CLINICAL STUDY PROTOCOL

A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study to Determine the Safety and Efficacy of a Single Dose of ASN100 for the Prevention of *Staphylococcus aureus* Pneumonia in Heavily Colonized, Mechanically Ventilated Subjects

Investigational Product: ASN100
Protocol Number: ASN100-201
IND Number: 128766
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SIGNATURE PAGE

STUDY TITLE: A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study to Determine the Safety and Efficacy of a Single Dose of ASN100 for the Prevention of *Staphylococcus aureus* Pneumonia in Heavily Colonized, Mechanically Ventilated Subjects

We, the undersigned, have read this protocol and agree that it contains all necessary information required to conduct the study.

Signature | Date

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INVESTIGATOR AGREEMENT

By signing below I agree that:

I have read this protocol. I approve this document and I agree that it contains all necessary details for carrying out the study as described. I will conduct this study in accordance with the design and specific provision of this protocol and will make a reasonable effort to complete the study within the time designated. I will provide copies of this protocol and access to all information furnished by Arsanis, Inc. (Arsanis) to study personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the study product and study procedures. I will let them know that this information is confidential and proprietary to Arsanis and that it may not be further disclosed to third parties. I understand that the study may be terminated or enrollment suspended at any time by Arsanis, with or without cause, or by me if it becomes necessary to protect the best interests of the study subjects.

I agree to conduct this study in full accordance with the United States Food and Drug Administration (FDA) Regulations, Institutional Review Board/Ethic Committee Regulations and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines for Good Clinical Practices.

_____ _____

Investigator's Signature Date

_____

Investigator's Printed Name

SYNOPSIS

TITLE: A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study to Determine the Safety and Efficacy of a Single Dose of ASN100 for the Prevention of *Staphylococcus aureus* Pneumonia in Heavily Colonized, Mechanically Ventilated Subjects

PROTOCOL NUMBER: ASN100-201

INVESTIGATIONAL PRODUCT: ASN100

PHASE: 2

INDICATION: The prevention of *Staphylococcus aureus* (*S. aureus*) pneumonia in mechanically ventilated subjects who are heavily colonized with *S. aureus*.

STUDY DEFINITION: For the purposes of this study, heavy colonization of *S. aureus* will be defined as a quantitative threshold of $\geq 10^5$ CFU/mL or 3+ to 4+ by semi-quantitative culture from an endotracheal aspirate.

OBJECTIVES:

The primary objective of this study is to evaluate the safety, tolerability, and efficacy of a single dose of ASN100 (administered as ASN-1 and ASN-2 components) versus placebo for the prevention of *S. aureus* pneumonia in mechanically ventilated subjects who are heavily colonized with *S. aureus.*

The secondary objectives of this study are:

- To compare the duration of mechanical ventilation post-treatment in subjects treated with ASN100 versus placebo;
- To compare the length of stay in the intensive care unit (ICU) post-treatment for subjects treated with ASN100 versus placebo;
- To determine the serum pharmacokinetics (PK) (i.e., maximum serum concentration [C_{max}], time to maximum serum concentration [T_{max}], area under the serum concentration-time curve [AUC], and terminal elimination half-life [$t_{1/2}$]) of ASN-1 and ASN-2 in mechanically ventilated subjects heavily colonized with *S. aureus*; and
- To compare 28-day all-cause mortality in subjects treated with ASN100 versus placebo.

The exploratory objectives are:

- To compare the incidence of hospital-acquired bacterial pneumonia (HABP) occurring >48 hours post-extubation up to but not including Day 22 in extubated subjects treated with ASN100 versus placebo;
- To compare the incidence of ventilator-associated bacterial pneumonia (VABP) up to but not including Day 22 in subjects treated with ASN100 versus placebo;

- To determine the immunogenicity of a single dose of ASN100;
- To compare the incidence of other *S. aureus*-associated infections which occur after dosing up to but not including Day 22 in subjects treated with ASN100 versus placebo; and
- To assess ASN-1 and ASN-2 levels in bronchoalveolar lavage (BAL) fluid and calculate a blood to epithelial lining fluid (ELF) ratio in those subjects participating in the BAL fluid PK sub-study.

POPULATION:

Inclusion Criteria

Subjects must meet all of the following criteria at the Screening and Randomization Visits in order to be eligible for randomization into the study:

1. Subject, legally authorized representative, or council of independent physicians (CIP) (if applicable) has provided written informed consent;
2. Subject is ≥18 years of age at the time of enrollment;
3. Subject is currently hospitalized and is mechanically ventilated endotracheally (i.e., orotracheal or nasotracheal) and, in the Investigator's opinion, will require ongoing ventilator support for at least 48 hours;

   NOTE: Subjects with a tracheostomy at the time of enrollment are not eligible. After enrollment, conversion to tracheostomy is permitted per standard of care.
4. Female subjects must not be pregnant or lactating. Female subjects of childbearing potential must have a documented negative pregnancy test at the Screening Visit. Female subjects may be enrolled on the basis of a negative urine pregnancy test, pending the result of a negative serum pregnancy test prior to randomization; and
5. Female subjects of childbearing potential and non-surgically sterile male subjects who are sexually active must agree to use an approved highly effective form of contraception from the time of informed consent until 165 days post-dose. Approved forms of contraception include hormonal intrauterine devices, hormonal contraceptives (oral birth control pill, depo, patch, or injectable) together with supplementary double barrier methods such as condoms or diaphragms with spermicidal gel or foam.

   NOTE: The following categories define women who are NOT considered to be of childbearing potential:

   - Premenopausal female with 1 of the following:
     - Documented hysterectomy,
     - Documented bilateral salpingectomy, or
     - Documented bilateral oophorectomy, or
   - Postmenopausal female, defined as having amenorrhea for at least 12 months without an alternative medical cause.

Exclusion Criteria

Subjects who meet any of the following criteria at the Screening and/or Randomization Visit will be excluded from participation in the study:

1. Subject has received intravenous (IV) immunoglobulin therapy within 4 months prior to the Screening Visit;
2. Subject has a chest X-ray or thoracic computed tomography (CT) scan that is definitive for a diagnosis of pneumonia;
3. Subject demonstrates both of the following:
   a. Need for acute changes in ventilator support to enhance oxygenation or to the amount of positive end-expiratory pressure; AND

      NOTE: Changes made in ventilator support due to day-to-day maintenance other than those changes made to improve oxygenation will not exclude a subject from participation in the study.

   b. New onset of purulent suctioned respiratory secretions;
4. Subject has a known and documented ETA culture showing heavy colonization with a Gram-negative organism at enrollment or at any time during the Screening period;

   NOTE: Randomization should not be delayed while waiting for Gram-negative results if heavy colonization with *S. aureus* has been confirmed.
5. Subject has been diagnosed with neutropenia (absolute neutrophil count <500/mm^3);
6. Subject has a severe non-pulmonary source of infection which, in the Investigator's opinion, would interfere with the conduct of the study or jeopardize the subject's safety;
7. Subject is currently on either continuous veno-venous hemodialysis or extracorporeal membrane oxygenation;
8. Subject has been previously exposed to ASN100 or ASN-1 or ASN-2, individually;
9. Subject has a known hypersensitivity to ASN100 or any of its excipients;
10. Subject has received any investigational product within 30 days prior to the Screening Visit (or 5 half-lives of the investigational product, whichever is longer);
11. Subject has, in the opinion of the Investigator, a high probability of death within 72 hours of enrollment;
12. Subject is, in the opinion of the Investigator, not able or willing to comply with the protocol;
13. Any condition that, in the opinion of the Investigator, would compromise the safety of the subject, the potential activity of the study drug, or the quality of the data; or
14. Subjects with a known history or current (suspected) diagnosis of cytokine release syndrome associated with the administration of peptides, proteins, and/or antibodies.[20]

    NOTE: Subjects with a prior history of sepsis are not excluded.

Randomization Criteria

At the Randomization Visit, subjects must meet all of the following requirements to be randomized into the study:

1. Subject continues to meet all of the inclusion criteria and none of the exclusion criteria required at the Screening Visit;

   NOTE: Subjects who undergo tracheostomy after enrollment are still eligible for randomization, provided they remain mechanically ventilated (see criterion 3 below).

2. Subject is heavily colonized with *S. aureus* as determined by either quantitative or semi-quantitative culture of an endotracheal aspirate (ETA);

   NOTE: The results of the qualifying ETA culture must allow randomization to occur within 48 hours (or up to 60 hours with Medical Monitor approval) from the time the ETA sample was collected. Subjects with ETA culture results obtained after 60 hours showing heavy colonization of *S. aureus* may not be randomized and will be discontinued from the study.

3. Subject remains mechanically ventilated and, in the Investigator's opinion, will require ongoing ventilator support for at least 48 hours; and

4. Subject has, in the opinion of the Investigator, a probability of survival beyond 72 hours post-randomization.

STUDY DESIGN AND DURATION:

This is a double-blind, randomized, single-dose, placebo-controlled study of ASN100 for the prevention of *S. aureus* pneumonia in mechanically ventilated subjects who are heavily colonized with *S. aureus*. Subjects will be screened for eligibility; once an Informed Consent Form is signed (or, in countries where it is applicable, a decision is made by the CIP), and all entry criteria are met, a subject is considered to be enrolled in the study. All enrolled subjects will undergo an observational stage evaluating endotracheal colonization. Subjects who are randomized will undergo a treatment, monitoring, and follow-up period.

Approximately 2250 eligible subjects who meet all of the inclusion criteria and none of the exclusion criteria will be screened daily (while mechanically ventilated) for up to 8 days to identify those subjects who are heavily colonized with *S. aureus*.

Screening for heavy *S. aureus* colonization by ETA culture will continue on Day 11 and Day 14 provided the subject remains mechanically ventilated, for a total Screening Period of up to 14 days. Additional ETA screening may occur on Days 9, 10, 12, and 13 at the discretion of the Investigator and providing the subject remains mechanically ventilated.

ETA specimens collected during Screening/Enrollment will be cultured by quantitative or semi-quantitative methods as required by the protocol. Sites are encouraged to perform semi-quantitative cultures using a chromogenic media that tests for both the presence of methicillin-susceptible *S. aureus* and methicillin-resistant *S. aureus* (e.g., CHROMagar™ *Staph aureus*) for more rapid and specific detection of *S. aureus*. If the site is not able to obtain chromogenic media for *S. aureus*, appropriate chromogenic media may be supplied by the Sponsor.

Semi-quantitative cultures using chromogenic media are preferred for determination of eligibility for randomization; however, any quantitative or semi-quantitative culture performed that indicates that a subject is heavily colonized with *S. aureus* is permitted if the culture results are available to randomize the subject within 48 hours (or up to 60 hours with Medical Monitor approval) from the time the ETA sample was collected.

Once an enrolled subject has an ETA culture reported positive for heavy *S. aureus* colonization and meets all of the inclusion and none of the exclusion criteria, they are eligible for randomization. If they are not randomized on this occasion according to the protocol, they are no longer eligible for continued ETA sampling and randomization, and will be discontinued from the study; however, if subjects are extubated and subsequently re-intubated/mechanically ventilated, they are eligible for re-screening as a new subject enrollment. Subjects who were previously randomized are not eligible to be re-consented and re-screened.

At the time of randomization, if a subject's screening ETA culture also shows heavy colonization of a Gram-negative organism (i.e., heavy co-colonization of both Gram-positive and Gram-negative organisms), the subject will not be randomized into the study; however, randomization should not be delayed while waiting for Gram-negative results if heavy colonization with *S. aureus* has been confirmed.

Nasal swab specimens (one from each nostril) will be obtained at the Randomization Visit and will be cultured by the central microbiology laboratory. Results from the nasal swab specimen cultures will be used to assess correlation with results of the qualifying ETA culture.

Upon determination of eligibility, approximately 354 subjects will be randomized in a 1:1 ratio to 1 of 2 treatment groups:

- ASN100 administered either sequentially or simultaneously as 2 separate IV infusions of ASN-1 and ASN-2, or
- Placebo administered either sequentially or simultaneously as 2 separate IV infusions.

To ensure balance among the treatment groups, randomization for this study will be stratified by receipt or non-receipt of concomitant anti-staphylococcal antibiotics at the time of randomization that are potentially active against *S. aureus* pneumonia, including, but not limited to, nafcillin, oxacillin, vancomycin, linezolid, telavancin, ceftaroline, ceftobiprole, and teicoplanin.

Following randomization on Day 1, subjects will receive either a single IV dose of 3600 mg of ASN100 (comprised of separate infusions each of 1800 mg ASN-1 and 1800 mg ASN-2) or matching placebos, per their assigned randomization scheme.

Randomized subjects will be monitored daily for the signs and symptoms of pneumonia and other *S. aureus*-associated infections while hospitalized up to Day 21.

All randomized subjects will undergo a study visit on Day 22 (+2 days). A follow-up visit will occur on Day 28 (+2 days) to assess mortality. This visit may be conducted via telephone if the subject is no longer hospitalized.

For subjects who remain hospitalized between Day 40 and Day 90 at the institution where they receive study treatment, a follow-up safety visit will be performed on the day of discharge.

All randomized subjects will return for a Safety Visit on Day 90 (±7 days) (if the subject is unable to return to the site, this visit may be conducted by telephone). Subjects who discontinue the study

prior to Day 22 will undergo an Early Termination Visit. The end of the study will occur upon completion of the last Day 90 visit by the last subject.

Safety assessments for this study will include adverse event monitoring, clinical laboratory assessments (including chemistry, hematology, coagulation, and urinalysis), physical examinations, vital sign measurements, 12-lead electrocardiograms (ECGs), chest X-rays and/or thoracic CT scans, and the determination of the presence of ASN-1 and ASN-2 anti-drug antibodies (ADAs). Additional safety assessments may be performed throughout the duration of the study if clinically indicated.

Procalcitonin testing will be performed at the Randomization Visit (Day 1 pre-dose), on the day of diagnosis of pneumonia (if applicable), and on Day 22 or Early Termination (if applicable).

Blood samples will be obtained for the measurement of ASN-1 and ASN-2 in serum. Blood samples for PK analysis will be collected immediately following the completion of the second infusion (+15 minutes) and at 6 hours (± 4 hours) and 24 hours (±6 hours) post-dose following the second infusion. In addition, a PK sample will be obtained from all subjects on Days 4 (±1 day), 7 (±1 day), 14 (±2 days), and 22 (+2 days) of the Monitoring Period. Additional PK samples will be collected on the day of discharge (if discharge occurs between Day 40 and Day 90), on Day 90 (if hospitalized or if the subject is able to return to the clinic), or Early Termination (if applicable).

Select sites will be invited to participate in a BAL fluid PK sub-study. Approximately 25 to 35 subjects who volunteer will participate in this sub-study and will have BAL fluid collected 48 hours (±36 hours) post-dose (on approximately Day 2 or Day 3, depending on time of study drug administration) to determine ASN-1 and ASN-2 levels and blood to ELF ratio. Subjects participating in the BAL fluid PK sub-study will have a blood sample collected for PK measurement ±1 hour relative to BAL fluid collection (including scheduled BAL fluid collection or if BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any time post-randomization).

Blood samples obtained for ADA and PK analysis purposes will also be used to assess the amount of pre-existing anti-*S. aureus* toxin antibodies and the toxin neutralizing titer of the serum sample prior to and after randomization. Only antigen-specific antibody titers will be determined and no genetic information will be extracted from these samples. Samples will be destroyed following the completion of all *S. aureus* anti-toxin antibody analyses.

DOSAGE FORMS AND ROUTE OF ADMINISTRATION:

ASN100 is a combination product with 2 separate human monoclonal antibody (mAb) components of the immunoglobulin G1 isotype. The specific mAbs are formulated separately and called ASN-1 and ASN-2. Each component is supplied as a sterile, colorless, clear liquid solution formulated ready for injection.

ASN-1 is a broadly cross-reactive anti-toxin mAb that targets alpha hemolysin and 3 F-components (gamma hemolysin B, leukocidin F, and leukocidin D) involved in forming 4 of the 5 bi-component leukocidins of *S. aureus*.

ASN-2 is directed against the fifth bi-component toxin of *S. aureus*, leukocidin GH.

ASN-1 and ASN-2 will be supplied in separate 10 mL vials as a sterile solution for infusion with an extractable volume of 10 mL and a nominal mAb concentration of 20 mg/mL. Each vial will

contain 200 mg of ASN-1 or 200 mg of ASN-2. Matched vials of ASN-1 placebo and ASN-2 placebo will also be provided.

