Ato*Bio

CLINICAL STUDY PROTOCOL

PHASE III, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PARALLEL-GROUP, STUDY OF AB103 AS COMPARED TO PLACEBO IN PATIENTS WITH NECROTIZING SOFT TISSUE INFECTIONS (NSTI)

Product: AB103

Protocol Number: ATB-202

Study Title: ACCUTE (AB103 Clinical Composite Endpoint Study in Necrotizing Soft Tissue Infections)

Development Phase: Phase 3

IND Number: 67,785

SPONSOR: Atox Bio Ltd.
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This clinical study will be conducted in accordance with current Good Clinical Practice (GCP) as directed by the provisions of the International Conference on Harmonization (ICH); United States (US) Code of Federal Regulations (CFR) and European Union (EU) Directives (as applicable in the region of the study); local country regulations; and the Sponsor's Standard Operating Procedures (SOPs).

Signature Page

The signature below constitutes approval of this protocol by the signatory on behalf of Atox Bio, Ltd.

Atox Bio, Ltd (the Atox Bio) agrees that it will arrange for the supply of the clinical study drug (investigational medicinal products [IMP]) described in this protocol and undertakes to report adverse events to the relevant authorities in compliance with the regulations. It further agrees to inform the Investigators of any information that would place the patients at risk by their continuing participation in the study.

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23AUG 2018

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INVESTIGATOR AGREEMENT

Version 4.0 Approval Date 23 August 2018

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein, in compliance with current Good Clinical Practice (GCP) and the applicable regulatory requirements and will make every reasonable effort to complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals under my responsibility who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the IMP and the conduct of the study.

I will use only the informed consent form (ICF) approved by Atox BioLtd. ("Atox Bio") and/or its representative and the Institutional Review Board/Independent Ethics Committee ("IRB/IEC") and will fulfill all responsibilities for submitting pertinent information to the IRB/IEC responsible for this study.

I agree that Atox Bio, its representatives, or regulatory authorities shall have access to any source documents from which case report form information may have been generated. I agree to maintain in a safe and secure location all required study documents and primary source documents until notified by Atox Bio that such documents may by discarded or transferred.

I further agree not to originate or use the name of Atox Bio, or any of its employees, and/or AB103 in any scientific publication, marketing or publicity material, news release or other public announcement, written or oral, whether to the public, press or otherwise, relating to this protocol, to any amendment hereto, or to the performance here under, without the prior written consent of Atox Bio. I further agree to keep in confidence and not disclose any confidential information provided by Atox Bio and/or related to the study or AB103.

Investigator's Signature	**Date**

Name of Investigator (Typed or Printed)

Investigator Title

Institution, Address*

Phone Number*

*If the address or phone number is changed during the course of the study, the Investigator will complete and provide a Form FDA 1572 to Atox Bio or its representatives, but will not require (a) protocol amendment(s). Should the Investigator plan to retire or transfer to another institution, he/she shall notify the Sponsor and arrange for the transfer of responsibility for all retained study materials and source records.

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PROTOCOL SYNOPSIS

<table>
<tr><td>Protocol Number</td><td>ATB-202</td></tr>
<tr><td>Protocol Title</td><td>Phase III, randomized, double-blind, placebo-controlled, parallel-group, study of AB103 as compared to placebo in patients with necrotizing soft tissue infections (NSTI)
ACCUTE (AB103 Clinical Composite Endpoint Study in Necrotizing Soft Tissue Infections)</td></tr>
<tr><td>Location(s)</td><td>A multicenter study, that will be conducted in approximately 75 qualified participating sites in the US and France</td></tr>
<tr><td>Phase of Development</td><td>Phase 3</td></tr>
<tr><td>Study population</td><td>Patients clinically diagnosed with necrotizing soft tissue infections (NSTI) who are scheduled for an urgent surgical intervention as part of their standard of care (SoC) for the treatment of their infection and who subsequently have a surgically confirmed diagnosis of NSTI.</td></tr>
<tr><td>Study Objectives</td><td>Primary objective:
To demonstrate the efficacy of AB103 as compared to placebo, in patients diagnosed with NSTI, using a clinical composite success endpoint:
The NSTI clinical composite score, NICCE (Necrotizing Infections Clinical Composite Endpoint) will be defined as the primary end point which will be composed of the following patient outcomes (i) Alive at Day 28, (ii) Day 14 debridements ≤3 (iii) No amputation done after the first debridement (iv) Day 14 modified SOFA (mSOFA) score ≤1 (mSOFA includes respiratory, cardiovascular, central nervous system (CNS), renal, and hematologic organ components but not hepatic/liver component) (v) Reduction of ≥3 score points between Baseline and Day 14 mSOFA score.
<table>
<tr><th>Component</th><th>NICCE</th></tr>
<tr><td>Survival</td><td>• Alive at Day 28</td></tr>
<tr><td>Local component</td><td>• ≤3 debridements throughout Day 14
• No amputation beyond first debridement</td></tr>
<tr><td>Systemic component</td><td>• mSOFA score of ≤1 at Day 14
• Reduction of ≥3 score points between Baseline and Day 14 mSOFA score</td></tr>
</table>
To be considered a success, patients must meet all components of the NICCE. A two-sided α=0.01 will be used to test superiority of AB103 relative to placebo for the primary endpoint (NICCE).</td></tr>
</table>

	Conditional co-primary end point: To demonstrate the efficacy of AB103 as compared to placebo, in patients diagnosed with NSTI, using a modified clinical composite success endpoint that includes (i) Alive at Day 28, (ii) Day 14 debridements ≤3 (iii) No amputation done after the first debridement. To be considered a success, patients must meet all 3 components of this modified composite endpoint. The conditional co-primary end point will only be tested if superiority is demonstrated for the primary endpoint at a two-sided α=0.01 significance level in order to account for multiple comparisons. A two-sided α=0.05 will be used to test superiority of AB103 relative to placebo for the co-primary endpoint. **Secondary objectives**: o To demonstrate the safety of AB103 when administered as a single dose of 0.50 mg/kg to patients diagnosed with NSTI o To demonstrate the efficacy of AB103 as compared to placebo, in patients diagnosed with NSTI, using the 5 component clinical composite endpoint but with mSOFA ≤1 at Days 21 and 28 o Comparison of the distribution of mSOFA scores between treatment groups at Days 14 and 21 o Kaplan-Meier analysis of the time to resolution of mSOFA score to ≤ 1 with censoring at Day 14 o To demonstrate the efficacy of AB103 compared to placebo as related to the individual components of the NICCE in patients with NSTI o To demonstrate efficacy of AB103 in relation to the critical care and hospital stay parameters: a. Intensive care unit (ICU) days b. ICU-free days c. Days on ventilator d. Ventilator free days e. Vasopressor days f. Vasopressor free days g. Hospital length of stay o To demonstrate the efficacy of AB103 in patients with septic shock. o To determine the incidence of Stage 2 and 3 acute kidney injury (AKI) (using the KDIGO criteria) and compare the rates of complete recovery by Day 28 between the AB103 treated patients and the placebo treated patients. o Additional AKI endpoints: ▪ To determine the incidence of Stage 2 and 3 AKI (using the KDIGO criteria) and compare the rates of complete and partial recovery by Day 28 between AB103 and placebo treated patients ▪ Determine the incidence of Stage 1, 2 and 3 AKI and compare the rates of complete recovery between the AB103 and placebo treated patients ▪ Determine the incidence of Stage 1, 2 and 3 AKI and compare the rates of complete and partial recovery between the AB103 and placebo treated patients

	▪ Calculate time to complete recovery from AKI ▪ Calculate time to partial recovery from AKI o To demonstrate the safety of AB103 in regard to secondary infections **Exploratory objectives:** o To conduct an exploratory evaluation of the clinical response by baseline pathogen o To evaluate microbiological outcome in the microbiological evaluable population o To conduct an exploratory evaluation of plasma and urinary biomarkers in patients with AKI o To conduct an exploratory evaluation of blood leukocyte transcriptome (RNA expression) profiling in patients with NSTI and compare genomic profile in patients treated with AB103 versus placebo o To evaluate health economic and financial outcomes associated with the treatment o To evaluate NICCE outcome in screen failure patients with baseline mSOFA = 2 and post-operative mSOFA ≥ 3. Note: These patients will not be included in the modified Intent-to-Treat (mITT) population analysis (they will not receive blinded study drug) and only considered to be an observational cohort o To evaluate the immunogenicity of Reltecimod (formerly AB103)
Study Hypothesis	The primary hypothesis of this study is that in addition to SoC, AB103 will demonstrate a clinically significant treatment benefit over placebo. This hypothesis will be addressed by measuring the effect of AB103 on a composite of clinical parameters associated with the disease course of patients with NSTI, using a responder analysis. A responding patient must meet **all** 5 parameters of the composite clinical success end point, while a non-responding patient can fail by not meeting any one of the parameters. These analyses are designed to demonstrate that in addition to being safe, one dose of 0.5 mg/kg of AB103 will: **Improve systemic signs of the infection by improving organ function of patients compared to placebo as measured by:** • Survival at Day 28 • mSOFA score on Day 14 and change from baseline to Day 14 ≥ 3. A Day 14 mSOFA score of ≤1 and a change from baseline (pre-treatment) to Day 14 ≥3 will be required for a patient to achieve the primary composite clinical success endpoint (NICCE) **Improve the local signs of the infection, as measured by:** • Reduced number of debridements, counted to Day 14. No more than 3 debridements to Day 14 will be required for a patient to achieve composite clinical success • No amputation after the first debridement (amputation on the first debridement is not considered a failure). A patient will be required to have had no amputations done after the first

