNR organisms in preterm infants with BPD, and in multiple other pediatric and adult respiratory conditions, is associated with respiratory morbidity and increased mortality.[10,11,16,18] Inhaled tobramycin has shown benefit from improving respiratory function and clinical outcomes in children CF and airway colonization with *P. aeruginosa* (the most common GNR detected in the airways of infants receiving invasive mechanical ventilation for BPD).[10,16,28] Based on these data, we anticipate that inhaled tobramycin may provide direct benefit to preterm infants with BPD who have a tracheal aspirate culture positive for a pathogenic GNR. Society may also benefit from the knowledge gained from this trial. This study will provide the first trial data on the safety, tolerability, and potential efficacy of inhaled tobramycin administered to preterm infants with BPD who require prolonged invasive mechanical ventilation. These data will inform the design of future phase 2 and 3 trials exploring this medication.

9.4.4 Risk-Benefit Assessment

Participants in the phase 1 drug trial will be exposed to greater than minimal risk but with the prospect of direct benefit through the expected treatment of GNR bacteria in the lungs. Given the many safeguards in place to mitigate the study risks, the potential benefits of treatment with inhaled tobramycin outweigh the risks associated with participation in this trial. Participation in the observational cohort is expected to be minimal risk.

9.5 Recruitment Strategy

All subjects will be recruited from the CHOP N/IICU. Unit census documents will be reviewed weekly to identify possible subjects. For subjects likely to be eligible, medical records will be further reviewed to assess eligibility (only the number of subjects screened for eligibility and no specific subject level data will be recorded prior to informed consent). The Investigator or a designated study team member will confirm eligibility with the potential subject's attending physician and request permission to approach the parent(s) or guardian(s). If appropriate, the parent(s) or guardian(s) of eligible subjects will be approached and details of the study will be discussed. If interested, the parent(s) or guardian(s) will be invited to participate. Study personnel will then review the information in the consent form, answer any questions, and obtain informed consent. A study team physician will obtain consent for participation in the phase 1 trial.

Only 2 participants will be allowed in the 14-day phase 1 trial at the same time. A new participant will not be enrolled into a higher tobramycin dose level until all participants enrolled at the lower dose have completed the 14-day trial or been withdrawn.

Most preterm infants with BPD who require prolonged invasive mechanical ventilation are followed by the CHOP Newborn and Infant Chronic Lung Disease (NeoCLD) Program. The NeoCLD program is comprised of physicians, respiratory therapists, and nurses who are jointly engaged in clinical care and research. Every year 60-70 infants are admitted to CHOP and cared for by the NeoCLD program. Another 15-20 admitted to the CHOP N/IICU require prolonged mechanical ventilation but do not receive care from the NeoCLD program. Together, these admission rates indicate up to 75-90 infants per year will be eligible to undergo a screening tracheal aspirate culture. During the past 2 years, over 60% of eligible infants were enrolled and retained in clinical trials conducted in the CHOP N/IICU. Based on this rate, we estimate that 30-50 infants will undergo a screening tracheal aspirate culture as part of this study each year. Our prior data show that 54-60% of infants in the BPD population at CHOP have tracheal aspirate cultures positive for pathogenic GNR bacteria. Accordingly, we expect to enroll 15-30 infants per year into the phase 1 trial or observational cohort. Based on these numbers we anticipate this study will require up to 3 years to complete. If all enrolled infants successfully complete the 14-day phase 1 trial, we anticipate the study will require up to 18 months to complete.

9.6 Informed Consent/Assent and HIPAA Authorization

After determining eligibility and obtaining permission from the attending physician, a study team member will contact a potential participant's parent(s) or guardian(s) to obtain informed consent and HIPAA authorization. This study will obtain informed consent in 2 phases. First, consent will be obtained to collect a screening tracheal aspirate culture to determine eligibility to enroll in the phase 1 trial of inhaled tobramycin or the untreated observational cohort. Second, for infants whose tracheal aspirate culture is positive for a pathogenic GNR organism, consent will be obtained to enroll in either the phase 1 trial or observational cohort.