ASN100 will be administered as separate infusions of 1800 mg ASN-1 and 1800 mg ASN-2 (9 vials of each to be pooled aseptically according to the Pharmacy Manual into separate infusion bags) delivered either sequentially within a 3-hour window from the time of initiation of the first infusion to the completion of the second infusion, or simultaneously.

Placebo will be administered as separate infusions of 9 vials of ASN-1 placebo and 9 vials of ASN-2 placebo (vials to be pooled aseptically according to the Pharmacy Manual into separate infusion bags) delivered either sequentially within a 3-hour window from the time of initiation of the first infusion to the completion of the second infusion, or simultaneously.

ANALYSIS POPULATIONS:

Intent-to-Treat (ITT) Population

The ITT Population includes all subjects who are randomized to receive study drug.

Modified Intent-to-Treat (MITT) Population

The MITT Population includes all subjects in the ITT Population who receive study drug who are heavily colonized with *S. aureus* as determined by quantitative or semi-quantitative culture of an ETA.

Per Protocol (PP) Population

The PP Population includes all subjects in the MITT Population who also meet the following criteria:

- Do not have any major protocol violations that would affect assessment of efficacy, and
- Complete an adequate number of Monitoring Period assessments through and including Day 22.

Pharmacokinetic (PK) Population

The PK Population includes all subjects in the MITT Population with at least 1 serum PK sample collected post-dose.

Safety Population

The Safety Population includes all subjects who receive any amount of study drug and have at least 1 post-treatment safety assessment.

EFFICACY VARIABLES:

The primary efficacy endpoint is the proportion of subjects in the MITT Population who develop *S. aureus* pneumonia up to but not including Day 22.

The secondary efficacy endpoints include:

- Duration of mechanical ventilation during the first 21 days post-randomization for subjects in the MITT, ITT, and PP Populations;

- Length of ICU stay during the first 21 days post-randomization for subjects in the MITT, ITT, and PP Populations;
- The C_{max}, T_{max}, AUC, and $t_{1/2}$ of ASN-1 and ASN-2 in serum following a single dose of ASN100 in the PK Population; and
- 28-day all-cause mortality in the MITT, ITT, and PP Populations.

Exploratory efficacy endpoints include:

- Proportion of subjects in the MITT and PP Populations with a diagnosis of HABP at >48 hours post-extubation up to but not including Day 22 in extubated subjects;
- Proportion of subjects in the MITT and PP Populations with development of VABP up to but not including Day 22;
- Incidence of all bacterial pneumonias in the MITT, ITT, and PP Populations;
- Incidence of other non-*S. aureus* pneumonias up to but not including Day 22 in the MITT, ITT, and PP Populations; and
- Incidence of other *S. aureus* infections acquired following study drug administration up to but not including Day 22 in the MITT, ITT, and PP Populations.

MICROBIOLOGICAL AND CLINICAL ASSESSMENTS:

ETA specimens will be collected for culture during Screening/Enrollment to determine the degree of *S. aureus* colonization. Additional respiratory specimens may be collected for culture throughout the Monitoring Period or Early Termination (if applicable) if clinically indicated according to standard of care.

If, as part of routine standard of care, additional respiratory and/or other microbiological specimens are collected for culture during the study, results will be documented within the electronic Case Report Form. As fully described within the study laboratory manual, bacterial isolates recovered from these specimens that are deemed pathogens by the Investigator (if retained and available at the study site local microbiology laboratory) will be sent to a central microbiology laboratory for confirmation of pathogen identification (all isolates) and susceptibility testing (*S. aureus* isolates only).

During the Monitoring Period, a diagnosis of *S. aureus* pneumonia will be determined if subjects have *S. aureus* identified from an adequate respiratory specimen and clinical signs and symptoms of pneumonia. For a diagnosis of *S. aureus* pneumonia, clinical signs and symptoms must occur ±2 days from the time of collection of an adequate respiratory specimen culture that is positive for *S. aureus*.

- For intubated subjects, a respiratory specimen obtained by BAL, non-bronchoscopic BAL, or protected specimen brush will be considered an adequate respiratory specimen; or
- For extubated subjects, an adequate expectorated/induced sputum specimen is defined as <10 squamous epithelial cells and >25 polymorphonuclear cells/100× field; and

- For both intubated and extubated subjects, adequate respiratory specimens will undergo either semi-quantitative or quantitative culture in accordance with the local laboratory's standard procedure.

Subjects will be assessed daily throughout the Monitoring Period for the following clinical signs and symptoms:

Clinical Signs and Symptoms

Mechanically Ventilated Subjects	Non-Mechanically Ventilated Subjects	
• Cough, • Rales, • Dullness on percussion,* • Bronchial breath sounds, • Egophony,* • Need for suctioning, • Need for ventilator support, and • Fever ≥38°C (≥100.4°F).	• Cough, • Rales, • Dullness on percussion,* • Bronchial breath sounds, • Egophony,*	• Dyspnea, • Tachypnea, • Respiratory rate, • Hypoxemia, and • Fever ≥38°C (≥100.4°F).
*If routinely performed.		

A diagnosis of pneumonia (HABP or VABP) will be determined as defined below:

Definition of Hospital-Acquired and Ventilator-Associated Bacterial Pneumonia

Indication	Definition
HABP	• A chest X-ray showing new or progressive infiltrates suggestive of pneumonia (as assessed by the Investigator); • At least ONE of the following: o Has been hospitalized for >48 hours, or o Developed clinical signs and symptoms within 7 days following hospital discharge; • At least ONE of the following: o New onset or worsening pulmonary signs/symptoms such as cough, dyspnea, tachypnea (e.g., respiratory rate >25 breaths per minute), expectorated sputum production, or the requirement for mechanical ventilation (if subject is not already ventilated); o Need for acute changes in ventilator support to enhance oxygenation or to the amount of PEEP; or o New onset of suctioned respiratory secretions; AND • At least ONE of the following clinical signs/symptoms: o Temperature >38°C or <35°C, o WBC count ≥10,000 cell/mm^3 or ≤4500 cell/mm^3, or o >15% immature neutrophils (bands) on peripheral blood smear.
VABP	• A chest X-ray showing new or progressive infiltrates suggestive of pneumonia (as assessed by the Investigator); • Subject has received mechanical ventilation via an endotracheal or nasotracheal tube for ≥48 hours; • At least ONE of the following: o New onset or worsening pulmonary signs/symptoms such as cough, dyspnea, tachypnea (e.g., respiratory rate >25 breaths per minute), expectorated sputum production, or the requirement for mechanical ventilation (if subject is not already ventilated); o Need for acute changes in ventilator support to enhance oxygenation or to the amount of PEEP; or o New onset of suctioned respiratory secretions; AND • At least ONE of the following clinical signs/symptoms: o Temperature >38°C or <35°C, o WBC count ≥10,000 cell/mm^3 or ≤4500 cell/mm^3, or o >15% immature neutrophils (bands) on peripheral blood smear.
HABP = hospital-acquired bacterial pneumonia; PEEP = positive end-expiratory pressure; VABP = ventilator-associated bacterial pneumonia; WBC = white blood cell.	

PHARMACOKINETIC VARIABLES:

The primary PK endpoints are the C_{max}, T_{max}, AUC, and $t_{1/2}$ of ASN-1 and ASN-2 in serum following a single IV dose of ASN100 in the PK Population.

Blood samples will be obtained for the measurement of ASN-1 and ASN-2 in serum. At the completion of the second infusion, blood samples for PK analysis will be collected immediately following the completion of the second infusion (+15 minutes) and at 6 hours (±4 hours) and 24 hours (±6 hours) post-dose.

In addition, a PK sample will be obtained from all subjects on Days 4 (±1 day), 7 (±1 day), 14 (±2 days), and 22 (+2 days) of the Monitoring Period. Additional PK samples will be collected on the day of discharge (if discharge occurs between Day 40 and Day 90), on Day 90 (if hospitalized or if the subject is able to return to the clinic), or Early Termination (if applicable). Subjects participating in the BAL fluid PK sub-study will have a blood sample collected for PK measurement ±1 hour relative to BAL fluid collection (including scheduled BAL fluid collection or if BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any time post-randomization).

In order to maintain the study blind, all subjects, including those randomized to receive placebo, will have PK samples obtained at the above time points.

The levels of ASN-1 and ASN-2 will be measured in BAL fluid in a PK sub-study (to include approximately 25 to 35 subjects who volunteer) which will allow the calculation of a blood to ELF ratio. Subjects participating in this sub-study will have BAL fluid collected 48 hours (±36 hours) post-dose (on approximately Day 2 or Day 3, depending on time of study drug administration) to determine ASN-1 and ASN-2 levels and blood to ELF ratio. If BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any other time post-randomization for any other reason, if possible, a sample for PK analysis will also be obtained.

SAFETY VARIABLES:

Safety variables for this study will include adverse event monitoring, clinical laboratory assessments (including chemistry, hematology, coagulation, and urinalysis), physical examinations, vital sign measurements, 12-lead ECGs, chest X-rays and/or thoracic CT scans, and the determination of the presence of ASN-1 and ASN-2 (i.e., ASN100) ADAs.

STATISTICAL ANALYSES:

Continuous variables will be summarized by using the number of non-missing observations, arithmetic mean, standard deviation, median, minimum, and maximum values as descriptive statistics. Categorical variables will be summarized by using the frequency count and the percentage of subjects in each category as descriptive statistics.

All statistical tests will be performed at the 0.05 significance level using 2-sided tests, except where otherwise noted.

The primary efficacy endpoint is whether the subject has developed *S. aureus* pneumonia up to but not including Day 22. The summary measure for efficacy is the proportion of subjects in the MITT Population who develop *S. aureus* pneumonia up to but not including Day 22. Analysis for

the primary endpoint will compare the proportion of subjects who develop *S. aureus* pneumonia in the ASN100 arm versus the placebo arm in the MITT Population. The statistical test for comparing 2 event rates is based on a 2-sided test with a false positive rate of 5%.

Analyses of the primary efficacy endpoint will also be performed separately for the ITT and PP Populations.

Duration of mechanical ventilation and length of hospital ICU stay during the first 21 days post-randomization will be compared between treatment groups using a Wilcoxon rank sum test.

Other efficacy endpoints will be summarized by treatment group.

All subjects in the Safety Population will be included in the safety analyses and analyzed based on the actual treatment received.

One interim analysis will be conducted during the study, when approximately 125 subjects have reached Day 22. The interim analysis is for futility assessment of the study, and for a potential study sample size adjustment based on an overall blinded efficacy evaluation pooled across the treatment arms. Based on this assessment, the sample size may be increased. The interim analysis of futility assessment will be reviewed by an independent Data Review Committee (DRC).

Adverse events will be coded using the Medical Dictionary for Regulatory Activities. Treatment-emergent adverse events (TEAEs) will be summarized by system organ class and preferred term. A TEAE is defined as an adverse event with a start date and time on or after the first dose of study treatment. Listings will be provided for serious adverse events and adverse events leading to drug discontinuation. In addition, all adverse events will be listed.

Safety laboratory data and vital signs will be presented by each scheduled time point and for change from baseline to each time point. The results of all laboratory tests, physical examination findings, vital signs, and ADAs will be presented in data listings.

Descriptive statistics will be provided for all PK concentration data and PK parameters. All PK analyses will be performed using the PK Population. Subject PK profiles will be determined using parameters such as C_{max}, T_{max}, AUC, and $t_{1/2}$ for each of ASN-1 and ASN-2 in serum. Epithelial lining fluid PK will also be analyzed and an ELF/serum PK ratio will be calculated.

SAMPLE SIZE DETERMINATION:

The estimated incidence of progression to *S. aureus* pneumonia in mechanically ventilated subjects with heavy colonization is approximately 25% within 22 days of randomization.

Assuming a 2-sided significance level of 0.05 and a desired power of 80% to detect a significant difference between *S. aureus* pneumonia incidence rates of 25% in the placebo group and 12.5% in the ASN100 treatment group (50% reduction), 152 evaluable subjects are required in each treatment group. A sample size of 304 subjects (152 subjects per treatment group) will yield 80% power to detect a 50% reduction in the incidence of *S. aureus* pneumonia with ASN100 treatment when assuming a 25% incidence rate of *S. aureus* pneumonia in placebo-treated subjects. Assuming a 14% non-evaluable rate, approximately 354 subjects (177 subjects per treatment group) will be randomized. Assuming the screen failure rate is about 84%, approximately 2250 subjects will be screened.

SITES: Approximately 70 global sites.

SPONSOR:

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
ADA	Anti-drug antibody
ADCC	Antibody-dependent cell-mediated cytotoxicity
ALT	Alanine aminotransferase
ASN-1	Broadly cross-reactive anti-toxin monoclonal antibody that targets alpha hemolysin (Hla) and 3 F-components (HlgB, LukF, LukD) involved in forming 4 of the 5 bi-component leukocidins of *Staphylococcus aureus*
ASN-2	Anti-toxin monoclonal antibody that targets the fifth bi-component toxin of *Staphylococcus aureus*, LukGH
ASN100	A combination of 2 fully human monoclonal antibodies (ASN-1 and ASN-2)
AST	Aspartate aminotransferase
AUC	Area under the serum concentration-time curve
BAL	Bronchoalveolar lavage
CFR	Code of Federal Regulations
CFU	Colony forming units
CIP	Council of independent physicians
C_{max}	Maximum serum concentration
CRA	Clinical research associate
CRO	Contract research organization
CT	Computed tomography
CTA	Clinical trial authorization
DRC	Data Review Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic data capture
ELF	Epithelial lining fluid
ET	Early Termination
ETA	Endotracheal aspirate
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HABP	Hospital-acquired bacterial pneumonia
Hla	Alpha hemolysin
HlgAB	Gamma hemolysin AB
HlgB	Gamma hemolysin B
HlgCB	Gamma hemolysin CB
ICF	Informed Consent Form

Abbreviation	Definition
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICU	Intensive care unit
IEC	Independent Ethics Committee
IgG1	Immunoglobulin G1
IRB	Institutional Review Board
IRT	Interactive Response Technology
ITT	Intent-to-Treat
IV	Intravenous
LAR	Legally authorized representative
LukD	Leukocidin D
LukED	Leukocidin ED
LukF	Leukocidin F
LukGH	Leukocidin GH
LukSF	Leukocidin SF or Panton-Valentine Leukocidin
mAb	Monoclonal antibody
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MITT	Modified Intent-to-Treat
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
NIMP	Non-investigational medical product
PCT	Procalcitonin
PD	Pharmacodynamic
PK	Pharmacokinetic
PMN	Polymorphonuclear cell
PP	Per Protocol
PT	Prothrombin time
PTT	Partial thromboplastin time
PVL	Panton-Valentine Leukocidin or leukocidin SF
RBC	Red blood cell
S. aureus	*Staphylococcus aureus*
SAE	Serious adverse event
$t_{1/2}$	Terminal elimination half-life
TEAE	Treatment-emergent adverse event
T_{max}	Time to maximum serum concentration
VABP	Ventilator-associated bacterial pneumonia
WBC	White blood cell

1 INTRODUCTION AND BACKGROUND INFORMATION

1.1 Background

Staphylococcus aureus (*S. aureus*) is a human pathogenic bacterium that causes severe infections, including pneumonia and sepsis. Hospital-acquired bacterial pneumonia (HABP) caused by *S. aureus*, including ventilator-associated bacterial pneumonia (VABP) in mechanically ventilated patients, is a significant public health threat despite efforts to optimize antibiotic treatment.

The primary exotoxins of *S. aureus* are alpha hemolysin (Hla), which damages lung epithelial cells, facilitating pneumonia and systemic bacterial invasion, and a group of exotoxins known as bi-component, pore-forming leukocidins.[1,2] *Staphylococcus aureus* isolates are capable of producing up to 5 potent leukocidins: 2 gamma hemolysins (gamma hemolysin AB [HlgAB] and gamma hemolysin CB [HlgCB]), the Panton-Valentine Leukocidin (PVL or leukocidin SF [LukSF]), leukocidin ED (LukED), and leukocidin GH (LukGH). Alpha hemolysin, the gamma hemolysins, and LukGH genes are present in all clinical isolates; however, LukSF and LukED are found in approximately 5% to 10% and approximately 60% of strains, respectively. The most prevalent community-acquired methicillin-resistant *S. aureus* (MRSA) strain in the United States is the USA300 pulsotype, which possesses genes for all 5 of these potent leukocidins.