	surgical procedure in order to achieve composite clinical success.
Number of Patients:	290 patients will be recruited into the study and randomized to receive either 0.5 mg/kg AB103 or placebo in a 1:1 ratio. Randomization will be stratified within center by the diagnosis of Fournier's Gangrene and mSOFA score category (3-4 vs >4) at screening. The study will be conducted with interim analyses for futility at 100 patients.
Inclusion Criteria:	1. Age: ≥12 years **2.** Surgical confirmation of NSTI by attending surgeon (e.g. presence of necrotic tissue, thrombosed vessels in the subcutaneous tissue, lack of bleeding and "dishwater" (cloudy, thin, gray) fluid) due to presumed bacterial infection (necrotizing cellulitis (most commonly group A strep), necrotizing fasciitis, necrotizing myositis and myonecrosis, NSTI of the perineum, bacterial synergistic gangrene, Clostridial gas gangrene***) that may be supported by specific signs and symptoms (e.g. tense edema outside area of compromised skin, pain disproportionate to appearance, skin discoloration, ecchymosis, blisters/bullae, necrosis, tense edema, crepitus and/or subcutaneous gas).*** • Patients with NSTI following intra-abdominal operation are eligible if adequate source control of intra-abdominal process has been established 3. mSOFA score ≥3 (in any one or combination of the 5 major components of SOFA score with any one organ component having a score of at least 2: cardiovascular, respiratory, renal, coagulation, CNS), measured as close as possible to the first debridement, (but before first debridement is performed). 4. IV drug administration within 6 hours from the clinical diagnosis and the decision at the study site, to have an urgent surgical exploration and debridement (drug should not be administered until surgical confirmation is established). 5. If a woman is of childbearing potential, she must consistently use an acceptable method of contraception from baseline through Day 28. Acceptable birth control methods include oral contraceptive medication, an intrauterine device (IUD), an injectable contraceptive (such as Depo-Provera®), a birth control patch, a barrier method (such as condom or diaphragm with spermicide) or abstinence. o Non-childbearing potential is defined as current tubal ligation, hysterectomy, or ovariectomy or post-menopause (1 year without menses with an appropriate clinical profile at the appropriate age e.g. >45 years). 6. If a male patient's sexual partner is of childbearing potential, the male patient must acknowledge that they will consistently use an acceptable method of contraception (defined above) from baseline through Day 28. 7. Signed and dated ICF as defined by the IRB and, if applicable, California Bill of Rights. By signing the ICF, the patient agrees to release any medical records pursuant to current Health Insurance Portability and Accountability Act (HIPAA) Guidelines. If patient is unable to comprehend or sign the ICF, patient's legally acceptable representative may sign the ICF

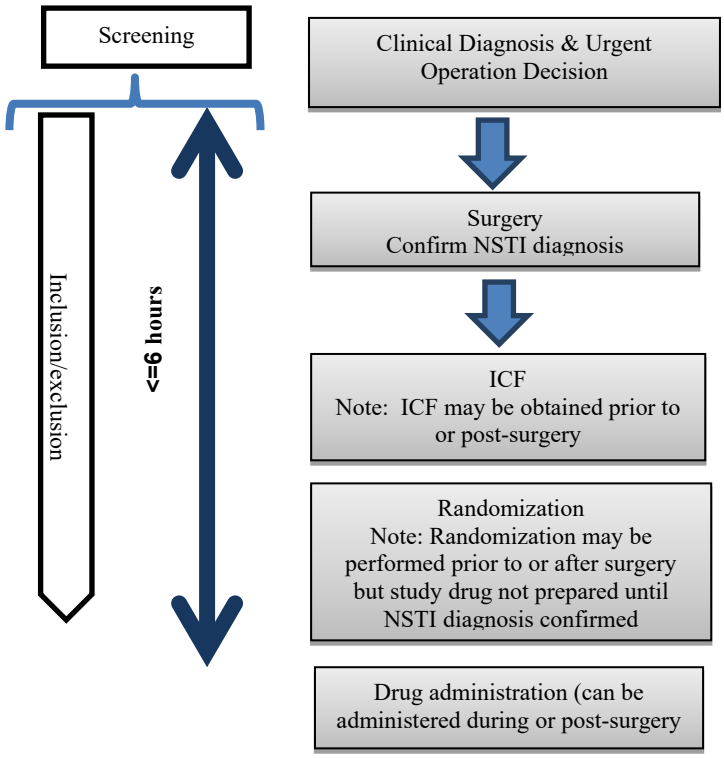
Exclusion Criteria:	1. BMI > 51; 2. Patient who has been operated at least once for the current NSTI infection and had a curative deep tissue debridement (patients who underwent prior diagnostic minor surgery are allowed to enter into the study); 3. Patients with overt peripheral vascular disease in the involved area - associated with ischemic wounds/ulcers or gangrene, and /or other significant symptoms of inadequate vascular supply or where limb amputation is considered likely within 7 days due to the peripheral vascular disease; 4. Diabetic patients with peripheral vascular disease who present with below the ankle infection; 5. Patient with burn wounds; 6. Current condition of: (a) Inability to maintain a mean arterial pressure > 50 mmHg and/or systolic blood pressure > 70 mmHg for at least 1 hour prior to screening despite the presence of vasopressors and IV fluids or (b) a patient with respiratory failure such that an SaO_2 of 80% cannot be achieved or (c) a patient with refractory coagulopathy (INR >5) or thrombocytopenia (platelet count <20,000) that does not partially correct with administration of appropriate factors or blood products, 7. Chronic neurological impairment that leads to a neuro mSOFA component ≥2 8. Recent cerebrovascular accident in the last 3 months. 9. Patients with cardiac arrest requiring cardiopulmonary resuscitation within the past 30 days; 10. Patient is not expected to survive throughout 28 days of study due to underlying medical condition, such as poorly controlled neoplasm (e.g. Stage III or IV cancer); 11. Patient or patient's family are not committed to aggressive management of the patient's condition, or the combination of necrotizing skin infection and underlying illness makes it unlikely that life support will be maintained; 12. Any concurrent medical condition, which in the opinion of the Investigator, may compromise the safety of the patient or the objectives of the study or the patient will not benefit from treatment such as: o CHF {NYHA class III-IV} o Severe COPD {GOLD stage III-IV. or chronic hypoxemia (PaO_2 <55 mmHg) on room air, or chronic use of home ventilation, or unable to climb stairs or perform household duties due to chronic obstructive disease resulting in severe exercise restriction, or use of continuous home oxygen prior to hospital admission (sleep apnoea treated with continuous positive airway pressure or biphasic positive airway pressure oxygen during sleep is acceptable)} o Liver dysfunction {Childs-Pugh class C} o Immunosuppression (see Appendix F, Section 15.6 for list of excluded immunosuppressive medications) o Neutropenia < 1,000 cells/mm^3not due to the underlying infection o Idiopathic Thrombocytopenic Purpura (ITP)

	○ Receiving or about to receive chemotherapy or biologic anti-cancer treatment although hormonal manipulation therapies for breast and prostate malignancies are permitted ○ Hematological and lymphatic malignancies in the last 5 years 13. Known HIV infection with CD4 count < 200 cells/mm3 or < 14% of all lymphocytes; 14. Patients with known chronic kidney disease (documented pre-illness creatinine value(s) ≥2.0) or patients receiving renal replacement therapy for chronic kidney disease: either hemodialysis, peritoneal dialysis, hemofiltration such as Continuous Veno-Venous Hemofiltration (CVVH) or hemodiafiltration 15. Patients that are treated with continuous hemofiltration (e.g. Continuous Veno-Venous Hemofiltration) for acute kidney dysfunction, not due to NSTI, starting prior to study drug administration. Exception: Patients with acute kidney dysfunction due to NSTI may be enrolled in the event that the patient is off the treatment from time of study drug administration and up to at least one hour post study drug administration. 16. Pregnant or lactating women; Women of childbearing potential must have a negative β-subunit hCG pregnancy test immediately prior to study entry 17. Previous enrollment in a clinical trial involving investigational drug or a medical device within 30 days before provision of written informed consent for the study or within five half-lives of the investigational drug, whichever is longer. 18. Previous enrollment in this protocol, ATB-202, or the Phase 2 NSTI trial of AB103, ATB-201, or the current Phase 2 SA-AKI trial of AB103, ATB-203.
Investigational Product: type, dose and mode of administration	AB103 is the sodium acetate salt of a 10 amino acids synthetic peptide that is homologous to specific amino acid residues of the T-lymphocyte receptor CD28. AB103 will be supplied in glass vials as a lyophilized powder to be reconstituted with sterile water for injection (WFI). Drug will be reconstituted on the day of its administration, in close proximity to infusion time, and several vials will be pooled together to compose the requested dose. Final volume for infusion will be calculated based on the patient's weight plus adequate priming volume of the IV line. Drug will be administered as an intravenous infusion, separate from other medications, over 10 minutes using a syringe pump (may be manually pushed if approved by the medical monitor). Volume of administration will be dependent on patients' actual weight. The dose of AB103 will be 0.50 mg/kg, administered as a one-time dose, either during the first surgery or immediately after the first surgery, all within the 6 hour time-window from clinical diagnosis and decision for urgent surgical exploration and debridement (study drug should only be administered after NSTI diagnosis is confirmed surgically). Blinded placebo will be sterile normal saline (0.9%)

	calculated similarly as for the active group according to the patient weight, plus adequate priming volume of the IV line.