During the initial consent conversation(s), the study team will provide a description of the goals and study procedures involved in the phase 1 drug trial and observational cohort and the need to obtain a screening tracheal aspirate culture to determine eligibility to participate in either study cohort. For families who express interest in potential participation, we will obtain consent to collect a research tracheal aspirate sample for culture to determine eligibility. Consent to participate in the phase 1 trial or observational cohort will not be obtained until after the results of the tracheal aspirate culture are available. Our initial consent discussions will be conducted in the N/IICU in a private setting or via the telephone. Before collection of a screening tracheal aspirate culture or medical/PHI, the screening consent form must be signed by a legally acceptable surrogate and the investigator-designated research professional obtaining the consent if consent is obtained in person. If consent for screening procedures is obtained via the telephone, the consent form will be signed by the investigator-designated research professional obtaining the consent and a study team member or clinician witnessing and verifying consent. Parents or guardians providing consent via telephone will be provided a copy of the consent form (either via email or left at the baby's bedside as per their preference).
For those infants whose screening tracheal aspirate culture is positive for a pathogenic GNR, a second consent discussion will take place to cover the goals, procedures, risks, and benefits of the phase 1 trial of inhaled tobramycin and the observational cohort. This conversation will take place in in a private setting in the N/IICU or via telephone. Enrollment

in either study cohort will be offered but an infant may only be enrolled in one of the study cohorts (phase 1 trial or observational cohort). Consent for the observational cohort will be obtained either in person or via telephone, through a waiver of documentation of consent.

Before any study procedures take place, all necessary consent forms must be signed by a legally acceptable surrogate and the investigator-designated research professional obtaining the consent.

Consent to participate in the phase 1 drug trial will be obtained by a physician member of the study team. Consent for the phase 1 drug trial will be obtained either through written consent or through electronic consent (also known as eConsent). Electronic consent will utilize the REDCap survey method. The 21 Code of Federal Regulations (CFR) Part 11 compliant version of REDCap will be used to obtain eConsent, and all associated regulations will be followed. If using the REDCap survey, the research team will email the eConsent to the parents. We will follow up with a telephone call or video conferencing to review the details of the trial and allow for all questions to be answered. Parents will then sign the eConsent, which is returned electronically back to the study team. PHI needed for emailing and returning the signed eConsent is only available in the PDF and not in the body of the email or the email subject header, thus maintaining subject confidentiality. REDCap's eConsent automatically returns a signed copy to the parent if he/she agrees to participate.

Parents/guardians with limited English proficiency will consent using the short form process. Short forms in the preferred language will be obtained from the CHOP IRB website. The Study Summary document will be signed by the study team member obtaining consent and the witness/interpreter. The short form document will be signed by the parent/guardian and the witness/interpreter. Consent will only occur in person with the study team, parent/guardian, and interpreter all present.

Whenever feasible, consent conversations will take place in person. At the conclusion of all consent discussions, parent(s) or guardian(s) will be provided the opportunity to ask questions about the study and to discuss the study with their family, friends, and/or other medical professionals. As there are no negative repercussions for lack of participation, we do not anticipate a significant risk of coercion or undue influence to participate.

9.6.1 Waiver of Assent

A waiver of assent is requested as all study participants are infants and incapable of providing assent under any circumstances.

9.7 Payment to Subjects/Families

Subjects and/or families will not receive financial compensation for participation in this study.

10 PUBLICATION

Following completion of this study, results and analyses will be presented at research meetings and published in in scholarly journals.

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APPENDIX

Appendix A: List of nephrotoxic medications[122]

Acyclovir

Ambisome

Amikacin

Captopril

Carboplatin

Cefotaxime

Ceftazidime

Cefuroxime

Cidofovir

Cisplatin

Colistimethate

Cyclosporine

Dapsone

Enalapril

Foscarnet

Gadopentetate dimeglumine

Ganciclovir

Gentamicin

Ibuprofen

Ifosfamide

Iodixanol

Iohexol

Iopamidol

Ioversol

Ketorolac

Lisinopril

Lithium

Mesalamine

Methotrexate

Nafcillin

Piperacillin

Sirolimus

Sulfasalazine

Tacrolimus

Ticarcillin/clavulanic acid

Tobramycin

Topiramate

Valacyclovir

Valganciclovir

Vancomycin

Zonisamide

Appendix B: List of neuromuscular blocking agents[1][2][3]

Altracurium

Cisatracurium

Doxacurium

Metrocurine

Mivacurium

Pancuronium

Pipercuronium

Rapacuronium

Rocuronium

Succinylcholine

Tubocurarine

Vecuronium

Appendix C: Inhaled tobramycin label

Appendix D: Aerogen ® Solo FDA approval letter

Appendix E: Aerogen ® Solo System Instruction Manual