Alpha hemolysin and the bi-component leukocidins bind to various cell surface receptors and this leads to cell type specific cytotoxicity. The combined effect of these exotoxins in staphylococcal pathogenesis is multifold:

- Hla destroys the epithelial barrier by direct cytolysis;
- Leukocidins target phagocytic cells for lysis, reducing the capacity of immune cells to eliminate *S. aureus*; and
- All cytotoxins induce inflammatory responses that lead to further tissue damage. Inflammatory signals further enhance the susceptibility of human polymorphonuclear cells (PMNs) to lysis by LukGH rendering this toxin dominant among the *S. aureus* leukocidins *in vitro*.[3]

Counteracting these functions of staphylococcal toxins by means of monoclonal antibodies (mAbs) may contribute to or induce the prevention and/or treatment of *S. aureus* pneumonia.

ASN100 is a combination of 2 fully human mAbs (ASN-1 and ASN-2, both of the immunoglobulin G1 [IgG1] subclass) that target these exotoxins of *S. aureus*. ASN-1 is a broadly cross-reactive anti-toxin mAb that targets Hla and 3 F-components (gamma hemolysin B [HlgB], leukocidin F [LukF], and leukocidin D [LukD]) involved in forming 4 of the 5 bi-component leukocidins of *S. aureus*, while ASN-2 is directed against the fifth bi-component toxin of *S. aureus*, LukGH.[4,5,6]

The 2 antibodies (i.e., ASN-1 and ASN-2) are dosed in a 1:1 ratio and operate as follows:

- ASN-1 binds to and neutralizes Hla and 4 bi-component *S. aureus* cytotoxins (leukocidins): HlgAB, HlgCB, LukED, and LukSF (also known as PVL); and
- ASN-2 binds to and neutralizes the fifth *S. aureus* leukocidin, LukGH.

While ASN-1 and ASN-2 each provide partial protection of human phagocytic cells against a combination of staphylococcal exotoxins, the 2 mAbs act in synergy to prevent lysis of human phagocytic cells in the presence of Hla and all 5 bi-component leukocidins. By neutralizing Hla

and inactivating HlgAB, HlgCB, LukED, and LukSF, ASN-1 prevents the lysis of granulocytes (PMNs) caused by these leukocidins. ASN-1 demonstrated superior efficacy compared to Hla-neutralizing mAbs against *S. aureus* pneumonia in a rabbit *in vivo* model.[7] By neutralizing LukGH, ASN-2 protects PMNs, monocytes, and macrophages from lysis.

ASN100 (i.e., ASN-1 and ASN-2) has demonstrated potency and efficacy against a wide range of *S. aureus* strains in *in vitro* studies and *in vivo* models of *S. aureus* pneumonia.[4,5] Collectively, these studies support the synergy of ASN-1 and ASN-2 and the need for both mAbs in ASN100 to protect patients in the clinical setting against the full range of *S. aureus* strains (methicillin-susceptible *S. aureus* [MSSA] and MRSA).

1.2 Rationale

Published studies have demonstrated that microbiologic screening of mechanically ventilated patients to determine those who are heavily colonized with *S. aureus* increases the probability of correctly predicting those patients who will eventually develop *S. aureus* pneumonia.[8,9] Approximately one-third of heavily colonized mechanically ventilated patients progressed to *S. aureus* pneumonia and these same studies demonstrated that patients still develop *S. aureus* pneumonia despite treatment with potentially effective anti-staphylococcal antibiotics. Secondary analyses of these studies have shown that antibiotic treatment is ineffective in reducing *S. aureus* colonization in the lower airways and preventing ventilator-associated tracheobronchitis or VABP, and *S. aureus* seems to be in competition for colonization with the normal respiratory flora.[10]

Staphylococcus aureus, particularly MRSA, is frequently identified as the causative pathogen of pneumonia and the incidence of healthcare-related MRSA pneumonia among hospitalized patients is increasing.[11,12,13] According to the American Thoracic Society and the Infectious Diseases Society of America, MRSA is associated with >50% of intensive care unit (ICU) infections in the United States.[14]

Despite the availability of microbiologically appropriate antibiotics, mortality in patients with *S. aureus* pneumonia remains high.[15] No specific prevention strategies have proven effective against *S. aureus* pneumonia in mechanically ventilated patients. While general pneumonia prevention measures demonstrate efficacy in many cases, not all ICU types benefit from these efforts.[17] Although reported pneumonia rates have decreased over time, the remaining *S. aureus* cases are unlikely to be prevented by non-specific measures.[17,18]

Data from retrospective observational studies indicate that mechanically ventilated patients who have moderate to heavy colonization with *S. aureus* as determined by culture of endotracheal aspirate (ETA) specimens (i.e., ≥3+ by semi-quantitative culture) have a higher risk of developing pneumonia.[8,19] As noted above, many of these patients still ultimately develop *S. aureus* pneumonia despite treatment with potentially effective antibiotics.

The USA300 pulsotype is the most prevalent community-acquired MRSA strain in the United States and is known to possess all 5 leukocidins produced by *S. aureus*. When Hla and the bi-component leukocidins bind to various cell surface receptors, cell type specific cytotoxicity results. In addition, Hla expression level of colonizing *S. aureus* MSSA strains have been observed as a biomarker for progression to VABP in ventilated patients.[19]

Monoclonal antibodies may counteract these toxin activities and thereby offer benefit in the prevention of *S. aureus* pneumonia. Together, the components of ASN100 (i.e., ASN-1 and

ASN-2) have demonstrated potency and efficacy against a wide range of *S. aureus* strains in *in vitro* studies and *in vivo* models of *S. aureus* pneumonia, positioning ASN100 to be a needed therapy for the prevention of *S. aureus* pneumonia. The design of this Phase 2 prevention study with ASN100 is based on an assumption of a 50% reduction in the incidence of *S. aureus* pneumonia in these subjects, irrespective of the concomitant use of potentially effective antibiotics.

A series of pharmacokinetic (PK) and pharmacodynamic (PD) experiments was conducted using the rabbit *S. aureus* pneumonia model and measured PK in both healthy rabbits and rabbits with pneumonia caused by several *S. aureus* strains. The range of effective doses for preventing *S. aureus* pneumonia in animal models was 2.5 to 10 mg/kg of each component of ASN100. The 20 mg/kg dose (10 mg ASN-1 and 10 mg ASN-2) translated into a 1600 mg dose for an 80 kg subject.

ASN100 was well tolerated up to 8000 mg (i.e., 4000 mg ASN-1 and 4000 mg ASN-2) in a single ascending dose study conducted in healthy human volunteers. There were no reported related serious adverse events (SAEs) and 2 related treatment-emergent adverse events (TEAEs) of headache and fatigue were reported in this study. These data and data obtained from the nonclinical program determined that ASN100 3600 mg (i.e., 1800 mg ASN-1 and 1800 mg ASN-2) will be an appropriate dose to assess safety and efficacy in this first study in mechanically ventilated subjects who are heavily colonized with *S. aureus*.

The significant unmet need, the lack of existing therapies to prevent *S. aureus* pneumonia in high-risk patients, and the demonstrated *in vitro* potency and *in vivo* efficacy of ASN100 provide sufficient rationale for this Phase 2 study in the prevention of *S. aureus* pneumonia in high-risk, mechanically ventilated subjects.

1.3 Risk/Benefit

In mechanically ventilated subjects heavily colonized with *S. aureus*, ASN100 may prevent *S. aureus* pneumonia, a condition associated with significant morbidity and mortality and representing a significant unmet clinical need.

The ASN100 drug components ASN-1 and ASN-2 are fully human mAbs belonging to the IgG1 immunoglobulin subclass glycoproteins. They share a high degree of sequence homology with endogenous IgGs of the same subclass (approximately 90% to 95%) and are manufactured using standard mammalian cell expression and purification technologies (including 4 orthogonal viral clearance steps); therefore, they are expected to carry mammalian glycosylation pattern and to continue to be well tolerated in humans.

The target toxins recognized by ASN-1 and ASN-2 are bacterial, non-human, and are not expressed in human tissues. Lack of off-target reactivity is supported by the results of human tissue cross-reactivity studies involving a panel of 33 tissue types that demonstrated no cross-reactivity with human tissues. The antibody function of ASN100 is based solely on neutralization; complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) mechanisms in the mode of action are irrelevant. ASN-1 and ASN-2 cannot bind to toxins already bound to human tissues and therefore unwanted ADCC reactions toward human cells and tissues is unlikely.

A single ascending dose study conducted with ASN100 in doses up to 8000 mg (i.e., 4000 mg ASN-1 and 4000 mg ASN-2) demonstrated that ASN100 was well tolerated with

no reported related SAEs and 2 reported possibly related adverse events of headache and fatigue. No dose-limiting toxicity was observed up to the highest dose tested.

The potential immunogenicity of the antibodies and resulting immune complex formation following the production of anti-drug immunoglobulin M and IgG antibodies by the host immune system cannot be excluded; however, the risk for this outcome is considered to be low.

ASN100 belongs to the class of mAbs targeting foreign (bacterial), non-human antigens and is expected to yield a low risk of adverse events resulting from immunogenicity due to the single-dose administration used in this study.

Subjects participating in this study will be closely monitored following study drug administration via means of adverse event monitoring, clinical laboratory assessments, vital sign measurements, physical examinations, 12-lead electrocardiograms (ECGs), chest X-rays and/or thoracic computed tomography (CT) scans, and the determination of the presence of ASN-1 and/or ASN-2 anti-drug antibodies (ADAs). Additional safety assessments may be performed throughout the duration of this study if clinically indicated. Concomitant antibiotics are permitted per standard of care throughout the duration of this study.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to evaluate the safety, tolerability, and efficacy of a single dose of ASN100 (administered as ASN-1 and ASN-2 components) versus placebo for the prevention of *S. aureus* pneumonia in mechanically ventilated subjects who are heavily colonized with *S. aureus*.

2.2 Secondary Objectives

The secondary objectives of this study are:

- To compare the duration of mechanical ventilation post-treatment in subjects treated with ASN100 versus placebo;
- To compare the length of stay in the ICU post-treatment for subjects treated with ASN100 versus placebo;
- To determine the serum PK (i.e., maximum serum concentration [C_{max}], time to maximum serum concentration [T_{max}], area under the serum concentration-time curve [AUC], and terminal elimination half-life [$t_{1/2}$]) of ASN-1 and ASN-2 in mechanically ventilated subjects heavily colonized with *S. aureus*; and
- To compare 28-day all-cause mortality in subjects treated with ASN100 versus placebo.

2.3 Exploratory Objectives

The exploratory objectives are:

- To compare the incidence of HABP occurring >48 hours post-extubation up to but not including Day 22 in extubated subjects treated with ASN100 versus placebo;
- To compare the incidence of VABP up to but not including Day 22 in subjects treated with ASN100 versus placebo;
- To determine the immunogenicity of a single dose of ASN100;
- To compare the incidence of other *S. aureus*-associated infections which occur after dosing up to but not including Day 22 in subjects treated with ASN100 versus placebo; and
- To assess ASN-1 and ASN-2 levels in bronchoalveolar lavage (BAL) fluid and calculate a blood to epithelial lining fluid (ELF) ratio in those subjects participating in the BAL fluid PK sub-study.

3 STUDY DESCRIPTION

3.1 Definition of Heavy Colonization

For the purposes of this study, heavy colonization of *S. aureus* will be defined as a quantitative threshold of $\geq 10^5$ CFU/mL or 3+ to 4+ by semi-quantitative culture from an endotracheal aspirate.

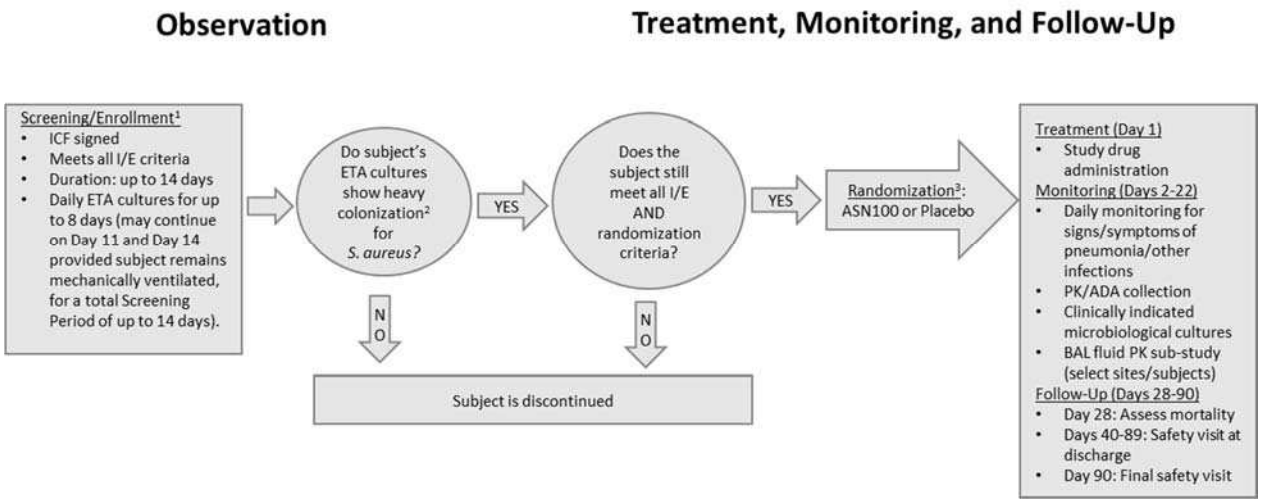
3.2 Summary of Study Design

This is a double-blind, randomized, single-dose, placebo-controlled study of ASN100 for the prevention of *S. aureus* pneumonia in mechanically ventilated subjects who are heavily colonized with *S. aureus*.

This will be a global study conducted at approximately 70 sites. Subjects will be screened for eligibility; once an Informed Consent Form (ICF) is signed (or, in countries where it is applicable, a decision is made by the council of independent physicians (CIP) [see Section 12.3]), and all entry criteria are met, a subject is considered to be enrolled in the study. All enrolled subjects will undergo an observational stage evaluating endotracheal colonization. Subjects who are randomized will undergo a treatment, monitoring, and follow-up period.

A schematic of the study is presented in Figure 1.

Figure 1. Study Schematic



1. Subjects do not need to be colonized to be enrolled.
2. For the purposes of this study, heavy colonization of *S. aureus* will be defined as a quantitative threshold of $\geq 10^5$ CFU/mL or 3+ to 4+ by semi-quantitative culture.
3. Once a subject has an ETA culture result signifying heavy growth of *S. aureus*, they must be randomized within 48 hours (or up to 60 hours with Medical Monitor approval) of sample collection or be discontinued from the study.

ADA = anti-drug antibody; BAL = bronchoalveolar lavage; ETA = endotracheal aspirate; ICF = Informed Consent Form; I/E = inclusion/exclusion; PK = pharmacokinetic; *S. aureus* = *Staphylococcus aureus*.

Approximately 2250 eligible subjects who meet all of the inclusion criteria and none of the exclusion criteria will be screened daily (while mechanically ventilated) for up to 8 days to identify those subjects who are heavily colonized with *S. aureus* (see Section 3.1).

Screening for heavy *S. aureus* colonization by ETA culture will continue on Day 11 and Day 14 provided the subject remains mechanically ventilated, for a total Screening Period of up to 14 days.

Additional ETA screening may occur on Days 9, 10, 12, and 13 at the discretion of the Investigator and providing the subject remains mechanically ventilated.

ETA specimens collected during Screening/Enrollment will be cultured by quantitative or semi-quantitative methods as required by the protocol. Sites are encouraged to perform semi-quantitative cultures using a chromogenic media that tests for both the presence of MSSA and MRSA (e.g., CHROMagar™ *Staph aureus*) for more rapid and specific detection of *S. aureus*. If the site is not able to obtain chromogenic media for *S. aureus*, appropriate chromogenic media may be supplied by the Sponsor.

Semi-quantitative cultures using chromogenic media are preferred for determination of eligibility for randomization; however, any quantitative or semi-quantitative culture performed that indicates that a subject is heavily colonized with *S. aureus* is permitted if the culture results are available to randomize the subject within 48 hours (or up to 60 hours with Medical Monitor approval) from the time the ETA sample was collected.

Once an enrolled subject has an ETA culture reported positive for heavy *S. aureus* colonization and meets all of the inclusion and none of the exclusion criteria, they are eligible for randomization. If they are not randomized on this occasion according to the protocol, they are no longer eligible for continued ETA sampling and randomization, and will be discontinued from the study; however, if subjects are extubated and subsequently re-intubated/mechanically ventilated, they are eligible for re-screening as a new subject enrollment. Subjects who were previously randomized are not eligible to be re-consented and re-screened.

At the time of randomization, if a subject's screening ETA culture also shows heavy colonization of a Gram-negative organism (i.e., heavy co-colonization of both Gram-positive and Gram-negative organisms), the subject will not be randomized into the study; however, randomization should not be delayed while waiting for Gram-negative results if heavy colonization with *S. aureus* has been confirmed.