Study design:

Flow chart: Study design from diagnosis to drug administration



Visit	Step/timing from drug administration
1	Screening
2	Day 1: Randomization & study drug administration (≤6 hr from clinical diagnosis); Begins with study drug administration and ends with the same calendar day
3	Day 2
4	Day 3
5	Day 7 (±1 day)
6	Day 10 (±1 day); perform only if patient is still hospitalized
7	Day 14 (±1 day)
8	Day 21 (±1 day)
9	Day 29 (+2 days)
10	Follow-up & Termination at 3 months (±5 days)

	This is a randomized, double-blind, placebo-controlled, parallel-group, multicenter study, to evaluate the efficacy and safety of a single dose of AB103 versus placebo (both in addition to SoC). Screening starts upon notification of study team about a patient with possible NSTI and lasts until study drug administration. Screened patients meeting inclusion/exclusion criteria and able to provide written informed consent (legal patient representative consent acceptable) may be randomized to one of 2 treatment arms (AB103 or placebo) in a 1:1 ratio. Treatment regimen consists of a single dose of 0.50 mg/kg AB103 or placebo, each administered as a single intravenous infusion over 10 minutes. Study drug administration may take place during or after the surgical exploration and debridement, but no later than 6 hours from clinical diagnosis of NSTI and a decision at the study site for urgent surgical wound exploration and debridement. All patients will receive SoC antimicrobial treatment and any other SoC treatment, as determined by the Investigator **SOFA score definition:** *Screening mSOFA*: Screening mSOFA score components are to be captured and the total score to be calculated prospectively and must be performed prior to first debridement: The screening mSOFA score should be evaluated any time after arrival at the hospital (may include referring hospital), although no more than 6 hours prior to anticipated first debridement. Screening mSOFA score must be ≥3, with one organ component having a score of at least 2, to be considered eligible for enrollment. Sites are encouraged to re-evaluate mSOFA score as close to surgery as feasible for patients not meeting mSOFA ≥3 inclusion criteria during initial screening. Screening mSOFA will include measurements of the score components in the following organ/systems: respiratory (to include evaluation of oxygenation either directly by arterial blood gas test or by calculation of PaO_2 from SpO_2, in case it is not possible to obtain arterial blood to determine the SOFA respiratory parameter), cardiovascular, renal, coagulation and CNS. *mSOFA score*: measurements of a 5 organ systems, including CNS. To be measured on days 1, 2, 3, 7, 10, 14, 21 and 28 and calculated retrospectively. *mSOFA respiratory parameter*: In case it is not possible to take arterial blood gases to determine the SOFA respiratory parameter, SpO2/FiO2 ratio can be imputed for PaO2/FiO2 ratio.

Outcome measures	**Primary Efficacy Measure:** • NICCE: Clinical composite success end point: Success is defined as meeting **all** 5 components of the composite score: Alive until Day 28, (ii) Day 14 debridements ≤3 (iii) No amputation done after the first debridement (iv) Day 14 mSOFA score ≤1 (v) Reduction of ≥3 score points between Baseline and Day 14 mSOFA score. **Conditional Co-Primary Efficacy Measure**: • Modified clinical composite endpoint: Success is defined as meeting all 3 components of the composite score: Alive until Day 28, (ii) Day 14 debridements ≤3 (iii) No amputation done after the first debridement **Safety Measures:** • Measures throughout Day 28: AEs (includes Serious Adverse Events (SAEs)), clinical safety laboratory (through Day 14), secondary infections, determination of survival through Day 28. Other measures: physical exam, vital signs, ECG (days 0 and 1); (specific times of these parameters are described in the table of study visits and procedures below) **Secondary Measures:** • 5 component clinical composite endpoint but with mSOFA ≤1 at Days 21 and 28 • Distribution of mSOFA scores at Days 14 and 21 • Kaplan-Meier analysis of the time to resolution of mSOFA score to ≤ 1 with censoring at Day 14 • Single components of the composite endpoint : o Alive at Day 28 o Number of patients with debridements by Day 14 ≤3 o Number of amputations (excision to a joint space) (done after first debridement) o mSOFA score on Day 14 ≤1 o Reduction of ≥3 score points between Baseline and Day 14 mSOFA score. • Critical care and hospital stay parameters, to be measured until Day 28: o ICU-free days o Days in ICU o Days on ventilator o Ventilator free days o Vasopressors days/ Vasopressors free days o Hospital length of stay (days) • Clinical local parameters: o Number of debridement to days 7, 10, 14 and 28 o Proportion of patients needing (up to Day 14): ▪ only one debridement to control the infection ▪ ≥2 debridements to control the infection ▪ ≥3 debridements to control the infection • Clinical systemic parameters: o Evaluation of organ function over time, using SOFA score o Recovery from AKI **<u>Exploratory Measures:</u>** • Evaluation of clinical response by baseline pathogen type

	• Evaluation of microbiological outcome in the microbiological evaluable population • Correlation of plasma and urinary biomarkers with incidence of AKI, AKI staging and recovery from AKI • Evaluation of blood leukocyte transcriptome (RNA expression) profiling in patients with NSTI and compare genomic profile in patients treated with AB103 versus placebo • Evaluation of NICCE outcome in screen failure patients with baseline mSOFA = 2 and post-operative mSOFA ≥ 3 • To evaluate the immunogenicity of Reltecimod (formerly AB103)
Health economic information	Details of health economic information will be collected throughout the hospital course and post hospital discharge. The health economic information will include several parameters: Hospital length of stay (includes both ICU and hospital ward stay), length of ICU stay, days on the ventilator, number of surgical operations, hospital readmission information, and mSOFA scores.
Duration of study	Overall, the study is expected to last approximately 48 months (from first patient in to last patient out).
Statistical analysis	**Primary analysis:** The primary efficacy comparison involves testing the following superiority hypotheses: **Ho:** $\pi_{0.50} - \pi_{Placebo} = 0$ **vs Ha:** $\pi_{0.50} - \pi_{Placebo} > 0$; where $\pi_{0.50}$ and $\pi_{Placebo}$ represents the true probability that a patient achieves specific composite clinical success criteria, NICCE, designed to be sensitive to both local and systemic drug effects. Each probability represents the proportion of subjects on each arm expected to respond according to the primary efficacy endpoint. **Sample Size Justification:** This trial will enroll 290 subjects that will be randomized in a ratio of 1:1 to either AB103 0.50 mg/kg (n=145) or placebo (n=145), each in addition to SoC). Sample size analysis was performed assuming that all patients will be evaluable for the primary endpoint. This assumption is justified since all patients in the Phase 2a trial and in the retrospective study were evaluable for NICCE. The primary efficacy hypothesis will be tested using an unadjusted χ^2 statistic with a 0.01 two-sided significance level. Statistical power was computed for a range of expected treatment group differences supported by the results of preliminary studies. For treatment group differences equal to 0.30, 0.25, and 0.20, statistical power will be equal to 99%, 95.9%, and 80.2%, respectively. These estimates were determined assuming an average success rate of 0.5 so that these power estimates are applicable across the range of expected response rates but may be conservative in some cases. The use of NICCE and this range of expected treatment effects is supported by preliminary studies. In the Phase 2a trial, 71.4% (5/7) patients with baseline mSOFA≥3 treated with AB103 0.50 mg/kg achieved NICCE. In contrast, 40% (2/5) placebo patients with baseline mSOFA≥3 achieved NICCE, a difference of 0.314. The retrospective study provided further support for the untreated response rate. Among 69 patients with baseline mSOFA≥3, 33.3% (23/69) achieved NICCE, difference of 0.381 relative to Phase 2a treated group. If the observed differences are equal to 0.35, 0.25, and 0.20 (centered about 0.5), then the corresponding 2-sided p-values will be $p<0.0001$, $p<0.0001$, and $p=0.0007$, respectively. The observed difference will need to be larger than about 0.16 (e.g., 0.58 vs 0.42) for $p<0.01$.

Based on a similar evaluation of the co-primary endpoint, the study is designed to demonstrate statistical significance at two-sided α=0.05 for co-primary success rates of 0.80 vs 0.60 in the AB103 and placebo groups, respectively. If these are the true success rates, then power will 96.4%. However, if the true rates are 0.78 vs 0.62 (difference = 0.16), then power is 84.8%. The sample size has also been selected to obtain sufficient enrollment on the treatment arm for relevant secondary effectiveness endpoints and for safety endpoints.

Futility Analysis:

A futility analysis will be performed based on the results of the first 100 patients (50 per group). The futility decision will be based on the predictive probability of eventual study success, conditioned on the data available at interim analysis. Independent, non-informative prior distributions will be used for each parameter: $\pi_{0.50} \sim \text{Beta}(1,1)$, $\pi_{\text{Placebo}} \sim \text{Beta}(1,1)$. The Bayesian predictive probability of study success (i.e., two-sided $p \leq 0.01$) when the remaining patients are finally observed will be determined. The trial will stop enrollment for futility if the predictive probability of study success is below a lower bound threshold of 10%. The following table summarizes what the observed predictive probabilities will be as a function size of the treatment group differences and assuming an observed control success rate of 0.40. If there are 20 success and 30 failures among placebo controls (40% success rate), then the futility boundary will be crossed if the number of active success is 22 (44%) or less.

Diff	0.000	0.020	0.040	0.060	0.080	0.100	0.120	0.140	0.160	0.180	0.200	0.220	0.240	0.260	0.280	0.300
PP	0.029	0.050	0.082	0.127	0.187	0.261	0.348	0.444	0.543	0.639	0.728	0.804	0.866	0.913	0.946	0.969

The futility bound is 'non-binding' in the sense that no effort was made to 'recover' alpha to increase power.

Independent Data Monitoring Committee (iDMC):

A detailed iDMC charter will be provided to clarify all relevant issues relating to the conduct of the futility analysis, including specific details regarding the operational procedures with fire-walls to protect against potential operational biases, decision rules, composition of the iDMC members and their conflict of interest statements.

Secondary Analyses:

Secondary endpoints have been specified from several domains including a similar composite endpoint but defined on the basis of Day 21 mSOFA, individual components of the primary composite endpoint, time to resolution of organ dysfunction, critical care and hospital stay parameters, clinical local parameters, and clinical systemic parameters. Analyses for these endpoints will generally be descriptive, with emphasis on characterizing clinical effect sizes. Nominal p-values will be presented. Categorical outcomes will be described using counts and percentages with nominal p-values determined through chi-square or exact methods. Critical care and hospital stay endpoints will be described using non-parametric approaches including using concordance statistics to characterize clinical effect size and Wilcoxon rank sum tests to determine nominal statistical significance. Methods appropriate for time-to-event endpoints including survival and life-table methods will be used for time-to-organ dysfunction resolution endpoints. Where appropriate, for continuous measures, statistical testing and estimation will be based on the results from a Mixed Model Repeated Measures (MMRM) analysis of covariance (ANCOVA) model. AKI endpoints will be assessed in several ways include incidence of Stage 2/3 AKI and among incident cases,

	resolution of AKI. A similar analysis will be performed based on any stage AKI. Treatment groups will be compared using counts and percentages with nominal p-values determined through chi-statistics. **Exploratory Analyses:** Exploratory subgroup analysis will be performed. For these exploratory analyses, active dose versus placebo differences in NICCE will be evaluated in several subgroups defined by (i) severity of disease at baseline (3-4 vs >4), (ii) presence of non-Fournier's gangrene (iii) involvement of suspected superantigen-producing bacteria and (iv) type of bacteria (Gram-positive vs. Gram-negative). Other similar comparisons may be performed. **Analysis Sets:** The following analysis sets are defined: • Intent-to-Treat (**ITT**): The ITT analysis set will include all randomized patients. • As-Treated (**AT**): The AT analysis set will include all randomized patients who were exposed to study medication (active or placebo). The AT analysis set will be used in primary safety analyses with patients assigned to actual treatment received. • Modified Intent-to-Treat (**mITT**): The mITT analysis set will include patients who were exposed to study medication and who had a definitive diagnosis of NSTI based on surgical verification. The mITT analysis set will be used in primary effectiveness analyses with patients assigned to their intended randomized assignment. • Per Protocol (**PP**): Optionally, a PP analysis set may be used in secondary effectiveness analyses. The PP analysis would include patients in the mITT analysis set assigned according to actual treatment received and excluding patients with either 1) significant violations of inclusion or exclusion criteria with potential to confound estimates of drug effects or 2) post randomization protocol violations with potential to confound estimates of treatment effects. Exclusions from the PP analysis set will be determined based on blinded clinical data. The PP analysis may be further restricted to include patients that survive at 3 least days when evaluation critical care variables. The safety profiles will be compared using descriptive statistics as appropriate for continuous and categorical safety variables including laboratory values and incidence of adverse events.

ABBREVIATIONS

Abbreviation	Description
AE	Adverse Event
AKI	Acute kidney injury
ALP	Alkaline phosphatase
ALT	Alanine transaminase
APC	Antigen-Presenting Cell
AST	Aspartate transaminase
ARDS	Acute respiratory distress syndrome
AT	As-Treated
AUC	Area under the curve
CBC	Complete blood count
CCS	Composite Clinical Success
CFR	Code of Federal Regulations
CI	Confidence Interval
CL	Clearance (plasma)
CLSI	Clinical and Laboratory Standards Institute
CNS	Central Nervous System
CRF	Case Report Form
CRO	Contract Research Organization
CRP	C-Reactive Protein
CVVH	Continuous Veno-Venous Hemofiltration
DVT	Deep vein thrombosis
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FDA	US Food and Drug Administration
FiO_2	Fraction of Inspired Oxygen
GAS	Group A Streptococcus
GCP	Good Clinical Practice
GGT	Gamma Glutamyl Transpeptidase
hCG	Human Chorionic Gonadotropin
hERG	Human Ether-a-go-go Related Gene
HEOR	Health economics and outcomes research
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
iDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP	Investigational Medicinal Product
IND	Investigational New Drug

Abbreviation	Description
IRB	Institutional Review Board
ITT	Intent-to-Treat
IV	Intravenous
IVIG	Intravenous Immunoglobulin G
IUD	Intrauterine device
IWR	Interactive Web Randomization
KLH	Keyhole Limpet Hemocyanin
LAR	Legally authorized representative
LOCF	Last observation carried forward
LPS	LipoPolySaccharide
LRINEC	Laboratory Risk Indicator for Necrotizing Fasciitis
MAR	Missing at random
MedDRA	Medical Dictionary for Regulatory Activities
MHC	Major Histocompatibility Complex
mITT	modified Intent-to-Treat
MMRM	Mixed Model Repeated Measures
MRSA	Methicillin-resistant Staphylococcus Aureus
mSOFA	Modified Sequential Organ Failure Assessment
MTD	Maximum Tolerated Dose
NICCE	Necrotizing Infections Clinical Composite Endpoint
NOAEL	No Observed Adverse Event Level
NSTI	Necrotizing Soft Tissue Infection/s
PAMP	Pathogen associated molecular patterns
PaO_2	Partial Pressure Oxygen in Arterial Blood
PBMC	Peripheral Blood Mononuclear Cell
PE	Physical Examination
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per Protocol
QTc	QT interval corrected
QTcF	QT interval corrected Fridericia
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SD	Standard Deviation
SoC	Standard of Care
SOC	System organ class
SOP	Standard Operating Procedure
SUSAR	Serious and Unexpected Suspected Adverse Reaction
TCR	T-cell Receptor
TEAE	Treatment Emergent Adverse Event
Th1	T Helper Cell 1 (CD4) subset
Th2	T Helper Cell 2 (CD4) subset
TNF	Tumor Necrosis Factor

Abbreviation	Description
VAC	Vacuum Assisted closure
WBC	White Blood Cell
WHO	World Health Organization
WFI	Water for Injection

1. INTRODUCTION

1.1 Background Information

1.1.1 Necrotizing Soft Tissue Infections (NSTI)

NSTI are rapidly progressing infections with significant local and systemic manifestations. They represent the most severe types of infections involving the skin, skin structures and soft tissue [1–9]. The key clinical feature of NSTI is the presence of necrosis confined to the subcutaneous fascial tissues and often also to the deep fascial layers, fat, nerves, arteries and veins. This may not always be apparent by physical examination (PE) or imaging studies[5]. Examples for NSTI include necrotizing fasciitis due to streptococcal infection (Group A or non-group A), bacterial synergistic gangrene, clostridial gas gangrene, Fournier's Gangrene, and haemolytic streptococcal gangrene. They all share the clinical features of severe local tissue necrosis, systemic toxaemia, bacteremia, and have a high mortality rate[5,6]. NSTI is a descriptive term which includes a variety of distinctive clinical diagnoses. While there is no unifying bacterial aetiology, the most frequently identified species in NSTI are *Staphylococcus aureus, Streptococcus pyogenes, Clostridia species*, enterobactereaciae, and non-clostridial anaerobes, sometimes as a mixed or multi-pathogen infection[1,2]. Although many specific variations of NSTI have been described on the basis of aetiology, microbiology and specific anatomical location of the infection, the initial approach to the diagnosis, antimicrobial treatment and decision to use operative management are similar for all forms and are more important than determining the specific variant[7].

NSTI develops when the initial, appropriate host response to an infection becomes amplified and is then dysregulated [10,11]. The pathogenesis of NSTI is thought to be related to the local release of inflammatory cytokines and bacterial toxins[8,9,12]. This has been shown in both, experimental animal models and clinical studies, particularly for infections caused by *S. pyogenes* (Group A strep; GAS) and *S. aureus*[10,13–16].