Nasal swab specimens (one from each nostril) will be obtained at the Randomization Visit and will be cultured by the central microbiology laboratory. Results from the nasal swab specimen cultures will be used to assess correlation with results of the qualifying ETA culture.

Upon determination of eligibility, approximately 354 subjects will be randomized in a 1:1 ratio to 1 of 2 treatment groups:

- ASN100 administered either sequentially or simultaneously as 2 separate intravenous (IV) infusions of ASN-1 and ASN-2, or
- Placebo administered either sequentially or simultaneously as 2 separate IV infusions.

To ensure balance among the treatment groups, randomization for this study will be stratified by receipt or non-receipt of concomitant anti-staphylococcal antibiotics at the time of randomization that are potentially active against *S. aureus* pneumonia (see Section 5.2).

Following randomization on Day 1, subjects will receive either a single IV dose of 3600 mg of ASN100 (comprised of separate infusions each of 1800 mg ASN-1 and 1800 mg ASN-2) or matching placebos, per their assigned randomization scheme.

If, as part of routine standard of care, additional respiratory and/or other microbiological specimens are collected for culture during the study, results will be documented within the electronic Case Report Form (eCRF). As fully described within the study laboratory manual, bacterial isolates

recovered from these specimens that are deemed pathogens by the Investigator (if retained and available at the study site local microbiology laboratory) will be sent to a central microbiology laboratory for confirmation of pathogen identification (all isolates) and susceptibility testing (*S. aureus* isolates only).

Randomized subjects will be monitored daily for the signs and symptoms of pneumonia (see Section 7.4.2 and Table 1) and other *S. aureus*-associated infections while hospitalized up to Day 21.

All randomized subjects will undergo a study visit on Day 22 (+2 days). A follow-up visit will occur on Day 28 (+2 days) to assess mortality. This visit may be conducted via telephone if the subject is no longer hospitalized.

For subjects who remain hospitalized between Day 40 and Day 90 at the institution where they receive study treatment, a follow-up safety visit will be performed on the day of discharge.

All randomized subjects will return for a Safety Visit on Day 90 (±7 days) (if the subject is unable to return to the site, this visit may be conducted by telephone). Subjects who discontinue the study prior to Day 22 will undergo an Early Termination Visit. The end of the study will occur upon completion of the last Day 90 visit by the last subject.

Safety assessments for this study will include adverse event monitoring, clinical laboratory assessments (including chemistry, hematology, coagulation, and urinalysis), physical examinations, vital sign measurements, 12-lead ECGs, chest X-rays and/or thoracic CT scans, and the determination of the presence of ASN-1 and ASN-2 ADAs. Additional safety assessments may be performed throughout the duration of the study if clinically indicated.

Procalcitonin testing will be performed at the Randomization Visit (Day 1 pre-dose), on the day of diagnosis of pneumonia (if applicable), and on Day 22 or Early Termination (if applicable).

Blood samples will be obtained for the measurement of ASN-1 and ASN-2 in serum. Blood samples for PK analysis will be collected immediately following the completion of the second infusion (+15 minutes) and at 6 hours (± 4 hours) and 24 hours (±6 hours) post-dose following the second infusion. In addition, a PK sample will be obtained from all subjects on Days 4 (±1 day), 7 (±1 day), 14 (±2 days), and 22 (+2 days) of the Monitoring Period. Additional PK samples will be collected on the day of discharge (if discharge occurs between Day 40 and Day 90), on Day 90 (if hospitalized or if the subject is able to return to the clinic), or Early Termination (if applicable).

Select sites will be invited to participate in a BAL fluid PK sub-study. Approximately 25 to 35 subjects who volunteer will participate in this sub-study and will have BAL fluid collected 48 hours (±36 hours) post-dose (on approximately Day 2 or Day 3, depending on time of study drug administration) to determine ASN-1 and ASN-2 levels and blood to ELF ratio. Subjects participating in the BAL fluid PK sub-study will have a blood sample collected for PK measurement ±1 hour relative to BAL fluid collection (including scheduled BAL fluid collection or if BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any time post-randomization).

Blood samples obtained for ADA and PK analysis purposes will also be used to assess the amount of pre-existing anti-*S. aureus* toxin antibodies and the toxin neutralizing titer of the serum sample prior to and after randomization. Only antigen-specific antibody titers will be determined and no

genetic information will be extracted from these samples. Samples will be destroyed following the completion of all *S. aureus* anti-toxin antibody analyses.

3.3 Study Indication

The prevention of *S. aureus* pneumonia in mechanically ventilated subjects who are heavily colonized with *S. aureus*.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Inclusion Criteria

Subjects must meet all of the following criteria at the Screening and Randomization Visits in order to be eligible for randomization into the study:

1. Subject, legally authorized representative (LAR), or CIP (if applicable) has provided written informed consent;
2. Subject is ≥18 years of age at the time of enrollment;
3. Subject is currently hospitalized and is mechanically ventilated endotracheally (i.e., orotracheal or nasotracheal) and, in the Investigator's opinion, will require ongoing ventilator support for at least 48 hours;

   NOTE: Subjects with a tracheostomy at the time of enrollment are not eligible. After enrollment, conversion to tracheostomy is permitted per standard of care.
4. Female subjects must not be pregnant or lactating. Female subjects of childbearing potential must have a documented negative pregnancy test at the Screening Visit. Female subjects may be enrolled on the basis of a negative urine pregnancy test, pending the result of a negative serum pregnancy test prior to randomization; and
5. Female subjects of childbearing potential and non-surgically sterile male subjects who are sexually active must agree to use an approved highly effective form of contraception from the time of informed consent until 165 days post-dose. Approved forms of contraception include hormonal intrauterine devices, hormonal contraceptives (oral birth control pill, depo, patch, or injectable) together with supplementary double barrier methods such as condoms or diaphragms with spermicidal gel or foam.

   NOTE: The following categories define women who are NOT considered to be of childbearing potential:

   - Premenopausal female with 1 of the following:
     - Documented hysterectomy,
     - Documented bilateral salpingectomy, or
     - Documented bilateral oophorectomy, or
   - Postmenopausal female, defined as having amenorrhea for at least 12 months without an alternative medical cause.

4.2 Exclusion Criteria

Subjects who meet any of the following criteria at the Screening and/or Randomization Visit will be excluded from participation in the study:

1. Subject has received IV immunoglobulin therapy within 4 months prior to the Screening Visit;
2. Subject has a chest X-ray or thoracic CT scan that is definitive for a diagnosis of pneumonia;

3. Subject demonstrates both of the following:
   a. Need for acute changes in ventilator support to enhance oxygenation or to the amount of positive end-expiratory pressure; AND

      NOTE: Changes made in ventilator support due to day-to-day maintenance other than those changes made to improve oxygenation will not exclude a subject from participation in the study.

   b. New onset of purulent suctioned respiratory secretions;
4. Subject has a known and documented ETA culture showing heavy colonization with a Gram-negative organism at enrollment or at any time during the Screening period;

   NOTE: Randomization should not be delayed while waiting for Gram-negative results if heavy colonization with *S. aureus* has been confirmed.
5. Subject has been diagnosed with neutropenia (absolute neutrophil count <500/mm^3);
6. Subject has a severe non-pulmonary source of infection which, in the Investigator's opinion, would interfere with the conduct of the study or jeopardize the subject's safety;
7. Subject is currently on either continuous veno-venous hemodialysis or extracorporeal membrane oxygenation;
8. Subject has been previously exposed to ASN100 or ASN-1 or ASN-2, individually;
9. Subject has a known hypersensitivity to ASN100 or any of its excipients;
10. Subject has received any investigational product within 30 days prior to the Screening Visit (or 5 half-lives of the investigational product, whichever is longer);
11. Subject has, in the opinion of the Investigator, a high probability of death within 72 hours of enrollment;
12. Subject is, in the opinion of the Investigator, not able or willing to comply with the protocol;
13. Any condition that, in the opinion of the Investigator, would compromise the safety of the subject, the potential activity of the study drug, or the quality of the data; or
14. Subjects with a known history or current (suspected) diagnosis of cytokine release syndrome associated with the administration of peptides, proteins, and/or antibodies.[20]

    NOTE: Subjects with a prior history of sepsis are not excluded.

4.3 Randomization Criteria

Following the Screening Visit, eligible subjects who meet all of the inclusion criteria and none of the exclusion criteria will be screened daily (while mechanically ventilated) for up to 8 days via quantitative or semi-quantitative culture of an ETA to identify those subjects who are heavily colonized with *S. aureus* (see Section 3.1).

Screening for heavy *S. aureus* colonization by ETA culture will continue on Day 11 and Day 14 provided the subject remains mechanically ventilated, for a total Screening Period of up to 14 days. Additional ETA screening may occur on Days 9, 10, 12, and 13 at the discretion of the Investigator and providing the subject remains mechanically ventilated.

Once an enrolled subject has an ETA culture reported positive for heavy *S. aureus* colonization and meets all of the inclusion and none of the exclusion criteria, they are eligible for randomization. If they are not randomized on this occasion according to the protocol, they are no longer eligible for continued ETA sampling and randomization, and will be discontinued from the study; however, if subjects are extubated and subsequently re-intubated/mechanically ventilated, they are eligible for re-screening as a new subject enrollment. Subjects who were previously randomized are not eligible to be re-consented and re-screened.

At the time of randomization, if a subject's screening ETA culture also shows heavy colonization of a Gram-negative organism (i.e., heavy co-colonization of both Gram-positive and Gram-negative organisms), the subject will not be randomized into the study.

NOTE: Randomization should not be delayed while waiting for Gram-negative results if heavy colonization with *S. aureus* has been confirmed.

At the Randomization Visit, subjects must meet all of the following requirements to be randomized into the study:

1. Subject continues to meet all of the inclusion criteria and none of the exclusion criteria required at the Screening Visit;

   NOTE: Subjects who undergo tracheostomy after enrollment are still eligible for randomization, provided they remain mechanically ventilated (see criterion 3 below).

2. Subject is heavily colonized with *S. aureus* as determined by either quantitative or semi-quantitative culture of an ETA (see Section 3.1);

   NOTE: The results of the qualifying ETA culture must allow randomization to occur within 48 hours (or up to 60 hours with Medical Monitor approval) from the time the ETA sample was collected. Subjects with ETA culture results obtained after 60 hours showing heavy colonization of *S. aureus* may not be randomized and will be discontinued from the study.

3. Subject remains mechanically ventilated and, in the Investigator's opinion, will require ongoing ventilator support for at least 48 hours; and

4. Subject has, in the opinion of the Investigator, a probability of survival beyond 72 hours post-randomization.

4.4 Withdrawal Criteria

Participation of a subject in this clinical study may be discontinued for any of the following reasons:

- The subject or LAR withdraws consent from the study for any reason;
- The subject or LAR requests discontinuation from the study for any reason;
- Occurrence of any medical condition or circumstance that exposes the subject to substantial risk and/or does not allow the subject to adhere to the requirements of the protocol;
- Any SAE, clinically significant adverse event, severe laboratory abnormality, intercurrent illness, or other medical condition which indicates to the Investigator that continued participation is not in the best interest of the subject;
- Pregnancy;

- Requirement of prohibited concomitant medication;
- Subject failure to comply with protocol requirements or study-related procedures; or
- Termination of the study by the Sponsor or the regulatory authority.

If a subject withdraws prematurely from the study due to the above criteria (except in the case of withdrawal of consent) or any other reason, study staff should make every effort to complete the full panel of assessments scheduled for the Early Termination Visit. The reason for subject withdrawal must be documented in the eCRF.

In the case of subjects lost to follow-up, attempts to contact the subject must be made and documented in the subject's medical records.

Randomized subjects who are withdrawn will not be replaced.

5 STUDY TREATMENTS

5.1 Treatment Groups

Following Screening/Enrollment and upon determination that a subject is heavily colonized with *S. aureus* as determined by a positive quantitative or semi-quantitative culture of an ETA (see Section 3.1), eligible subjects will be randomized in a 1:1 ratio to 1 of 2 treatment groups:

- ASN100 administered either sequentially or simultaneously as 2 separate IV infusions of ASN-1 and ASN-2, or
- Placebo administered either sequentially or simultaneously as 2 separate IV infusions.

Whether ASN-1, ASN-2, or placebo are administered sequentially or simultaneously will be determined by operational considerations of IV access at the time of dosing. If 2 IV lines are available for simultaneous administration this is preferable; however, if IV access or other considerations support sequential administration, this is allowed provided that both infusions are completed within 3 hours.

Following randomization on Day 1, subjects will receive either a single IV dose of 3600 mg of ASN100 (comprised of separate infusions each of 1800 mg ASN-1 and 1800 mg ASN-2) or matching placebos, per their assigned randomization scheme.

5.2 Stratification

To ensure balance among the treatment groups, randomization for this study will be stratified by receipt or non-receipt of concomitant anti-staphylococcal antibiotics at the time of randomization that are potentially active against *S. aureus* pneumonia, including, but not limited to, nafcillin, oxacillin, vancomycin, linezolid, telavancin, ceftaroline, ceftobiprole, and teicoplanin.

5.3 Rationale for Dosing

A series of PK and PD experiments was conducted using the rabbit *S. aureus* pneumonia model and measured PK in both healthy rabbits and rabbits with pneumonia caused by several *S. aureus* strains. The range of effective doses for preventing *S. aureus* pneumonia in animal models was 2.5 to 10 mg/kg of each component of ASN100. The 20 mg/kg dose (10 mg ASN-1 and 10 mg ASN-2) translated into a 1600 mg dose for an 80 kg subject.

ASN100 was well tolerated up to 8000 mg (i.e., 4000 mg ASN-1 and 4000 mg ASN-2) in a single-ascending dose study conducted in healthy human volunteers. There were no reported related SAEs and 2 related TEAEs of headache and fatigue were reported in this study. These data and data obtained from the nonclinical program determined that ASN100 3600 mg (i.e., 1800 mg ASN-1 and 1800 mg ASN-2) will be an appropriate dose to assess safety and efficacy in this first study in mechanically ventilated subjects who are heavily colonized with *S. aureus*.

This study did not identify a maximum tolerated dose. Based on these observations from the single ascending dose study, as well as data obtained from the nonclinical program conducted to date, it is reasonable to select a dose of ASN100 of 3600 mg (i.e., 1800 mg ASN-1 and 1800 mg ASN-2) as an appropriate dose to assess safety and efficacy in this first study in mechanically ventilated subjects who are heavily colonized with *S. aureus*.

5.4 Randomization and Blinding

Randomization will occur at the Randomization Visit following determination of the subject's eligibility. Subjects will be randomized via a centralized Interactive Response Technology (IRT) system in a 1:1 ratio to receive a single dose (2 infusions) of either ASN100 or matching placebo.

The study follows a double-blind, placebo-controlled design. All study personnel, including investigators, site personnel, site pharmacist, Sponsor and contract research organization (CRO) staff involved in the conduct of the study (e.g., clinical research associate [CRA]/monitor), and subjects will be blinded to treatment assignment. ASN100 and placebo will be identical in appearance to preserve blinding.

5.5 Breaking the Blind

Randomization data will be kept strictly confidential until the time of unblinding and will not be accessible by subjects, Investigators, or anyone performing assessments and having access to study data until unblinding occurs. Individual subject unblinding will only occur in the case of subject emergencies, with complete unblinding at the conclusion of the study.

Emergency breaking of the assigned treatment code should only be undertaken when it is essential that knowledge of the treatment assignment is necessary to treat the subject's emergency safely and effectively. Emergency treatment code breaks will be performed using the IRT. When the Investigator contacts the system to break a treatment code for a subject, he/she must provide the requested subject's identifying information and confirm the necessity to break the treatment code for the subject. The Investigator will then receive details of the investigational drug treatment for the specified subject and a fax or email confirming this information. The system will automatically inform the Sponsor and Medpace study personnel that the code has been broken.

5.6 Drug Supplies

5.6.1 Formulation and Packaging

ASN100 is a combination product with 2 separate human mAb components of the IgG1 isotype. The specific mAbs are formulated separately and called ASN-1 and ASN-2.

ASN-1 is a broadly cross-reactive anti-toxin mAb that targets Hla and 3 F-components (HlgB, LukF, and LukD) involved in forming 4 of the 5 bi-component leukocidins of *S. aureus*.

ASN-2 is directed against the fifth bi-component toxin of *S. aureus*, LukGH.

Each component is supplied as a sterile, colorless, clear liquid solution formulated ready for injection.

ASN-1 and ASN-2 will be supplied in separate 10 mL vials as a sterile solution for infusion with an extractable volume of 10 mL and a nominal mAb concentration of 20 mg/mL. Each vial will contain 200 mg of ASN-1 or 200 mg of ASN-2. Matched vials of ASN-1 placebo and ASN-2 placebo will also be provided.

Additional details regarding formulation and packaging will be provided in the Pharmacy Manual and the Investigator's Brochure.

5.6.2 Study Drug Preparation and Dispensing

Blinded study drug and reference preparations will be delivered to the study site. The site pharmacist will prepare the study drug for administration by extracting all volume from each set of 9 vials and inserting into an empty IV bag for each set, using aseptic technique according to the Pharmacy Manual. These will be delivered to the study site research staff in 2 blinded, ready-to-use infusion bags.

Additional details regarding study drug preparation and dispensing will be provided in the Pharmacy Manual.

5.6.3 Study Drug Administration

Subjects will receive ASN100 or matching placebo via 2 IV infusions over a duration of approximately 50 to 60 minutes per infusion.

Both ASN100 and placebo will be administered as 2 separate infusions (either sequentially or simultaneously). The last infusion will be completed within 5 hours from initiation of drug preparation in the pharmacy.

Infusions delivered simultaneously must be administered via distinct IV lines (either peripheral or central lines; in the instance that a multi-port central line is used, infusions will be delivered through separate ports) such that the infusions do not mix before entering the blood stream.

In the occasion that the 2 infusion bags cannot be hung simultaneously, the blinded IV bags will indicate which bag should be infused first and which bag should be infused second. If administered sequentially, no more than a 3-hour window should elapse from the time of initiation of the first infusion to the completion of the second infusion.

ASN100 will be administered as separate infusions of 1800 mg ASN-1 and 1800 mg ASN-2 (9 vials of each to be pooled aseptically according to the Pharmacy Manual into separate infusion bags) delivered either sequentially within a 3-hour window from the time of initiation of the first infusion to the completion of the second infusion, or simultaneously.

Placebo will be administered as separate infusions of 9 vials of ASN-1 placebo and 9 vials of ASN-2 placebo (vials to be pooled aseptically according to the Pharmacy Manual into separate infusion bags) delivered either sequentially within a 3-hour window from the time of initiation of the first infusion to the completion of the second infusion, or simultaneously.

The infusion catheter and tubing will be flushed with sterile saline for injection immediately before and following completion of the infusion.

Either infusion may be discontinued if deemed medically necessary at any time.

If the first infusion is stopped at any point due to the concern of an adverse event, the second infusion will not be administered.

Refer to the Pharmacy Manual for additional details regarding study drug administration.

5.6.4 Treatment Compliance

Study drug will be administered at the clinical site by blinded study personnel. The date and time of administration, including start and completion times of each infusion, will be accurately logged in the source documentation. Due to the single dose design of this study, treatment compliance is anticipated to be high.

5.6.5 Storage and Accountability

Study drug (ASN-1, ASN-2, and matching placebos) will be stored at 2°C to 8°C, will be protected from light, and will be kept in a secure area with access limited to authorized personnel. The primary packaging (vials) is to be kept within the secondary packaging (carton box).

Investigational site personnel (e.g., study pharmacist) will perform an ongoing inventory of study drug products. At the end of the study, a full reconciliation of drug inventory will be performed and the results of the inventory will be recorded in the drug accountability log. Any unused vials should be returned for destruction.

Additional details regarding study drug storage and accountability will be provided in the Pharmacy Manual.

5.7 Prior and Concomitant Medications and/or Procedures

5.7.1 Excluded Medications and/or Procedures

The use of any investigational product within 30 days prior to the Screening Visit (or 5 half-lives of the investigational product, whichever is longer) is prohibited. Any immunoglobulin preparation for any medical indication is prohibited through follow-up Day 90 of the study.

Prior use of ASN100, ASN-1, or ASN-2 is prohibited.

5.7.2 Documentation of Prior and Concomitant Medication Use

All prior medications received by the subject within 14 days prior to study drug administration and any concomitant medications used throughout the duration of the study will be recorded in the source documents and on the appropriate eCRF. The medication name, route of administration, dose, frequency, indication, and duration of treatment (start and stop dates) will be recorded.

6 STUDY PROCEDURES

6.1 Informed Consent

Written informed consent will be obtained from all subjects prior to any study-specific procedures being performed. A separate consent will be obtained from those subjects who participate in the BAL fluid PK sub-study.

See Section 12.3 for additional details regarding informed consent.

6.2 Screening/Enrollment (Up to Day -14)

Screening/Enrollment may be performed up to 14 days prior to randomization. Subjects who meet all of the inclusion criteria and none of the exclusion criteria will be screened daily (while mechanically ventilated) for up to 8 days to identify those subjects who are heavily colonized with *S. aureus* (see Section 3.1).

Screening for heavy *S. aureus* colonization by ETA culture will continue on Day 11 and Day 14 provided the subject remains mechanically ventilated, for a total Screening Period of up to 14 days. Additional ETA screening may occur on Days 9, 10, 12, and 13 at the discretion of the Investigator and providing the subject remains mechanically ventilated.

Once an enrolled subject has an ETA culture reported positive for heavy *S. aureus* colonization and meets all of the inclusion and none of the exclusion criteria, they are eligible for randomization. If they are not randomized on this occasion according to the protocol, they are no longer eligible for continued ETA sampling and randomization, and will be discontinued from the study; however, if subjects are extubated and subsequently re-intubated/mechanically ventilated, they are eligible for re-screening as a new subject enrollment. Subjects who were previously randomized are not eligible to be re-consented and re-screened.

The following procedures will be performed during Screening/Enrollment:

- Obtain informed consent;
- Review inclusion and exclusion criteria;
- Record medical history;
- Record demographics;
- Record antibiotic and other medication history;
- Obtain height and body weight measurements;
- Perform complete physical examination;
- Obtain vital sign measurements, including oxygenation status;
- Perform pregnancy test for female subjects of childbearing potential only. Female subjects may be enrolled on the basis of a negative urine pregnancy test, pending the result of a negative serum pregnancy test prior to randomization;
- Assess ventilator device status;
- Perform chest X-ray or thoracic CT scan. A chest X-ray or thoracic CT scan obtained as part of standard of care within 24 hours prior to the initial Screening Visit is sufficient provided

there are no changes in the subject's clinical status (e.g., no change in ventilation status, secretions, or signs/symptoms that may be suggestive of pneumonia) within the 24 hours following imaging;

- Obtain ETA for culture;
- Assess for other *S. aureus*-associated infections; and
- Perform adverse event assessment (adverse events that occur between the time the subject signs the ICF and the Randomization Visit will only be recorded if related to a study-specific procedure).

6.3 Randomization Visit (Day 1 Pre-Dose)

The Randomization Visit will occur pre-dose on Day 1. At the time of randomization, if a subject's screening ETA culture also shows heavy colonization of a Gram-negative organism (i.e., heavy co-colonization of both Gram-positive and Gram-negative organisms), the subject will not be randomized into the study; however, randomization should not be delayed while waiting for Gram-negative results if heavy colonization with *S. aureus* has been confirmed.

The following procedures will be performed at the Randomization Visit:

- Review inclusion, exclusion, and randomization criteria;
- Review and record medical history (if changes occurred since the Screening Visit);
- Review and record antibiotic and other medication history (if changes occurred since the Screening Visit);
- Perform limited physical examination (to include, at a minimum, a pulmonary examination [including auscultation]);
- Obtain vital sign measurements, including oxygenation status;
- Perform clinical laboratory assessments, including chemistry, hematology, coagulation, and urinalysis;
- Obtain a blood sample for procalcitonin testing;
- Assess ventilator device status;
- Perform chest X-ray or thoracic CT scan. A chest X-ray or thoracic CT scan obtained within 24 hours of the Randomization Visit is sufficient, provided there are no changes in the subject's clinical status (e.g., no change in ventilation status, secretions, or signs/symptoms that may be suggestive of pneumonia) since the time of imaging;
- Assess for clinical signs/symptoms of pneumonia (Section 7.4.2; Table 1);
- Assess for other *S. aureus*-associated infections;
- Obtain nasal swab specimens for culture (one from each nostril);
- Randomize to study treatment;
- Obtain a blood sample for ADA and assessment of pre-existing anti-*S. aureus* toxin antibodies;
- Perform 12-lead ECG;

- Record concomitant medications; and
- Perform adverse event assessment.

6.4 Treatment Period (Day 1)

The following procedures will be performed on Day 1 following administration of study drug:

- Obtain vital sign measurements, including oxygenation status;
- Assess ventilator device status;
- Assess for clinical signs/symptoms of pneumonia (Section 7.4.2; Table 1);
- Assess for other *S. aureus*-associated infections;
- Administer study drug;
- Obtain a blood sample for PK measurement and assessment of anti-*S. aureus* toxin antibodies (to be collected immediately following the completion of the second infusion [+15 minutes] and at 6 hours [±4 hours] and 24 hours [±6 hours] post-dose);
- Track days on ventilator from randomization;
- Track days in ICU (or other observation area) from randomization;
- Record concomitant medications; and
- Perform adverse event assessment.

6.5 Monitoring Period

6.5.1 Day 2/Day 3 – Bronchoalveolar Lavage Fluid Pharmacokinetic Sub-Study

The following procedures will be performed 48 hours (±36 hours) post-dose (Day 2 or Day 3 depending on the time of study drug administration) for those subjects participating in the BAL fluid PK sub-study only or if BAL fluid is collected as part of a standard of care procedure for any subject at any other time post-randomization for any other reason:

- Obtain vital sign measurements, including oxygenation status;
- Assess ventilator device status;
- Perform chest X-ray or thoracic CT scan;
- Assess for clinical signs/symptoms of pneumonia (Section 7.4.2; Table 1);
- Assess for other *S. aureus*-associated infections;
- Obtain BAL fluid to determine ASN-1 and ASN-2 concentrations (48 hours [±36 hours] post-dose) for those subjects participating in the BAL fluid PK sub-study (if BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any other time post-randomization for any other reason, if possible, a sample for PK analysis will also be obtained);

- Obtain a blood sample for PK measurement ±1 hour relative to BAL fluid collection (including scheduled BAL fluid collection or if BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any time post-randomization);
- Track days on ventilator from randomization;
- Track days in ICU (or other observation area) from randomization;
- Record concomitant medications; and
- Perform adverse event assessment.

6.5.2 Day 2 to Day 21

The following procedures will be performed daily (unless otherwise indicated) from Day 2 to Day 21 while the subject is hospitalized. For subjects participating in the BAL fluid PK sub-study, procedures noted for the Day 2 visit only need to be performed 1 time:

- Perform limited physical examination (to include, at a minimum, a pulmonary examination [including auscultation]);
- Obtain vital sign measurements, including oxygenation status;
- Perform clinical laboratory assessments, including chemistry, hematology, coagulation, and urinalysis. Blood samples for chemistry, hematology, and coagulation will be obtained on Days 2 (+1 day), 4 (±1 day), and 6 (±1 day) of the Monitoring Period, and twice weekly during Week 2 (Day 8 through Day 14) and/or Week 3 (Day 15 through Day 21) of the Monitoring Period while the subject remains hospitalized. Urine for urinalysis will be obtained on Day 6 (±1 day) of the Monitoring Period only;
- Obtain a blood sample for procalcitonin testing (on day of diagnosis of pneumonia only, if applicable);
- Assess ventilator device status (if applicable);
- Perform chest X-ray or thoracic CT scan (required during the Monitoring Period for those subjects presenting with signs and symptoms of a respiratory infection such as dyspnea, tachypnea, fever, or cough);
- Obtain adequate respiratory specimen for culture (if clinically indicated);
- Assess for clinical signs/symptoms of pneumonia (Section 7.4.2; Table 1);
- Assess for other *S. aureus*-associated infections;
- Obtain a blood sample for ADAs (to be obtained only upon discharge from the ICU, if discharge occurs prior to Day 22);
- Obtain a blood sample for PK measurement and assessment of anti-*S. aureus* toxin antibodies (Days 4 [±1 day], 7 [±1 day], and 14 [±2 days] only);
- Track days on ventilator from randomization;
- Track days in ICU (or other observation area) from randomization;

- Record concomitant medications; and
- Perform adverse event assessment.

6.5.3 Day 22

The following procedures will occur on Day 22 (+2 days):

- Perform limited physical examination (if subject is hospitalized) (to include, at a minimum, a pulmonary examination [including auscultation]);
- Obtain vital sign measurements, including oxygenation status;
- Perform pregnancy test for female subjects of childbearing potential only (additional pregnancy testing may occur throughout the duration of the study, per applicable country requirements);
- Perform clinical laboratory assessments including chemistry, hematology, coagulation, and urinalysis;
- Obtain a blood sample for procalcitonin testing;
- Assess ventilator device status (if applicable);
- Perform chest X-ray or thoracic CT scan (required during the Monitoring Period for those subjects presenting with signs and symptoms of a respiratory infection such as dyspnea, tachypnea, fever, or cough);
- Obtain adequate respiratory specimen for culture (if clinically indicated);
- Assess for clinical signs/symptoms of pneumonia (Section 7.4.2; Table 1);
- Assess for other *S. aureus*-associated infections;
- Obtain a blood sample for ADAs;
- Perform 12-lead ECG;
- Assess for survival and discharge disposition;
- Obtain a blood sample for PK measurement and assessment of anti-*S. aureus* toxin antibodies;
- Track days on ventilator from randomization;
- Track days in ICU (or other observation area) from randomization;
- Record concomitant medications; and
- Perform adverse event assessment.

6.6 Follow-Up Period

6.6.1 Day 28 (+2 Days) Follow-Up

The following procedures will occur during a follow-up telephone call or visit, if the subject remains hospitalized, on Day 28 (+2 days):

- Assess ventilator device status (if applicable); and
- Assess for survival and discharge disposition.

6.6.2 Day 40 to Day 89 (Follow-Up Safety Visit)

For subjects who remain hospitalized between Day 40 and Day 90 at the institution where they receive study treatment, a follow-up safety visit will be performed on the day of discharge.

The following procedures will occur on the day of hospital discharge for subjects who are discharged between Day 40 and Day 90:

- Perform limited physical examination (to include, at a minimum, a pulmonary examination [including auscultation]);
- Obtain vital sign measurements, including oxygenation status;
- Assess ventilator device status (if applicable);
- Obtain a blood sample for ADAs;
- Perform 12-lead ECG;
- Assess for survival and discharge disposition;
- Obtain a blood sample for PK measurement and assessment of anti-*S. aureus* toxin antibodies;
- Track days on ventilator from randomization (if applicable);
- Track days in ICU (or other observation area) from randomization;
- Record concomitant medications; and
- Perform adverse event assessment.

6.6.3 Safety Visit (Day 90 [±7 Days])

The following procedures will be performed at the Safety Visit on Day 90 (±7 days). If the subject is unable to return to the site, this visit may be conducted by telephone, with procedures performed as appropriate (i.e., concomitant medications and adverse events collected):

- Perform limited physical examination (to include, at a minimum, a pulmonary examination [including auscultation]);
- Obtain vital sign measurements, including oxygenation status;
- Assess ventilator device status (if applicable);
- Obtain a blood sample for ADAs;
- Perform 12-lead ECG;

- Assess for survival and discharge disposition;
- Obtain a blood sample for PK measurement and assessment of anti-*S. aureus* toxin antibodies;
- Track days on ventilator from randomization;
- Track days in ICU (or other observation area) from randomization;
- Record concomitant medications; and
- Perform adverse event assessment.

6.7 Early Termination Visit and Withdrawal Procedures

For subjects who are withdrawn from the study prior to Day 22, the following procedures will be performed at an Early Termination Visit:

- Perform limited physical examination (to include, at a minimum, a pulmonary examination [including auscultation]);
- Obtain vital sign measurements, including oxygenation status;
- Perform pregnancy test for female subjects of childbearing potential only;
- Perform clinical laboratory assessments including chemistry, hematology, coagulation, and urinalysis (if clinically indicated);
- Obtain a blood sample for procalcitonin testing;
- Assess ventilator device status (if applicable);
- Obtain adequate respiratory specimen for culture (if clinically indicated);
- Assess for clinical signs/symptoms of pneumonia (Section 7.4.2; Table 1);
- Assess for other *S. aureus*-associated infections;
- Obtain a blood sample for ADAs;
- Perform 12-lead ECG;
- Assess for survival and discharge disposition;
- Obtain a blood sample for PK measurement and assessment of anti-*S. aureus* toxin antibodies;
- Track days on ventilator from randomization;
- Track days in ICU (or other observation area) from randomization;
- Record concomitant medications; and
- Perform adverse event assessment.