These bacteria produce a variety of exotoxins, including among others, toxins known as superantigens which bypass the normal immune response for processing bacterial antigens, are a major inducer of the inflammatory response, and can spread into the systemic circulation. This leads to massive polyclonal expansion with the release of pro-inflammatory cytokines, causing a T helper 1 "cytokine storm" and subsequent refractory shock and multi-organ failure.

NSTI is a rare disease, with an estimated prevalence of 4.5 cases per 100,000 individuals in Europe and 6.8 cases per 100,000 in the US. Risk factors appear to be diabetes, obesity, intravenous drug use, peripheral vascular disease and immunosuppression. However, a large proportion of NSTI patients have no predisposition for their infection [3–5,8].

NSTI is different from milder superficial skin infections with regard to clinical presentation, treatment strategies, coexisting systemic manifestations and its fatal outcome[7]. Although the survival of patients with NSTI has improved throughout recent

years[17–21], mortality rates are still unacceptable, with up to 17% according to reports from 2006 to 2011[1,2,8,22].

Patients with NSTI require emergent operative and repeated exploration with wide debridement of all necrotic tissue, with acute resuscitation along with administration of broad spectrum antibiotics with specific coverage for methicillin-resistant *Staphylococcus aureus* (MRSA), beta haemolytic Streptococcus and clostridia, and patients with extensive necrosis of an extremity may require amputation. The duration of intensive care unit (ICU) stay is usually dependent on the degree of organ dysfunction and the need for mechanical ventilation for respiratory failure. The duration of hospital stay is dependent on the duration of antibiotic therapy and wound management issues.

The current mainstay of therapy includes multiple surgical debridements performed during the first 7-14 days of the patient's hospital stay, along with broad spectrum antibiotics and critical care support. To date no drug products have been approved for treatment of NSTI and the standard of care (SoC) is unsatisfactory. Patients with NSTI suffer from decreased physical function and pose a significant burden on health care. Following a retrospective review, a substantial number of patients discharged from hospital (30%) have a mild to severe functional limitation[23]. The frequent need for debridements may lead to loss of function at the site of infection, the creation of large disfiguring wounds requiring reconstruction, and amputations in 10-20% of patients when extremities are involved. Moreover, the systemic manifestations of the disease process can result in the development of multi-organ failure.

Other morbidities: In patients with colonic and rectal involvement, colostomy (performed in 90% of these patients, and in 22.2 % of overall patients) is often required[8,24] and in cases of groin involvement (Fournier's Gangrene), genital or perineal changes are reported, which may lead to loss of function[5]. In cervical necrotizing fasciitis, complications include airway obstruction, jugular venous thrombosis, rupture of the great vessels, aspiration pneumonia, mediastinitis, empyema and lung abscess[5]. To recover from their acute illness, many of these patients (34%) are disposed to centers of rehabilitation, nursing homes, other sub-acute centers such as short term hospitals, or even burn units for proper wound care[23,25]; many of them require physical and occupational therapy support[23] and psychological help to overcome their inconveniences. These sets of morbidities constitute a substantial economic burden on the hospitals and health care system.

As neither surgical debridement nor antibacterial therapy directly addresses the immunological pathogenesis of NSTI, reducing the host inflammatory response by targeted immune therapies could lead to important clinical benefits in both morbidity and mortality. In many cases, the diagnosis of NSTI is not established when the patient first presents with acute symptoms, and the infection progresses rapidly despite the use of antibiotics. Delay in diagnosis, and thus delay in surgical debridement of necrotic tissue, is an independent variable for mortality. Since treatment of NSTI patients is often delayed due to the challenging diagnosis and inevitable delays in scheduling major surgery, a potential benefit of a therapy for NSTI is the speed with which it could be administered.

1.1.2 Indication

The treatment indication is adjunctive treatment of NSTI. The Investigational Medicinal Product (IMP) AB103 acts as an immunomodulator to attenuate CD28 activation of Th1 lymphocytes. AB103 is being developed for the treatment of NSTI in conjunction with SoC including surgical debridement, antibiotic therapy and supportive care.

The current study, ATB-202, is a pivotal Phase 3 clinical trial that is designed to demonstrate improved efficacy and safety of AB103 over placebo in conjunction with SoC for the treatment of NSTI

1.1.3 Investigational Medicinal Product

AB103 (also known as p2TA; Reltecimod) is the acetate salt of the synthetic peptide that consists of 10 amino acids. AB103 has homology to amino acid residues 8-15 of the co-stimulatory receptor CD28 and has D-Ala residues abutted to N- and C-termini to render them more protease resistant. Binding of AB103 to CD28 is postulated to prevent activation of CD28 by B7 on the antigen-presenting cell. AB103 has broad spectrum activity in animal models and was shown to inhibit pro-inflammatory cytokine induction without any evidence of agonist activity (up-regulation of cytokines) in response to bacterial toxins.

AB103 is a lyophilized powder, formulated to contain 1 mg/mL of the AB103 peptide, 30 mg/mL mannitol, and 3.6 mg/mL sodium chloride after reconstitution with sterile water for injection (WFI). The drug product to be used in clinical trials will be supplied in 20 mL vials filled to deliver 10 mL after reconstitution, or 10 mg of AB103. The reconstituted drug product is administered directly, and is not diluted prior to administration. It will be administered at a dose of 0.5 mg/kg as an intravenous infusion given over 10 minutes

As NSTI is a complex and rare disease for which no approved therapy exist, AB103 has been granted Fast Track and Orphan Drug designations from FDA. Encouraging results from non-clinical (See Section 1.1.3.2) and clinical studies (Phase 1 and 2; see Section 1.1.3.3) indicate a good safety profile with no reported toxicities in animals or humans as well as preliminary efficacy in this patient population.

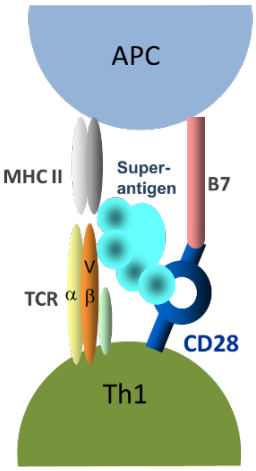
1.1.3.1 Mechanism of action

There is growing appreciation that T-cell and antigen-presenting cell (APC) interactions play a critical role in inflammatory response of severe infections, and link the adaptive and innate immune systems. CD28 is a co-stimulatory receptor, expressed mostly on CD4+ and CD8+ lymphocytes. The CD28-mediated co-stimulatory signal is involved in the induction of many pro-inflammatory cytokines by T-cells, and is required to avoid an apoptotic or anergic response by the lymphocyte. CD28 is the receptor for APC molecules B7.1 (CD80) and B7.2 (CD86). CD28 signal is mediated by dimerization and binding to its ligands on the APC, and deliver a strong activation signal to the effector cell population. The role that CD28 plays in the adaptive immune system is well accepted as a co-stimulatory molecule; however, it also plays a significant role in immune surveillance and innate immune responses.

Major inducers of inflammatory response during bacterial infection are superantigens, stable proteins that stimulate virtually all T-cells, without a need for processing by antigen-presenting cells (APC)[26–28]. Bypassing the restricted presentation of conventional antigens, superantigens can activate 30-50% of T-cells to divide and produce cytokines. In contrast to conventional immune response, during which 0.01% of T-cells react with antigens to orchestrate immune attack without harming healthy tissue, superantigens activate the cellular immune response at least 5,000-fold more strongly than do ordinary antigens. Toxic shock results from a sudden and massive induction of Th1 cytokines including interleukin-2 (IL-2), interferon gamma (IFN-γ), and tumor necrosis factor (TNF-β)[29–31].

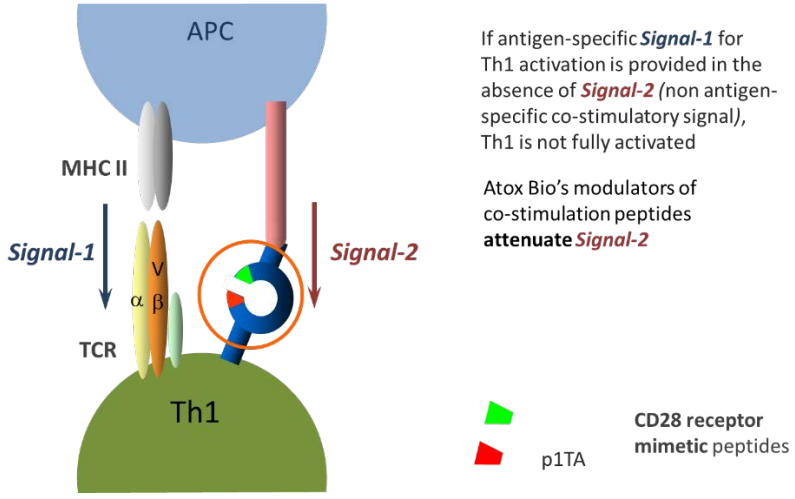
Induction of these Th1 cytokines can occur through the binding of a superantigen (produced by Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*) to the major histocompatibility class II (MHC II) complex (located on the surface of an APC), and the T-cell receptor (TCR, located on the surface of a T helper [Th]1 cell). However, this interaction is insufficient for activation. Superantigens must also bind to the dimer interface of CD28. By engaging all 3 receptors simultaneously, the superantigen overcomes its inherently limited affinity for each, allowing it to deliver a strong activation signal (Figure 1).