7 EFFICACY ASSESSMENTS

7.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of subjects in the MITT Population who develop *S. aureus* pneumonia as defined in Section 7.4 up to but not including Day 22.

7.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints include:

- Duration of mechanical ventilation during the first 21 days post-randomization for subjects in the MITT, Intent-to-Treat (ITT), and Per Protocol (PP) Populations;
- Length of ICU stay during the first 21 days post-randomization for subjects in the MITT, ITT, and PP Populations;
- The C_{max}, T_{max}, AUC, and $t_{1/2}$ of ASN-1 and ASN-2 in serum following a single dose of ASN100 in the PK Population; and
- 28-day all-cause mortality in the MITT, ITT, and PP Populations.

7.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints include:

- Proportion of subjects in the MITT and PP Populations with a diagnosis of HABP at >48 hours post-extubation up to but not including Day 22 in extubated subjects;
- Proportion of subjects in the MITT and PP Populations with development of VABP up to but not including Day 22;
- Incidence of all bacterial pneumonias in the MITT, ITT, and PP Populations;
- Incidence of other non-*S. aureus* pneumonias up to but not including Day 22 in the MITT, ITT, and PP Populations; and
- Incidence of other *S. aureus* infections acquired following study drug administration up to but not including Day 22 in the MITT, ITT, and PP Populations.

7.4 Microbiological and Clinical Assessments

7.4.1 Microbiological Assessment

ETA specimens will be collected for culture during Screening/Enrollment to determine the degree of *S. aureus* colonization. Additional respiratory specimens may be collected for culture throughout the Monitoring Period or Early Termination (if applicable) if clinically indicated according to standard of care.

If, as part of routine standard of care, additional respiratory and/or other microbiological specimens are collected for culture during the study, results will be documented within the eCRF. As fully described within the study lab manual, bacterial isolates recovered from these specimens that are deemed pathogens by the Investigator (if retained and available at the study site local microbiology laboratory) will be sent to a central microbiology laboratory for confirmation of pathogen identification (all isolates) and susceptibility testing (*S. aureus* isolates only).

During the Monitoring Period, a diagnosis of *S. aureus* pneumonia will be determined if subjects have *S. aureus* identified from an adequate respiratory specimen (Section 7.4.1) and clinical signs and symptoms of pneumonia. For a diagnosis of *S. aureus* pneumonia, clinical signs and symptoms must occur ±2 days from the time of collection of an adequate respiratory specimen culture that is positive for *S. aureus* (Section 7.4.2).

- For intubated subjects, a respiratory specimen obtained by BAL, non-bronchoscopic BAL, or protected specimen brush will be considered an adequate respiratory specimen; or
- For extubated subjects, an adequate expectorated/induced sputum specimen is defined as <10 squamous epithelial cells and >25 PMNs/100× field; and
- For both intubated and extubated subjects, adequate respiratory specimens will undergo either semi-quantitative or quantitative culture in accordance with the local laboratory's standard procedure.

7.4.2 Assessment of Clinical Signs and Symptoms

Subjects will be assessed daily throughout the Monitoring Period for the following clinical signs and symptoms presented in Table 1.

Table 1. Clinical Signs and Symptoms

Mechanically Ventilated Subjects	Non-Mechanically Ventilated Subjects	
• Cough, • Rales, • Dullness on percussion,* • Bronchial breath sounds, • Egophony,* • Need for suctioning, • Need for ventilator support, and • Fever ≥38°C (≥100.4°F).	• Cough, • Rales, • Dullness on percussion,* • Bronchial breath sounds, • Egophony,*	• Dyspnea, • Tachypnea, • Respiratory rate, • Hypoxemia, and • Fever ≥38°C (≥100.4°F).
*If routinely performed.		

A diagnosis of pneumonia (HABP or VABP) will be determined as defined in Table 2.

Table 2. Definition of Hospital-Acquired and Ventilator-Associated Bacterial Pneumonia

Indication	Definition
HABP	• A chest X-ray showing new or progressive infiltrates suggestive of pneumonia (as assessed by the Investigator); • At least ONE of the following: o Has been hospitalized for >48 hours, or o Developed clinical signs and symptoms within 7 days following hospital discharge; • At least ONE of the following: o New onset or worsening pulmonary signs/symptoms such as cough, dyspnea, tachypnea (e.g., respiratory rate >25 breaths per minute), expectorated sputum production, or the requirement for mechanical ventilation (if subject is not already ventilated); o Need for acute changes in ventilator support to enhance oxygenation or to the amount of PEEP; or o New onset of suctioned respiratory secretions; AND • At least ONE of the following clinical signs/symptoms: o Temperature >38°C or <35°C, o WBC count ≥10,000 cell/mm^3 or ≤4500 cell/mm^3, or o >15% immature neutrophils (bands) on peripheral blood smear.
VABP	• A chest X-ray showing new or progressive infiltrates suggestive of pneumonia (as assessed by the Investigator); • Subject has received mechanical ventilation via an endotracheal or nasotracheal tube for ≥48 hours; • At least ONE of the following: o New onset or worsening pulmonary signs/symptoms such as cough, dyspnea, tachypnea (e.g., respiratory rate >25 breaths per minute), expectorated sputum production, or the requirement for mechanical ventilation (if subject is not already ventilated); o Need for acute changes in ventilator support to enhance oxygenation or to the amount of PEEP; or o New onset of suctioned respiratory secretions; AND • At least ONE of the following clinical signs/symptoms: o Temperature >38°C or <35°C, o WBC count ≥10,000 cell/mm^3 or ≤4500 cell/mm^3, or o >15% immature neutrophils (bands) on peripheral blood smear.

HABP = hospital-acquired bacterial pneumonia; PEEP = positive end-expiratory pressure; VABP = ventilator-associated bacterial pneumonia; WBC = white blood cell.

8 PHARMACOKINETIC ASSESSMENTS

8.1 Pharmacokinetic Endpoint

The primary PK endpoints are the C_{max}, T_{max}, AUC, and $t_{1/2}$ of ASN-1 and ASN-2 in serum following a single IV dose of ASN100 in the PK Population.

The levels of ASN-1 and ASN-2 will be measured in BAL fluid in a PK sub-study which will allow the calculation of a blood to ELF ratio.

8.2 Pharmacokinetic Assessments

Samples for PK analysis will be obtained to determine the PK of ASN-1 and ASN-2 in serum and BAL fluid (for subjects participating in the BAL fluid PK sub-study only). Instructions for the collection, handling, and storage of biological samples will be provided in a separate Laboratory Manual.

In order to maintain the study blind, all subjects, including those randomized to receive placebo, will have PK samples obtained at the specified time points. The actual date and time of each sample will be recorded.

Drug concentration information that may unblind the study will not be reported to investigative sites or blinded Sponsor or CRO personnel until the study has been unblinded.

8.2.1 Serum Pharmacokinetic Sampling

Blood samples will be obtained for the measurement of ASN-1 and ASN-2 in serum. At the completion of the second infusion, blood samples for PK analysis will be collected immediately following the completion of the second infusion (+15 minutes) and at 6 hours (±4 hours) and 24 hours (±6 hours) post-dose.

In addition, a PK sample will be obtained from all subjects on Days 4 (±1 day), 7 (±1 day), 14 (±2 days), and 22 (+2 days) of the Monitoring Period. Additional PK samples will be collected on the day of discharge (if discharge occurs between Day 40 and Day 90), on Day 90 (if hospitalized or if the subject is able to return to the clinic), or Early Termination (if applicable).

The following serum PK parameters will be assessed:

- Maximum serum concentration (C_{max}),
- Time to maximum serum concentration (T_{max}),
- Area under the serum concentration-time curve (AUC), and
- Terminal elimination half-life ($t_{1/2}$).

Subjects participating in the BAL fluid PK sub-study will have a blood sample collected for PK measurement ±1 hour relative to BAL fluid collection (including scheduled BAL fluid collection or if BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any time post-randomization).

8.2.2 Bronchoalveolar Lavage Fluid Pharmacokinetic Sampling

Select sites will be invited to participate in a BAL fluid PK sub-study. Approximately 25 to 35 subjects who volunteer will participate in this sub-study and will have BAL fluid collected 48 hours (±36 hours) post-dose (on approximately Day 2 or Day 3, depending on time of study drug administration) to determine ASN-1 and ASN-2 levels and blood to ELF ratio. If BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any other time post-randomization for any other reason, if possible, a sample for PK analysis will also be obtained.

8.2.3 Analysis of Anti-*Staphylococcus aureus* Toxin Antibody Titers

Blood samples obtained for ADA and PK analysis purposes will also be used to assess the amount of pre-existing anti-*S. aureus* toxin antibodies and the toxin neutralizing titer of the serum sample prior to and after randomization. Only antigen-specific antibody titers will be determined and no genetic information will be extracted from these samples. Samples will be destroyed following the completion of all *S. aureus* anti-toxin antibody analyses.

9 SAFETY ASSESSMENTS

9.1 Adverse Events

An adverse event is defined as any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and/or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product. All adverse events, including observed or volunteered problems, complaints, or symptoms, are to be recorded on the appropriate eCRF.

Adverse events that occur between the time the subject signs the ICF and the Randomization Visit will only be recorded if related to a study-specific procedure. Beginning with randomization all adverse events, which include clinical laboratory test and physical examination variables, will be monitored and documented until study participation is complete. Subjects should be instructed to report any adverse event that they experience to the Investigator. Site personnel will ensure each adverse event is recorded on the appropriate adverse event eCRF.

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the Investigator and recorded on the eCRF. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the Investigator, it should be recorded as a separate adverse event on the eCRF. Additionally, the condition that led to a medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, or transfusion) should be recorded as an adverse event, not the procedure.

Any medical condition already present at the time of randomization should not be reported as an adverse event unless the medical condition or signs or symptoms present at baseline change in severity or seriousness at any time during the study. In this case, it should be reported as an adverse event.

Clinically significant abnormal laboratory or other examination (e.g., ECG) findings that are detected during the study or are present at the time of randomization and significantly worsen during the study should be reported as adverse events. The Investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Clinically significant abnormal laboratory values occurring during the clinical study will be followed until repeat tests return to normal, stabilize, or are no longer clinically significant. Any abnormal test that is determined to be an error does not require reporting as an adverse event.

Lack of efficacy (i.e., development of pneumonia as defined in Section 7.4) will be captured as an efficacy measure and in general will not be considered an adverse event; however, new onset of specific pulmonary symptoms that do not meet the study-specific definition of pneumonia or worsening of baseline signs and symptoms should be captured as adverse events. Events that meet the criteria for an SAE (Section 9.2) should follow the procedures for SAE reporting as detailed in Section 9.3.

9.1.1 Adverse (Drug) Reaction

All noxious and unintended responses to a medicinal product related to any dose should be considered an adverse drug reaction. "Responses" to a medicinal product mean that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

9.1.2 Unexpected Adverse Drug Reaction

An Unexpected Adverse Drug Reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

The reference safety information for ASN100 is included in the current version of the Investigator's Brochure. The reference safety information will be reviewed yearly and the periodicity of the review will be harmonized with the reporting period of the Development Safety Update Report.

9.1.3 Assessment of Adverse Events by the Investigator

The Investigator will assess the severity (intensity) of each adverse event as mild, moderate, or severe, and will also categorize each adverse event as to its potential relationship to study drug using the categories of yes or no.

Assessment of Severity:

Mild – An event that is easily tolerated and generally not interfering with normal daily activities.

Moderate – An event that is sufficiently discomforting to interfere with normal daily activities.

Severe – An event that is incapacitating with inability to work or perform normal daily activities.

Causality Assessment:

The relationship of an adverse event to the administration of the study drug is to be assessed according to the following definitions:

No (unrelated, not related, no relation) – The time course between the administration of study drug and the occurrence or worsening of the adverse event rules out a causal relationship and another cause (concomitant drugs, therapies, complications, etc.) is suspected.

Yes (related) – The time course between the administration of study drug and the occurrence or worsening of the adverse event is consistent with a causal relationship and no other cause (concomitant drugs, therapies, complications, etc.) can be identified.

The definition implies a reasonable possibility of a causal relationship between the event and the study drug. This means that there are facts (evidence) or arguments to suggest a causal relationship.

The following factors should also be considered:

- The temporal sequence from study drug administration:
  - The event should occur after the study drug is given. The length of time from study drug exposure to event should be evaluated in the clinical context of the event;
- Underlying, concomitant, intercurrent diseases:
  - Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have;
- Concomitant drugs:
  - The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them might be recognized to cause the event in question;
- Known response pattern for this class of study drug:
  - Clinical and/or preclinical data may indicate whether a particular response is likely to be a class effect;
- Exposure to physical and/or mental stresses:
  - The exposure to stress might induce adverse changes in the recipient and provide a logical and better explanation for the event; and
- The pharmacology and PK of the study drug:
  - The known pharmacologic properties (absorption, distribution, metabolism, and excretion) of the study drug should be considered.

9.2 Serious Adverse Events

An adverse event or adverse reaction is considered serious if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- A life-threatening adverse event;
  - NOTE: An adverse event or adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an event that, had it occurred in a more severe form, might have caused death.
- Requires hospitalization or prolongation of existing hospitalizations;
  - NOTE: Any hospital admission with at least 1 overnight stay will be considered an inpatient hospitalization. An emergency room visit without hospital admission will not be recorded as a SAE under this criterion, nor will hospitalization for a procedure scheduled or planned before signing of informed consent. However, unexpected complications and/or prolongation of hospitalization that occur during elective surgery should be recorded as adverse events and assessed for seriousness. Admission to the hospital for social or

situational reasons (i.e., no place to stay, live too far away to come for hospital visits) will not be considered inpatient hospitalizations.

- A persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions;
- A congenital anomaly/birth defect; or
- An important medical event.
    - NOTE: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalizations, or the development of drug dependency.

9.3 Serious Adverse Event Reporting − Procedures for Investigators

Initial Reports

Serious adverse events related to a study-specific procedure that occur between the Screening Visit and the Randomization Visit, and all SAEs occurring from the time of randomization until the Day 90 Safety Visit, must be reported to Medpace Clinical Safety within 24 hours of the knowledge of the occurrence (this refers to any adverse event that meets any of the aforementioned serious criteria). All SAEs that the Investigator considers related to study drug occurring after the Day 90 Safety Visit must be reported to the Sponsor.

To report the SAE, complete the SAE form electronically in the electronic data capture (EDC) system for the study. When the form is completed, Medpace Safety personnel will be notified electronically and will retrieve the form.

If the event meets serious criteria and it is not possible to access the EDC system, send an email to Medpace Safety at Medpace-safetynotification@medpace.com or call the Medpace SAE hotline (phone number listed below), and fax the completed paper SAE form to Medpace (fax number listed below) within 24 hours of awareness. When the EDC system becomes available, the SAE information must be entered within 24 hours of the system becoming available.

Safety Contact Information: Medpace Clinical Safety
Medpace SAE hotline – USA:
Telephone: +1-800-730-5779, Dial 3 or +1-513-579-9911, Dial 3
Facsimile: +1-866-336-5320 or +1-513-579-0444
Email: medpace-safetynotification@medpace.com

Medpace SAE hotline – Europe:
Telephone: +49-89-89-55-718-44
Facsimile: +49-89-89-55-718-104
Email: medpace-safetynotification@medpace.com

Follow-Up Reports

The Investigator must continue to follow the subject until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the subject dies.

Within 24 hours of receipt of follow-up information, the Investigator must update the SAE form electronically in the EDC system for the study and submit any supporting documentation (e.g., subject discharge summary or autopsy reports) to Medpace Clinical Safety via fax or email. If it is not possible to access the EDC system, refer to the procedures outlined above for initial reporting of SAEs.

9.4 Pregnancy Reporting

If the subject or partner of a subject participating in the study becomes pregnant from the time of randomization until the Day 90 Safety Visit, the Investigator should report the pregnancy to Medpace Clinical Safety within 24 hours of being notified. Medpace Clinical Safety will then forward the Exposure In Utero form to the Investigator for completion.

The subject or partner should be followed by the Investigator until completion of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the Investigator should notify Medpace Clinical Safety. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for reporting an SAE.

9.5 Expedited Reporting

The Sponsor will report all relevant information about suspected unexpected serious adverse reactions that are fatal or life-threatening as soon as possible to the FDA, applicable competent authorities in all the Member States concerned, and to the Central Ethics Committee, and in any case no later than 7 days after knowledge by the Sponsor of such a case, and that relevant follow-up information will subsequently be communicated within an additional 8 days.