Figure 1: Th1 induction



AB103 is a synthetic short peptide, that is capable of modulating the interaction between host T-cells (host factors) and pathogen associated molecular patterns (PAMPs) such as superantigens and endotoxins. AB103 is homologous to specific amino acid 8-15 residues of CD28, and belongs to a family of peptide modulators that mimic the dimer interface of CD28, and was shown to affect the downstream signaling of CD28.

Figure 2: AB103 function



AB103 was originally designed and developed towards creating a broad spectrum countermeasure directed against the superantigen toxin family, for the treatment of Gram-positive bacterial superantigen toxicity. Indeed, AB103 prevents the binding of superantigens to CD28 and subsequent activation of the downstream cellular cascade. As superantigens play a critical role in the pathogenesis caused by Gram-positive bacteria, AB103 could modulate the interaction between the host and pathogen factors, inhibit their harmful effects and provide a clinical benefit.

Nevertheless, binding of AB103 to CD28 was found to intervene also with the downstream signaling of CD28 independently of superantigens: AB103 binds directly to CD28 on the opposing monomer, and can therefore intervene with the downstream signaling of CD28 in cases that are not mediated by superantigens. As such, AB103 was found to be a potent attenuator of lethal inflammatory signaling induced by multiple pathogens, including Gram-positive and Gram-negative bacteria as well as viral pathogens. The role of the co-stimulatory pathway in the interaction of bacterial endotoxins with host cells has not been fully characterized, however evidence exists that it has a role in LPS-mediated effects in endotoxin-induced Gram-negative infections[32–35]. Further support for the role of the co-stimulatory pathway in Gram-negative infection in animals and in sepsis patients was demonstrated using CD28 knock out mice[36,37].

Thus, AB103 is both a superantigen and a CD28 antagonist, its activity is not pathogen specific, and it is a host-oriented, broad spectrum attenuator of cytokine storm. AB103 can therefore be employed to treat infections from various sources having a substantial inflammatory component, such as NSTI that involve mixed infections.

1.1.3.2 Non-clinical studies

In safety pharmacology studies, AB103 at a concentration of 10 µg/mL had no significant effect on human ether-a-go-go related gene (hERG) tail current. In pigs, AB103 at up to 5.0 mg/kg did not cause toxicologic effects on electrocardiogram (ECG) interval durations. The no observed adverse effect level (NOAEL) of AB103 for neurobehavioral measures of

a central nervous system (CNS) screen in mice was greater than 5 mg/kg (the highest dose tested).

Immunotoxicology study was performed in mice where AB103 was administered by a slow bolus IV injection weekly for 4 weeks at doses of 1.25, and 5 mg/kg. On Day 15, the potent T-cell immunogen, keyhole limpet hemocyanin (KLH), was administered by intraperitoneal (IP) injection. Mice were observed for clinical signs and death. Blood immunophenotyping was conducted at scheduled necropsies as well as primary IgM and IgG antibody responses to KLH. AB103 given weekly for 4 weeks at doses of 1.25, and 5 mg/kg/dose were well tolerated. No drug-related changes were seen in immunophenotyping measures or anti-KLH antibody responses. Statistically significant decreases in thymic weights in males given ≥1.25 mg/kg/dose of peptide AB103 were observed, which may have been related to the test article; these had no histologic correlate and were reversible. In addition, higher thymus weights in females, as compared to males, are regarded as a normal phenomenon for mice, which was also consistent with the historical data and published observations. Thus, the high dose of 5 mg/kg/dose was concluded to be the NOAEL

Non-clinical toxicology: The non-clinical toxicology and safety pharmacology of AB103 has been tested in vitro and in vivo in single dose and repeat-dose toxicology studies performed in mice and pigs. Single doses of AB103 given intravenously (IV) at up to 16 mg/kg to CD-1 mice were well tolerated with no toxicologically important clinical signs and no dose-related effects on body weight or food consumption. The maximum tolerated dose (MTD) is expected to be higher than 16 mg/kg body weight in mice.

In repeat-dose toxicology studies in pigs and mice with doses up to 5 mg/kg/day given for 14 days, no AB103-related clinically relevant changes were seen, indicating that the NOAEL in pigs and mice is greater than 5 mg/kg.

The efficacy of AB103 was examined in several mice models of bacterial infections from various sources, including Gram-positive infections (S. pyogenes and S. pneumoniae), Gram-negative infections (E. coli) and mixed infections (intra-abdominal polymicrobial infection). It was tested either as a stand-alone treatment or in combination with antibiotics. Overall, AB103 was shown to be efficacious in protecting mice from lethal shock in these models as evident by increased survival [38,39]. The effect of AB103 was tested also in a quantitative pig model for superantigen-induced incapacitation where by AB103 reduced the severity of both vomiting and diarrhea. In all models evaluated, a substantially high treatment benefit was demonstrated, indicating that AB103 has a broad spectrum of activity against infections. In addition, AB103 ameliorated disease symptoms both systemically and locally at the site of infection[38]. Moreover, treatment with AB103 did not compromise the host adaptive immune response or the humoral and long-term activities of the immune system[38].

Dose response studies were performed in various mice models of bacterial infections where AB103 was given as single dose. In all models evaluated, the optimal doses that provided the highest efficacy were within the same range of 2.5-5 mg/kg.

1.1.3.3 Clinical studies

AB103 has been evaluated in 2 safety and pharmacokinetic (PK) clinical studies, a Phase 1 study in healthy volunteers (study no. ATB-101) and a Phase 2 first-in-patients, proof of concept study (study no. ATB-201).

1.1.3.3.1 Phase I study ATB-101

The Phase 1 single center, randomized, double-blind, placebo-controlled, sequential-dose escalation study was conducted in 25 healthy volunteers. Four different escalating dose levels of AB103 were evaluated: 7.5 µg/kg, 37.5 µg/kg, 150 µg/kg and 450 µg/kg with a placebo control (1 or 2 subjects) included in each cohort; each subject received a single intravenous (IV) infusion of AB103 or placebo (saline) control. Blood was collected for PK and flow cytometry assessments of leukocyte subsets after infusions. Subjects were assessed 1-day and 6- to 8-days after the infusions for adverse events (AEs), vital signs, and clinical laboratory parameters. The primary objective of this study was to establish the safety profile and MTD of AB103 given as a single IV infusion in healthy volunteers. The secondary objective was to determine the PK profile of AB103 in humans after a single IV infusion. In addition, lymphocyte profiling was performed on subjects from the high dose cohort 4. Peripheral blood mononuclear cells (PBMCs) were obtained and subjected to flow cytometric analysis, for phenotypic analysis and intracellular cytokine expression. Study duration was 14 days.

A total of 22 AEs were reported during the conduct of the trial, of which 21 (95.5%) were mild and only one was moderate in severity. Of all the subjects in the study, 7 had AEs and 18 had no AEs. None of the AEs were considered related to Investigational Product. There were no dose-limiting toxicities nor were there any SAEs. There were no clinically meaningful changes in vital signs, clinical laboratory parameters, ECG parameters or lymphocyte subsets.

Lymphocyte profiling: Although variability between subjects was apparent, for the most part within a subject over time, the percentages of positive cells and absolute counts remained similar to those at baseline

PK in healthy subjects: Non-parametric pharmacokinetic parameters estimates are shown below:

Table 1: Summary of ATB-103 pharmacokinetic parameters in healthy volunteers

Dose µg/kg	**C_{max} ng/mL**	**AUC_{0-t} ng□min/mL**	**$T_{1/2}$ min**	**CL mL/min/kg**
7.5	10.14 ± 2.22	61 ± 19	ND	ND
37.5	56.67 ± 30.28	523 ± 248	1.36 ± 0.51	85 ± 50
150	208.80 ± 51.37	2137 ± 370	1.26 ± 0.28	72 ± 11
450	707.74 ± 269.03	6082 ± 2022	1.34 ± 0.21	80 ± 22

ND = not determined, insufficient data to calculate

Plasma AB103 concentrations in all cohorts peak near the end of the infusion and decline rapidly with a $T_{1/2}$ of a little over one minute. The apparent elimination $T_{1/2}$ was very similar

across dose levels. Systemic exposure to AB103 as measured by C_{max} and area under the curve (AUC) appeared to be dose-proportional. Consequently, plasma clearance (CL), which was derived from AUC and dose, was similar for all doses. The apparent volume of distribution, approximately 200 mL/kg, is much larger than plasma volume, which is consistent with distribution to sites outside the plasma compartment.

Summary: Overall, AB103 given IV at doses up to 450 μg/kg was very well tolerated in healthy volunteers. Following a 10-minute IV infusion of AB103 to healthy volunteers, plasma concentrations peaked at the end of infusion and then decline rapidly with an apparent elimination half-life of a little over one minute. Exposure was dose-proportional.