All other suspected unexpected serious adverse reactions will be reported to the FDA, applicable competent authorities concerned, and to the Central Ethics Committee concerned as soon as possible but within a maximum of 15 days of first knowledge by the Sponsor.

The Sponsor will also inform all Investigators as required.

Expedited reporting of suspected unexpected serious adverse reactions related to non-investigational medical products (NIMPs) used in this study (and any other NIMPs) will not be necessary. Listings of cases related to NIMPs will be included in the Development Safety Update Report.

9.6 Clinical Laboratory Evaluations

Standard clinical laboratory profiles for safety assessments (including chemistry, hematology, coagulation, and urinalysis) will be collected and evaluated at the Randomization Visit (Day 1 pre-dose).

Blood samples for chemistry, hematology, and coagulation will be obtained on Days 2 (+1 day), 4 (±1 day), and 6 (±1 day) of the Monitoring Period, or Early Termination (if applicable and if

clinically indicated). During Week 2 (Day 8 through Day 14) and/or Week 3 (Day 15 through Day 21) of the Monitoring Period, clinical laboratory assessments of chemistry, hematology, and coagulation will be performed twice a week while the subject remains hospitalized.

Urine for urinalysis will be obtained at the Randomization Visit (Day 1 pre-dose), on Day 6 (±1 day) of the Monitoring Period, or Early Termination (if applicable and if clinically indicated).

Procalcitonin testing will be performed at the Randomization Visit (Day 1 pre-dose), on the day of diagnosis of pneumonia (if applicable), and on Day 22 or Early Termination (if applicable).

Study required laboratory samples will be sent to the central laboratory for analysis. Subject care should be directed by local laboratory testing.

See Appendix B for a list of clinical laboratory analytes to be assessed.

9.7 Radiographs and Imaging

A chest X-ray or thoracic CT scan is required on 4 occasions during the study as defined below for (1) initial Screening Visit, (2) Randomization Visit, (3) Post-BAL sampling for those subjects participating in the optional BAL sub-study, and (4) Monitoring Period (Day 2 through Day 22) for those subjects presenting with signs/symptoms suggestive of a respiratory infection.

- Initial Screening Visit: A chest X-ray or thoracic CT scan will be obtained at the initial Screening Visit to ensure that the subject does not have pneumonia. A chest X-ray or thoracic CT scan obtained as part of standard of care within 24 hours of the initial Screening Visit is sufficient provided there are no changes in the subject's clinical status (e.g., no change in ventilation status, secretions, or signs/symptoms that may be suggestive of pneumonia) within the 24 hours since the imaging procedure.
- Randomization Visit: A chest X-ray or thoracic CT scan will be obtained at the Randomization Visit to ensure that the subject does not have pneumonia. A chest X-ray or thoracic CT scan obtained as part of standard of care within 24 hours of the Randomization Visit is sufficient provided there are no changes in the subject's clinical status (e.g., no change in ventilation status, secretions, or signs/symptoms that may be suggestive of pneumonia) since the time of imaging.
- BAL sub-study: A chest X-ray or thoracic CT scan will be obtained on Day 2/Day 3 post-BAL sampling for all subjects participating in the optional BAL fluid PK sub-study.
- Monitoring Period (Day 2 through Day 22): A chest X-ray or thoracic CT scan is required during the Monitoring Period for those subjects presenting with signs and symptoms of a respiratory infection such as dyspnea, tachypnea, fever, or cough.

Imaging should be conducted per institutional guidelines and assessed by the Investigator. The results of radiographs or imaging obtained at the Screening Visit and any subsequent visits should be documented on the appropriate eCRF. Radiograph or imaging reports and/or films will be collected for future analysis and will be captured within the EDC system.

9.8 Vital Signs

Vital sign measurements will be obtained at the Screening Visit, the Randomization Visit (Day 1 pre-dose), on Day 1 following administration of study drug, daily during the Monitoring Period (if subject is hospitalized), on Day 22, on the day of hospital discharge (for subjects who are

discharged between Day 40 and Day 90), at the Day 90 Safety Visit (if the visit occurs onsite), or Early Termination (if applicable).

Vital sign measurements will include temperature, systolic and diastolic blood pressure, pulse, respiratory rate, and oxygenation status (as measured by pulse oximetry or arterial blood gas). Vital sign measurements will be recorded on the appropriate eCRF.

Blood pressure and pulse will be assessed via an automated device. Manual techniques are only to be used if an automated device is not available.

Oxygenation status will be determined at the Screening and Randomization Visits via measurement of arterial blood gas, provided the subject has an arterial line placed. If no arterial line is present, pulse oximetry may be used. Oxygenation status may be determined at subsequent visits by either measurement of arterial blood gas or pulse oximetry, if no arterial line is present.

9.9 Electrocardiograms

A 12-lead ECG will be performed for all eligible subjects at the Randomization Visit (Day 1 pre-dose), on Day 22, on the day of hospital discharge (for subjects who are discharged between Day 40 and Day 90), at the Day 90 Safety Visit (if the visit occurs onsite), or Early Termination (if applicable).

9.10 Physical Examinations

A complete physical examination will be performed at the Screening Visit and as deemed necessary by the Investigator and will include, at a minimum, a pulmonary examination (including auscultation) and assessments of the skin, abdomen, cardiovascular, gastrointestinal, and neurological systems. Body weight and height will also be measured.

A limited physical examination will be performed at the Randomization Visit, daily during the Monitoring Period, on Day 22, on the day of hospital discharge (for subjects who are discharged between Day 40 and Day 90), at the Day 90 Safety Visit (if the visit occurs onsite), or Early Termination (if applicable). The limited physical examination will include, at a minimum, a pulmonary examination (including auscultation).

9.11 Immunogenicity

Samples to evaluate ADA response including neutralizing antibodies to ASN-1 and ASN-2 will be collected from all subjects at the Randomization Visit (Day 1 pre-dose), upon discharge from the ICU (if discharge occurs prior to Day 22), on Day 22, on the day of hospital discharge (for subjects who are discharged between Day 40 and Day 90), and at the Day 90 Safety Visit (if the visit occurs onsite).

Subjects who discontinue early or are withdrawn from the study should also have samples collected as part of their Early Termination Visit. These samples will be sent to the central laboratory for analysis.

Samples will be screened for antibodies binding to ASN-1 and ASN-2 and the titer of confirmed positive samples will be reported. Other analyses may be performed to verify the stability of antibodies to ASN-1 or ASN-2 and/or to further characterize the immunogenicity of ASN-1 and ASN-2.

The impact of ADAs on safety, efficacy, and PK will be addressed in the Statistical Analysis Plan.

10 STATISTICS

10.1 Analysis Populations

Intent-to-Treat (ITT) Population

The ITT Population includes all subjects who are randomized to receive study drug.

Modified Intent-to-Treat (MITT) Population

The MITT Population includes all subjects in the ITT Population who receive study drug who are heavily colonized with *S. aureus* as determined by quantitative or semi-quantitative culture of an ETA.

Per Protocol (PP) Population

The PP Population includes all subjects in the MITT Population who also meet the following criteria:

- Do not have any major protocol violations that would affect assessment of efficacy, and
- Complete an adequate number of Monitoring Period assessments through and including Day 22.

Pharmacokinetic (PK) Population

The PK Population includes all subjects in the MITT Population with at least 1 serum PK sample collected post-dose.

Safety Population

The Safety Population includes all subjects who receive any amount of study drug and have at least 1 post-treatment safety assessment.

10.2 Statistical Methods

All statistical analyses will be provided in the Statistical Analysis Plan. Continuous variables will be summarized by using the number of non-missing observations, arithmetic mean, standard deviation, median, minimum, and maximum values as descriptive statistics. Categorical variables will be summarized by using the frequency count and the percentage of subjects in each category as descriptive statistics.

All statistical tests will be performed at the 0.05 significance level using 2-sided tests, except where otherwise noted. All confidence intervals will have a confidence level of 95%.

10.2.1 Disposition

The number of subjects randomized, treated, completed, and discontinued early from the study and the reasons for discontinuation will be summarized descriptively. In addition, reasons leading to study discontinuation will be summarized for each treatment group. The number and percentage of subjects for the randomized subjects included in each analysis population will also be presented.

10.2.2 Demographic and Baseline Characteristics

Summary statistics will be provided by treatment group for demographics (e.g., age, gender, race, and ethnicity) and for baseline characteristics.

10.2.3 Analysis of Efficacy

The primary efficacy endpoint is whether the subject has developed *S. aureus* pneumonia up to but not including Day 22. The summary measure for efficacy is the proportion of subjects in the MITT Population who develop *S. aureus* pneumonia up to but not including Day 22. Analysis for the primary endpoint will compare the proportion of subjects who develop *S. aureus* pneumonia in the ASN100 arm versus the placebo arm in the MITT Population. The statistical test for comparing 2 event rates is based on a 2-sided test with a false positive rate of 5%.

Analyses of the primary efficacy endpoint will also be performed separately for the ITT and PP Populations.

Duration of mechanical ventilation and length of hospital ICU stay during the first 21 days post-randomization will be compared between treatment groups using a Wilcoxon rank sum test.

Other efficacy endpoints will be summarized by treatment group.

10.2.4 Analysis of Safety

All subjects in the Safety Population will be included in the safety analyses and analyzed based on the actual treatment received.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Treatment-emergent adverse events will be summarized by system organ class and preferred term. A TEAE is defined as an adverse event with a start date and time on or after the first dose of study treatment. Listings will be provided for SAEs and adverse events leading to drug discontinuation. In addition, all adverse events will be listed.

Safety laboratory data and vital signs will be presented by each scheduled time point and for change from baseline to each time point. The results of all laboratory tests, physical examination findings, vital signs, and ADAs will be presented in data listings.

10.2.5 Analysis of Pharmacokinetics

Descriptive statistics will be provided for all PK concentration data and PK parameters. All PK analyses will be performed using the PK Population. Subject PK profiles will be determined using parameters such as C_{max}, T_{max}, AUC, and $t_{1/2}$ for each of ASN-1 and ASN-2 in serum. Epithelial lining fluid PK will also be analyzed and an ELF/serum PK ratio will be calculated.

A description of the PK parameter analysis will be included in the Statistical Analysis Plan.

10.2.6 Sample Size Determination

The estimated incidence of progression to *S. aureus* pneumonia in mechanically ventilated subjects with heavy colonization is approximately 25% within 22 days of randomization.

Assuming a 2-sided significance level of 0.05 and a desired power of 80% to detect a significant difference between *S. aureus* pneumonia incidence rates of 25% in the placebo group and 12.5% in the ASN100 treatment group (50% reduction), 152 evaluable subjects are required in each treatment group. A sample size of 304 subjects (152 subjects per treatment group) will yield 80% power to detect a 50% reduction in the incidence of *S. aureus* pneumonia with ASN100 treatment when assuming a 25% incidence rate of *S. aureus* pneumonia in placebo-treated subjects. Assuming a 14% non-evaluable rate, approximately 354 subjects (177 subjects per treatment

group) will be randomized. Assuming the screen failure rate is about 84%, approximately 2250 subjects will be screened.

10.2.7 Interim Analysis

One interim analysis will be conducted during the study, when approximately 125 subjects have reached Day 22. The interim analysis is for futility assessment of the study, and for a potential study sample size adjustment based on an overall blinded efficacy evaluation pooled across the treatment arms. Based on this assessment, the sample size may be increased. The interim analysis of futility assessment will be reviewed by an independent Data Review Committee (DRC).

11 DATA MANAGEMENT AND RECORD KEEPING

11.1 Data Management

11.1.1 Data Handling

Data will be recorded at the site on eCRFs and reviewed by the CRA during monitoring visits. The CRAs will verify data recorded in the EDC system with source documents. All corrections or changes made to any study data must be appropriately tracked in an audit trail in the EDC system. An eCRF will be considered complete when all missing, incorrect, and/or inconsistent data has been accounted for.

11.1.2 Computer Systems

Data will be processed using a validated computer system conforming to regulatory requirements.

11.1.3 Data Entry

Data must be recorded using the EDC system as the study is in progress. All site personnel with a role designated for data entry must log into the system using their secure user name and password in order to enter, review, or correct study data. These procedures must comply with Title 21 of the Code of Federal Regulations (21 CFR Part 11) and other appropriate international regulations. All passwords will be strictly confidential.

11.1.4 Medical Information Coding

For medical information, the following thesauri will be used:

- MedDRA for adverse events, and
- World Health Organization Drug Dictionary for prior and concomitant medications.

11.1.5 Data Validation

Validation checks programmed within the EDC system, as well as supplemental validation performed via review of the downloaded data, will be applied to the data in order to ensure accurate, consistent, and reliable data. Data identified as erroneous, or data that are missing, will be referred to the investigative site for resolution through data queries.

The eCRFs must be reviewed and electronically signed by the Investigator.

11.2 Record Keeping

Records of subjects, source documents, monitoring visit logs, eCRFs, inventory of study product, regulatory documents, and other Sponsor correspondence pertaining to the study must be kept in the appropriate study files at the site. Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the evaluation and reconstruction of the clinical study. Source data are contained in source documents (original records or certified copies). These records will be retained in a secure file for the period as set forth in the Clinical Study Agreement. Prior to transfer or destruction of these records, the Sponsor must be notified in writing and be given the opportunity to further store such records.

12 INVESTIGATOR REQUIREMENTS AND QUALITY CONTROL

12.1 Ethical Conduct of the Study

Good Clinical Practice (GCP) is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical study data are credible.

12.2 Institutional Review Board/Independent Ethics Committee

The IRB/IEC will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of subjects. The study will only be conducted at sites where IRB/IEC approval has been obtained. The protocol, Investigator's Brochure, ICF, advertisements (if applicable), written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC by the Investigator.

Federal regulations and ICH require that approval be obtained from an IRB/IEC prior to participation of subjects in research studies. Prior to study onset, the protocol, any protocol amendments, ICFs, advertisements to be used for subject recruitment, and any other written information regarding this study to be provided to a subject or subject's legal guardian must be approved by the IRB/IEC. Where applicable, the requirements of the CIP, as well as the decision-making process regarding inclusion of subjects into the study, must be approved by the IEC.

No drug will be released to the site for dosing until written IRB/IEC authorization has been received by the Sponsor.

It is the responsibility of the Sponsor or CRO to obtain the approval of the responsible ethics committees according to the national regulations.

The study will only start at the respective sites once the respective committee's written approval has been given.

12.3 Informed Consent

The ICF, the ICF for the BAL fluid PK sub-study, and any changes to the ICFs made during the course of the study must be agreed to by the Sponsor or designee and the IRB/IEC prior to use and must be in compliance with all ICH GCP, local regulatory requirements, and legal requirements.

The Investigator must ensure that each study subject is fully informed about the nature and objectives of the study and possible risks associated with participation and must ensure that the subject has been informed of his/her rights to privacy. The Investigator will obtain written informed consent from each subject or the subject's legal representative (if applicable) before any study-specific activity is performed and should document in the source documentation that consent was obtained prior to enrollment in the study. In the case that the subject is not fully conscious, a shortened version of informed consent will be signed by the subject and a complete version of informed consent will be signed, dependent on the country-specific requirements, by the subject's legal representative or close person (e.g., husband/wife, child, or sibling), or by the CIP (if applicable), by an impartial witness, and by the Investigator prior to any study-specific procedure

being performed. A subject who is not fully conscious will be required to sign the complete version of informed consent once fully conscious. The original signed copy of the ICF must be maintained by the Investigator and is subject to inspection by a representative of the Sponsor, their representatives, auditors, the IRB/IEC, and/or regulatory agencies. A copy of the signed ICF will be given to the subject.

In countries only where it is applicable, for subjects who are unable to read and sign either version of the ICF (i.e., shortened or complete version), decisions regarding the subject's enrollment will be made by the CIP. The decision of the CIP to include the subject in the study must be documented in the minutes of the CIP meeting, signed by the members of the CIP, and the decision must be entered in the medical records of the subject. The minutes of the CIP meeting should contain the names of all physicians included in the composition of the CIP, the subject's diagnosis, the subject's condition at the time the decision was made, the interpretation of clinical, laboratory, instrumental, and other data, and the decision of the CIP. The documents supporting the CIP decision must be maintained by the Investigator.

12.4 Study Monitoring Requirements

It is the responsibility of the Investigator to ensure that the study is conducted in accordance with the protocol, ICH GCP, Directive 2001/20/EC, applicable regulatory requirements, and the Declaration of Helsinki (Seoul 2008), and that valid data are entered into the eCRFs.