1.1.3.3.2. Phase II study ATB-201

The Phase 2 study (ATB-201) was a multicenter, randomized, double blinded, first-in-patients exploratory study in subjects who had a clinical diagnosis of NSTI and scheduled to undergo urgent surgical exploration and debridement. The study was conducted in 7 clinical sites in the US, and included 40 patients. Patients were randomized to receive either AB103 as a single intravenous (IV) infusion of 0.50 mg/kg or 0.25 mg/kg, or placebo (saline), over 10 minutes. Study drug or placebo was administered within 6 hours from clinical diagnosis, in addition to SoC (including prompt and repeated aggressive surgical debridement, aggressive resuscitation and physiologic support, and antimicrobial drugs).

The study objectives were to determine safety and PK of AB103 and the potential treatment benefit compared to placebo, by clinical benefit (measured by resolution of systemic inflammatory parameters, resolution of organ dysfunction or failure, and the need for repeated surgeries for the primary infection), assess critical care/ pharmacoeconomic benefit by hospital length of stay, ICU length of stay/ICU-free days, vasopressors free days, and mechanical ventilation days/ free days, and define potential surrogate biomarkers. Study duration was 28 days.

Safety results: Safety was evaluated in the ITT population, comprised of all study participants who were randomized and received study drug or placebo (n=43). Due to the natural severity of the underlying disease, a high incidence of adverse events was expected in the study population. Patients in the High dose group presented with a more severe state of the disease, as exemplified by a higher rate of AEs, SAEs, and abnormal labs on Day 0 (before study drug administration). Post Day 0 (after drug administration) and over the course of the study, AEs were reported in 94.1%, 93.3% and 81.8% of patients in the High dose, Low dose and Placebo groups, respectively, with no statistically significant difference. No study drug-related AEs were reported. Most of the AEs were mild or moderate. Severe AEs were reported in 2 patients (11.8%) of the High dose group, 2 patients (13%) of the Low dose group and 3 patients (27.3%) of the Placebo group.

Five patients (29.4%), 8 patients (53.3%), and 4 patients (36.4%) in the high dose, Low dose, and placebo groups, respectively experienced an SAE after Day 0. All SAEs (except one SAE of acute renal failure in a patient of the placebo group that had a background condition of chronic renal disease) were assessed as related to the underlying disease of NSTI, and none was regarded as study drug-related.

Four patients died during the study; one patient in each of the high and low dose groups and 2 patients in the placebo group. In both patients of the AB103-treated groups, the cause of death was multi-organ failure related to the underlying disease of NSTI, and in the placebo group one patient died of respiratory failure and cardiac arrest, and the second patient died of septic shock that started already at Day 0 and multi-organ failure related to NSTI.

In addition, there were no significant trends or findings related to laboratory abnormalities that could be attributed to study drug administration.

An ECG was performed on Day 0 prior to study drug administration and within 6 hours post study drug administration on Day 1. Comparator groups demonstrated similar means and distribution of QTcF values. Treatment groups did not have higher mean change in QTc than placebo and outliers were similar in number and scale.

In summary, no safety concerns were detected.

PK in patients with NSTI: Following a 10-minute intravenous infusion of AB103 to NSTI patients before, during, or after surgery, plasma drug concentrations peaked at the end of infusion. Plasma AB103 concentrations declined with a half-life of approximately 5 minutes in the high and low dose groups. Systemic AB103 exposure, as measured by C_{max} and AUC, appeared to be dose-proportional when comparing these parameters across the 2 dose groups (Table 2). There were no obvious differences in plasma AB103 concentrations between samples collected from venous lines compared to those collected from arterial lines. There also did not appear to be a clear effect of dosing time relative to surgery. Plasma AB103 concentrations in patients treated before, during, or after surgery did not show any apparent differences.

Table 2: Non-parametric AB103 pharmacokinetic parameters in NSTI patients

Non-parametric PK Parameters						
Treatment group	**Statistic**	**C_{max} ng/mL**	**T_{max} min**	**$AUC_{0-\infty}$ ng-min/mL**	**$T_{1/2}$ min**	**CL mL/min/kg**
0.50 mg/kg	Mean	1,503	10.60	16,020	5.75	39.89
	SD	655	4.82	7,086	3.64	24.46
	Median	1,352	10.00	16,921	4.89	29.55
0.25 mg/kg	Mean	961	6.73	7,733	4.80	43.17
	SD	681	2.80	4,021	6.30	26.68
	Median	899	5.00	8,497	2.61	29.42

The PK studies in human confirm the short elimination half-life of the product, and that the systemic clearance (CLs) values demonstrate that the clearance processes involved are of high capacity and rate.

Efficacy results: Analysis of results from the Phase 2a study, indicated that patients in the AB103 treatment groups had a substantial improvement in their clinical status across

multiple endpoints when compared to patients in the placebo group. Patients in the AB103 0.50 mg/kg and AB103 0.25 mg/kg treatment groups had a continuous resolution of organ dysfunction over time without subsequent worsening. Although not statistically significant, AB103 treated patients had a trend of spending fewer days in ICUs, requiring fewer days of assisted ventilation, and needing fewer surgical procedures to remove necrotic tissue. In addition, treatment of patients with AB103 reduced the levels of both systemic and local tissue cytokines and chemokines in a dose dependent manner. Tissue biomarkers, especially in the margins of the lesion, had reduced levels of cytokines, suggesting that administration of high dose AB103 may reduce progression of inflammation in marginally involved tissues. The overall tissue and systemic biomarkers are consistent with the mechanism of action of AB103, as attenuating the inflammatory response and possibly reducing the zone of tissue injury. The summary of the results is presented in Table 3 below:

Table 3: Consistent response across multiple clinically relevant end points

	Placebo	***0.25 mg/kg***	***0.5 mg/kg***
Days in ICU	8.9	4.9	5.4
Days on Ventilator	5.2	3.1	2.7
SOFA score Day 14*	2.7	1.1	0.7
% patients with organ failure at Day 14 (defined as SOFA score ≥3)	40	14.3	7.1
% patients with organ dysfunction at Day 14 * (defined as SOFA score ≥2)	66.7	37.5	11.1
% of patients with only 1 debridement	20	26	33
% of patients with ≥4 debridement	30	20	13
% Mortality	20	6.7	6.7

*P<0.05

Based on the reproducibility of the dose response in the various parameters, the 0.5 mg/kg dose was chosen for further clinical development.

1.1.3.3.3 A retrospective, non-interventional analysis study ATB-201A

This study was set up to better understand and characterize the clinical course of the disease under SoC treatment. The main goal was to evaluate potential clinical outcome parameters and to assess their suitability for the pivotal Phase 3 study program. Within this context, the study reviewed clinical data from 198 patients receiving SoC in 2013 at 12 US clinical sites. In a second step, the results of this retrospective putative placebo population were compared to those from the placebo- and AB103-treated population in the Phase 2 Study AB-201. The retrospective analysis employed a new composite endpoint, defined as necrotizing infection composite clinical endpoint (NICCE). Responders to the NICCE are (i) alive at Day 28, (ii) have Day 14 debridements ≤3 (iii) had no amputation done after the first debridement, and (iv) have a Day 14 mSOFA (modified Sequential Organ Failure Assessment [SOFA] evaluating 5 organ systems not including the liver organ component) score ≤1. The retrospective study demonstrated that early development of systemic organ dysfunction in patients with NSTI is associated with higher morbidity and mortality. Failure of resolution of organ dysfunction by Day 14 is also associated with poor outcome. mSOFA

score may be a useful marker for patient selection for inclusion in interventional trials and the resolution of organ dysfunction by d14 may be an important clinical endpoint. The study confirmed that the suggested composite endpoint NICCE is a clinically relevant endpoint which is highly correlated with several meaningful and relevant clinical patient outcome. In addition, the analysis determined that a more severe NSTI population (defined by a baseline mSOFA score of ≥3) demonstrate worse outcome compared to All-comers and would likely benefit most from AB103 treatment.

1.2 Risks and Benefit

Efficacy results of the Phase 2 study ATB-201 suggest that patients treated with AB103 in addition to the SoC can experience improved clinical outcomes as compared to SoC alone.

Patients randomized to receive placebo in the study will not benefit from the potential therapeutic effect of AB103, however they stand to benefit from the safety measures and close observation included in the study as detailed below.

No compensation, monetary or otherwise is offered to study participants except to cover customary cost of travel and/or meals associated with any study required visits that may occur after hospital discharge.

Safety of AB103 has been studied extensively in a set of non-clinical studies (section 1.1.3.2) in Phase 1 study of human healthy volunteers and in Phase 2a in NSTI patients (see section 1.1.3.3). In both clinical studies AB103 has demonstrated a good safety profile, and no specific drug-related safety concern has been identified to date.