To achieve this objective, the monitor's duties are to aid the Investigator and, at the same time, the Sponsor in the maintenance of complete, legible, well-organized, and easily retrievable data. Before the enrollment of any subject in this study, the Sponsor or their designee will review with the Investigator and site personnel the following documents: protocol, Investigator's Brochure, eCRFs and procedures for their completion, informed consent process, and the procedure for reporting SAEs.

The Investigator will permit the Sponsor or their designee to monitor the study as frequently as deemed necessary to determine that data recording and protocol adherence are satisfactory. During the monitoring visits, information recorded on the eCRFs will be verified against source documents and requests for clarification or correction may be made. After the eCRF data is entered by the site, the CRA will review the data for safety information, completeness, accuracy, and logical consistency. Computer programs that identify data inconsistencies may be used to help monitor the clinical study. If necessary, requests for clarification or correction will be sent to investigators. The Investigator and his/her staff will be expected to cooperate with the monitor and provide any missing information, whenever possible.

All monitoring activities will be reported and archived. In addition, monitoring visits will be documented at the investigational site by signature and date on the study-specific monitoring log.

12.5 Protocol Deviations

Deviations from the protocol are not permitted. If a protocol deviation occurs, the CRA will ensure the deviation is appropriately documented. Substantial deviations will be compiled into a listing for inclusion in the final clinical study report and minor deviations will be recorded in the source documents at the site. Investigators must comply with all applicable IRB/IEC and local requirements in the reporting of protocol deviations.

12.6 Disclosure of Data

Data generated by this study must be available for inspection by the FDA, the Sponsor or their designee, applicable national or regional health authorities, and the IRB/IEC as appropriate. Subjects or their legal representatives may request their medical information be given to their personal physician or other appropriate medical personnel responsible for their welfare.

Subject medical information obtained during the study is confidential and disclosure to third parties other than those noted above is prohibited.

12.7 Retention of Records

To enable evaluations and/or audits from regulatory authorities or the Sponsor, the Investigator will keep records, including the identity of all participating subjects (sufficient information to link records, e.g., CRFs and hospital records), all original signed ICFs, copies of all eCRFs, SAE forms, source documents, and detailed records of treatment disposition. The records should be retained by the Investigator according to specifications in the ICH guidelines, local regulations, or as specified in the Clinical Study Agreement, whichever is longer. The Investigator must obtain written permission from the Sponsor before disposing of any records, even if retention requirements have been met.

If the Investigator relocates, retires, or for any reason withdraws from the study, the Sponsor should be prospectively notified. The study records must be transferred to an acceptable designee, such as another Investigator, another institution, or to the Sponsor.

12.8 Publication Policy

Following completion of the study, the data may be considered for publication in a scientific journal or for reporting at a scientific meeting. Each Investigator is obligated to keep data pertaining to the study confidential. The Investigator must consult with the Sponsor before any study data are submitted for publication. The Sponsor reserves the right to deny publication rights until mutual agreement on the content, format, interpretation of data in the manuscript, and journal selected for publication are achieved. However, the Investigator will be afforded the right to publish their individual results after the end of the trial if no agreement on the publication can be achieved within 2 years of database lock.

12.9 Financial Disclosure

Investigators are required to provide financial disclosure information to the Sponsor to permit the Sponsor to fulfill its obligations under 21 CFR §54. In addition, Investigators must commit to promptly updating this information if any relevant changes occur during the study and for a period of 1 year after the completion of the study.

12.10 Insurance and Indemnity

In accordance with the relevant national regulations, the Sponsor has taken out subject liability insurance for all subjects who have given their consent to the clinical study. This cover is designed for the event that a fatality, physical injury, or damage to health occurs during the clinical study's execution.

12.11 Legal Aspects

The clinical study is submitted to the relevant national competent authorities in all participating countries to achieve a clinical trial authorization (CTA).

The study will commence (i.e., initiation of study centers) when the CTA and favorable Ethics opinion have been received from those bodies governing those study centers.

13 STUDY ADMINISTRATIVE INFORMATION

13.1 Protocol Amendments

Any amendments to the study protocol will be communicated to the Investigators by Medpace or the Sponsor. All protocol amendments will undergo the same review and approval process as the original protocol. A protocol amendment may be implemented after it has been approved by the IRB/IEC, unless immediate implementation of the change is necessary for subject safety. In this case, the situation must be documented and reported to the IRB/IEC within 5 working days.

13.2 Address List

13.2.1 Sponsor

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13.2.3 Drug Safety

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APPENDIX A: SCHEDULE OF PROCEDURES

Study Period	Screening/ Enrollment	Randomization	Treatment	Monitoring Period			Follow-Up Period			ET
Study Day Study Procedures	Up to Day -14[a]	Day 1 pre-dose	Day 1	Day 2/3[b] (BAL PK sub-study)	Day 2 to Day 21[c]	Day 22 (+2 days)	Day 28 (+2 days)[d]	Day 40 to Day 89[e]	Day 90 (±7 days) Safety Visit[f]	Unsched[g]
Informed consent	X									
Inclusion/exclusion/ randomization criteria	X[h,i]	X[j]								
Medical history	X[h]	X[k]								
Demographics	X[h]									
Antibiotic/medication history[l]	X	X								
Height/weight	X[h]									
Physical examination[m]	X[h]	X			X	X		X	X	X
Vital signs[n]	X[h]	X	X	X	X	X		X	X	X
Pregnancy test[o]	X[h]					X				X
Clinical laboratory assessments (chemistry, hematology, and coagulation)		X			X[p]	X				X[q]
Urinalysis		X			X[r]	X				X[q]
Procalcitonin (analysis performed at central lab)		X			X[s]	X				X
Ventilator device status	X	X	X	X	X[t]	X[t]	X[t]	X[t]	X[t]	X[t]
Chest X-ray/thoracic CT scan	X[u]	X[v]		X	X[w]	X[w]				
Nasal swab specimens for culture		X								
ETA for culture[x]	X									
Collection/culture of additional microbiological specimens[y]					X	X				X
Clinical assessment of signs/symptoms of pneumonia		X	X	X	X	X				X
Other *S. aureus* infection assessment	X	X	X	X	X	X				X

Footnotes begin on the next page.

APPENDIX A: SCHEDULE OF PROCEDURES (Continued)

Study Period	Screening/ Enrollment	Randomization	Treatment	Monitoring Period			Follow-Up Period			ET
Study Day **Study Procedures**	**Up to Day -14[a]**	**Day 1 pre-dose**	**Day 1**	**Day 2/3[b] (BAL PK sub-study)**	**Day 2 to Day 21[c]**	**Day 22 (+2 days)**	**Day 28 (+2 days)[d]**	**Day 40 to Day 89[e]**	**Day 90 (±7 days) Safety Visit[f]**	**Unsched[g]**
Randomization		X								
Blood collection for ADA and anti-*S. aureus* toxin antibodies		X			X[z]	X		X	X	X
12-lead ECG		X				X		X	X	X
Assess survival and discharge disposition						X	X	X	X	X
Study drug administration[aa]			X							
BAL for PK[bb]				X						
Blood collection for PK sampling and anti-*S. aureus* toxin antibodies			X[cc]	X[dd]	X[ee]	X[ee]		X[ee]	X	X
Track days on ventilator from randomization			X	X	X	X		X	X	X
Track days in ICU (or other observation area)			X	X	X	X		X	X	X
Concomitant medications[l]		X	X	X	X	X		X	X	X
Adverse event assessment	X[ff]	X	X	X	X	X		X	X	X

a. Eligible subjects will be screened daily (while mechanically ventilated) for up to 8 days to identify those subjects who are heavily colonized with *S. aureus* (see Section 3.1). Screening for heavy *S. aureus* colonization by ETA culture will continue on Day 11 and Day 14 provided the subject remains mechanically ventilated, for a total Screening Period of up to 14 days. Additional ETA screening may occur on Days 9, 10, 12, and 13 at the discretion of the Investigator and providing the subject remains mechanically ventilated.

b. Procedures are to be performed 48 hours (±36 hours) post-dose on approximately Day 2 or Day 3, depending on time of study drug administration, for those subjects participating in the BAL fluid PK sub-study only or if BAL fluid is collected as part of a standard of care procedure for any subject at any other time post-randomization for any other reason.

c. Procedures are to be performed daily (unless otherwise indicated) from Day 2 to Day 21 while the subject is hospitalized. For subjects participating in the BAL fluid PK sub-study, procedures noted for the Day 2 visit only need to be performed 1 time.

d. A follow-up visit will occur on Day 28 (+2 days) to assess mortality. This visit may be conducted via telephone if the subject is no longer hospitalized.

e. For subjects who remain hospitalized between Day 40 and Day 90 at the institution where they receive study treatment, a follow-up safety visit will be performed on the day of discharge.

f. If the subject is unable to return to the site, this visit may be conducted by telephone, with procedures performed as appropriate (i.e., concomitant medications and adverse events collected).

g. An ET visit will be conducted for subjects who are withdrawn from the study prior to Day 22.

h. To be performed at the initial Screening Visit only.
i. At the Screening Visit, subjects must meet all of the inclusion criteria and none of the exclusion criteria in order to be eligible to return for the Randomization Visit.
j. At the Randomization Visit, subjects must continue to meet all of the inclusion criteria and none of the exclusion criteria. Subjects must also meet all randomization criteria to be eligible to be randomized into the study.
k. Review and record medical history (if changes occurred since the Screening Visit).
l. All prior medications received by the subject within 14 days prior to study drug administration and any concomitant medications used throughout the duration of the study will be recorded in the source documents and on the appropriate eCRF.
m. A complete physical examination will be performed at the Screening Visit and as deemed necessary by the Investigator and will include, at a minimum, a pulmonary examination (including auscultation) and assessments of the skin, abdomen, cardiovascular, gastrointestinal, and neurological systems. A limited physical examination will be performed at the Randomization Visit, daily during the Monitoring Period (if the subject is hospitalized), on Day 22 (if the subject is hospitalized), at the time of hospital discharge (if discharge occurs between Day 40 and Day 90), at the Safety Visit (Day 90) if the visit occurs onsite, and at ET (if applicable). The limited physical examination will include, at a minimum, a pulmonary examination (including auscultation).
n. Vital sign measurements will include temperature, systolic and diastolic blood pressure, pulse, respiratory rate, and oxygenation status. Blood pressure and pulse will be assessed via an automated device. Manual techniques are only to be used if an automated device is not available. Oxygenation status will be determined via measurement of arterial blood gas, provided the subject has an arterial line placed. If no arterial line is present, pulse oximetry may be used.
o. Female subjects of childbearing potential must have a documented negative pregnancy test at the Screening Visit. Female subjects may be enrolled on the basis of a negative urine pregnancy test, pending the result of a negative serum pregnancy test prior to randomization. A pregnancy test will also be performed on Day 22 or ET (if applicable). Additional pregnancy testing may occur throughout the duration of the study, per applicable country requirements.
p. Blood samples for chemistry, hematology, and coagulation will be obtained on Days 2 (+1 day), 4 (±1 day), and 6 (±1 day) of the Monitoring Period, and twice weekly during Week 2 (Day 8 through Day 14) and/or Week 3 (Day 15 through Day 21) of the Monitoring Period while the subject remains hospitalized.
q. To be obtained if clinically indicated.
r. Urinalysis to be obtained on Day 6 (±1 day) of the Monitoring Period only.
s. Procalcitonin testing to occur on the day of diagnosis of pneumonia, if applicable.
t. If applicable.
u. A chest X-ray or thoracic CT scan is to be obtained 1 time at the initial Screening Visit. A chest X-ray or thoracic CT scan obtained as part of standard of care within 24 hours prior to the initial Screening Visit is sufficient provided there are no changes in the subject's clinical status (e.g., no change in ventilation status, secretions, or signs/symptoms that may be suggestive of pneumonia) within the 24 hours following imaging.
v. A chest X-ray or thoracic CT scan obtained within 24 hours of the Randomization Visit is sufficient, provided there are no changes in the subject's clinical status (e.g., no change in ventilation status, secretions, or signs/symptoms that may be suggestive of pneumonia) since the time of imaging.
w. A chest X-ray or thoracic CT scan is required during the Monitoring Period for those subjects presenting with signs and symptoms of a respiratory infection such as dyspnea, tachypnea, fever, or cough.
x. ETA specimens collected during Screening/Enrollment will be cultured by quantitative or semi-quantitative methods as required by the protocol. Sites are encouraged to perform semi-quantitative cultures using a chromogenic media that tests for both the presence of MSSA and MRSA (e.g., CHROMagar™ *Staph aureus*) for more rapid and specific detection of *S. aureus*. If the site is not able to obtain chromogenic media for *S. aureus*, appropriate chromogenic media may be supplied by the Sponsor. Semi-quantitative cultures using chromogenic media are preferred for determination of eligibility for randomization; however, any quantitative or semi-quantitative culture performed that indicates that a subject is heavily colonized with *S. aureus* is permitted if the culture results are available to randomize the subject within 48 hours (or up to 60 hours with Medical Monitor approval) from the time the ETA sample was collected. If subjects are extubated and subsequently re-intubated/mechanically ventilated, they are eligible for re-screening as a new subject enrollment. Subjects who were previously randomized are not eligible to be re-consented and re-screened. Subjects with a screening ETA culture that also shows heavy colonization of a Gram-negative organism (i.e., heavy co-colonization of both Gram-positive and Gram-negative organisms), will not be randomized into the study; however, randomization should not be delayed while waiting for Gram-negative results if heavy colonization with *S. aureus* has been confirmed.
y. Adequate respiratory specimen or other microbiological specimens to be obtained if clinically indicated. If, as part of routine standard of care, additional respiratory and/or other microbiological specimens are collected for culture during the study, results will be documented within the eCRF.
z. Obtain sample for ADA (to be obtained only upon discharge from the ICU, if discharge occurs prior to Day 22).

aa. Study drug will be administered in a double-blind manner. Subjects will receive ASN100 or matching placebo via 2 IV infusions over a duration of approximately 50 to 60 minutes per infusion. Both ASN100 and placebo will be administered as 2 separate infusions (either sequentially or simultaneously). The last infusion will be completed within 5 hours from initiation of drug preparation in the pharmacy.
bb. Obtain BAL fluid 48 hours (±36 hours) post-dose to determine ASN-1 and ASN-2 levels and blood to ELF ratio. If BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any other time post-randomization for any other reason, if possible, a sample for PK analysis will also be obtained.
cc. Blood samples for PK analysis will be collected immediately following the completion of the second infusion (+15 minutes) and at 6 hours (±4 hours) and 24 hours (±6 hours) post-dose.
dd. Obtain a blood sample for PK measurement ±1 hour relative to BAL fluid collection (including scheduled BAL fluid collection or if BAL fluid is collected as part of a standard of care procedure from any subject enrolled in the sub-study at any time post-randomization).
ee. A PK sample will be obtained from all subjects on Days 4 (±1 day), 7 (±1 day), 14 (±2 days), and 22 (+2 days) of the Monitoring Period. Additional PK samples will be collected on the day of discharge (if discharge occurs between Day 40 and Day 90).
ff. Adverse events that occur between the time the subject signs the ICF and the Randomization Visit will only be recorded if related to a study-specific procedure.

ADA = anti-drug antibody; BAL = bronchoalveolar lavage; CT = computed tomography; ECG = electrocardiogram; eCRF = electronic Case Report Form; ELF = epithelial lining fluid; ET = Early Termination; ETA = endotracheal aspirate; ICF = Informed Consent Form; ICU = intensive care unit; IV = intravenous; MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-susceptible *S. aureus*; PK = pharmacokinetic; *S. aureus* = *Staphylococcus aureus*; Unsched = unscheduled.

APPENDIX B: CLINICAL LABORATORY ANALYTES

Chemistry

Alanine aminotransferase (ALT)
Albumin
Alkaline phosphatase
Aspartate aminotransferase (AST)
Bicarbonate
Blood urea nitrogen
Calcium
Chloride
Creatinine
Direct bilirubin
Glucose
Phosphorus
Potassium
Sodium
Total bilirubin
Total protein

Hematology

Hematocrit
Hemoglobin
Platelet count
Red blood cell (RBC) count
White blood cell (WBC) count with differential (basophils, eosinophils, lymphocytes, monocytes, and neutrophils)
RBC indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), %Reticulocytes

Coagulation

Prothrombin time (PT)
Partial thromboplastin time (PTT)

Urinalysis

pH
Blood
Ketones
Specific Gravity
Leukocyte esterase
Bilirubin
Glucose
Nitrite
Protein

Other Screening Tests as Needed (for female subjects of childbearing potential only)

Urine and/or serum pregnancy test

Other Tests

Procalcitonin (PCT)