This study protocol details several safety measures:

- Detailed exclusion criteria (Section 3.2) including:
    - Any concurrent medical condition, which in the opinion of the Investigator, may compromise the safety of the patient or the objectives of the study or the patient will not benefit from treatment such as:
        - CHF {NYHA class III-IV}
        - Severe COPD {GOLD stage III-IV. or chronic hypoxemia (PaO2 <55 mmHg) on room air, or chronic use of home ventilation, or unable to climb stairs or perform household duties due to chronic obstructive disease resulting in severe exercise restriction, or use of continuous home oxygen prior to hospital admission (sleep apnoea treated with continuous positive airway pressure or biphasic positive airway pressure oxygen during sleep is acceptable)}
        - Liver dysfunction {Childs-Pugh class C}
        - Immunosuppression (see Appendix F- Section 15.6 for list of excluded immunosuppressive medications)
        - Neutropenia < 1,000 cells/mm3not due to the underlying infection
        - Idiopathic Thrombocytopenic Purpura (ITP)
        - Receiving or about to receive chemotherapy or biologic anti-cancer treatment although hormonal manipulation therapies for breast and prostate malignancies are permitted

- Hematological and lymphatic malignancies in the last 5 years
  - Patients that are treated with continuous hemofiltration (e.g. CVVH) for acute kidney dysfunction, not due to NSTI, starting prior to study drug administration.
- Pregnant or lactating women; Female of childbearing potential must have a negative βsubunit hCG pregnancy test immediately prior to study entry.
- Frequent follow-up visits
- A medical monitor and Independent Data Monitoring Committee (iDMC) will monitor the safety aspects of the study (see sections 8.6).

1.3 Study Treatment

This is a blinded randomized clinical study in which each patient will receive a single treatment of either 0.50 mg/kg AB103 or placebo (0.9% Sodium Chloride sterile solution) administered intravenously at one of 2 time points: (A) during surgery or (B) shortly after surgery, as long as the administration will be at the earliest time possible and within 6 hours from the clinical diagnosis. Administration will be based on clinical diagnosis of bacterial NSTI at the study site and a decision for urgent surgical wound debridement, and upon confirming the diagnosis of NSTI during the debridement.

The intravenous infusion will be delivered by a syringe pump (may be manually pushed if approved by the medical monitor) over 10 minutes in a separate catheter than that used to deliver other medications. The syringe will identify the contents as “study drug” for protocol ATB-202. All study personnel and clinical staff, as well as the patient, shall remain blinded as to the actual composition of the study drug administered to a given patient. Only the assigned study pharmacist or authorized qualified designate preparing the drug shall be unblinded to the treatment.

1.4 Compliance Statement

This clinical study will be conducted according to the Declaration of Helsinki. The study will be conducted in compliance with this protocol, GCP (CPMP/ICH/135/95), designated SOPs, and with local laws and regulations relevant to the use of new therapeutic agents in the country of conduct.

1.5 Study Population

The study population will consist of patients with a clinical diagnosis of NSTI scheduled to undergo urgent surgical exploration and debridement. At the time of surgery, the diagnosis of NSTI shall be confirmed.

290 patients will be recruited into the study and randomized to either 0.5 mg/kg AB103 or placebo in a 1:1 ratio. Randomization will be stratified by the diagnosis of Fournier’s Gangrene and magnitude of mSOFA score at screening categorized as 3-4 versus >4. In the retrospective study analysis set with baseline mSOFA ≥ 3 (N=69), the median value was 4 and 46.6% had values larger than 4.

2. STUDY OBJECTIVES

2.1 Primary Objective

To demonstrate the efficacy of AB103 as compared to placebo, in patients diagnosed with NSTI, using a clinical composite success endpoint:

The NSTI clinical composite score, NICCE will be defined as the primary end point which will be composed of the following patient outcomes (i) Alive at Day 28, (ii) Day 14 debridements ≤3 (iii) No amputation done after the first debridement (iv) Day 14 modified SOFA (mSOFA) score ≤1 (mSOFA includes respiratory, cardiovascular, CNS, renal, and hematologic organ components but not hepatic/liver component) (v) Reduction of ≥3 score points between Baseline and Day 14 mSOFA score.

Table 4: NICCE Components

Component	NICCE
Survival	• Alive at Day 28
Local component	• ≤3 debridements throughout Day 14 • No amputation beyond first debridement
Systemic component	• mSOFA score of ≤1 at Day 14 • Reduction of ≥3 score points between Baseline and Day 14 mSOFA score

To be considered a success, patients must meet all components of the NICCE. A two-sided α=0.01 will be used to test superiority of AB103 relative to placebo for the primary endpoint (NICCE).

Conditional co-primary end point:

To demonstrate the efficacy of AB103 as compared to placebo, in patients diagnosed with NSTI, using a modified clinical composite success endpoint that includes (i) Alive at Day 28, (ii) Day 14 debridements ≤3 (iii) No amputation done after the first debridement. To be considered a success, patients must meet all 3 components of this modified composite endpoint.

The conditional co-primary end point will only be tested if superiority is demonstrated for the primary endpoint at a two-sided α=0.01 significance level in order to account for multiple comparisons. A two-sided α=0.05 will be used to test superiority of AB103 relative to placebo for the co-primary endpoint.

2.2 Secondary Objectives:

- To demonstrate the safety of AB103 when administered as a single dose of 0.50 mg/kg to patients diagnosed with NSTI
- To demonstrate the efficacy of AB103 as compared to placebo, in patients diagnosed with NSTI, using the 5 component clinical composite endpoint but with mSOFA ≤1 at Days 21 and 28

- Comparison of the distribution of mSOFA scores between treatment groups at Days 14 and 21
- Kaplan-Meier analysis of the time to resolution of mSOFA score to ≤ 1 with censoring at Day 14
- To demonstrate the efficacy of AB103 compared to placebo as related to the individual components of the NICCE in patients with NSTI
- To demonstrate efficacy of AB103 in relation to the critical care and hospital stay parameters:
  a. ICU days
  b. ICU-free days
  c. Days on ventilator
  d. Ventilator free days
  e. Vasopressor days
  f. Vasopressor free days
  g. Hospital length of stay
- To demonstrate the efficacy of AB103 in patients with septic shock
- To determine the incidence of Stage 2 and 3 acute kidney injury (AKI) (using the KDIGO criteria) and compare the rates of complete recovery by Day 28 between the AB103 treated patients and the placebo treated patients
- Additional AKI endpoints:
  - To determine the incidence of Stage 2 and 3 AKI (using the KDIGO criteria) and compare the rates of complete and partial recovery by Day 28 between AB103 and placebo treated patient
  - Determine the incidence of Stage 1, 2 and 3 AKI and compare the rates of complete recovery between the AB103 and placebo treated patients
  - Determine the incidence of Stage 1, 2 and 3 AKI and compare the rates of complete and partial recovery between the AB103 and placebo treated patients
  - Calculate time to complete recovery from AKI
  - Calculate time to partial recovery from AKI
- To demonstrate the safety of AB103 in regard to secondary infections

2.3 Exploratory Objectives:

- To conduct an exploratory evaluation of the clinical response by baseline pathogen
- To evaluate microbiological outcome in the microbiological evaluable population
- To conduct an exploratory evaluation of plasma and urinary biomarkers in patients with AKI
- To conduct an exploratory evaluation of blood leukocyte transcriptome (RNA expression) profiling in patients with NSTI and compare genomic profile in patients treated with AB103 versus placebo
- To evaluate health economic and financial outcomes associated with the treatment
- To evaluate NICCE outcome in screen failure patients with baseline mSOFA = 2 and post-operative mSOFA ≥ 3. Note: These patients will not be included in the

MITT population analysis (they will not receive blinded study drug) and only considered to be an observational cohort

- To evaluate the immunogenicity of Reltecimod (formerly AB103)

2.4 Study Hypothesis

The primary hypothesis of this study is that in addition to SoC, AB103 will demonstrate a clinically significant treatment benefit over placebo.

This hypothesis will be addressed by measuring the effect of AB103 on a composite of clinical parameters associated with the disease course of patients with NSTI, using a responder analysis. A responding patient must meet all the 5 parameters of the composite clinical success end point, while a non-responding patient can fail by not meeting any one of the parameters. These analyses are designed to demonstrate that in addition to being safe, one dose of 0.5 mg/kg of AB103 will:

Improve systemic signs of the infection by improving organ function of patients compared to placebo as measured by:

- Survival at Day 28
- mSOFA score on Day 14 and change from baseline to Day 14 ≥ 3. Day 14 mSOFA score of ≤ 1 and a change from baseline (pre-treatment) to Day 14 ≥ 3 will be required for a patient to achieve the primary composite clinical success endpoint (NICCE)

Improve the local signs of the infection, as measured by:

- Reduced number of debridements, counted to Day 14. No more than 3 debridements to Day 14 will be required for a patient to achieve composite clinical success
- No amputation after the first debridement (amputation on the first debridement is not considered a failure). A patient will be required to have had no amputations done after the first surgical procedure in order to achieve composite clinical success.

A full response will be considered as meeting **all** the above systemic and local criteria for response, in addition to being alive at Day 28.

A conditional co-primary endpoint will be evaluated only if the two-sided p-value for the primary responder analysis is ≤ 0.01.

The conditional co-primary endpoint also involves a responder analysis that involves being alive at Day 28 and demonstrating improvement in the local signs of infection as measured by:

- A reduced number of debridements, counted to Day 14. A patient with ≤ 3 debridements to Day 14 will be considered a responding patient
- No amputation after the first debridement (amputation during the first debridement is not considered a failure). A patient with no amputations performed after the first surgical procedure will be considered a responding patient in relation to this parameter.

3. SELECTION OF STUDY POPULATION

To be enrolled in the study, patients must meet ALL of the inclusion criteria and NONE of the exclusion criteria.

3.1 Inclusion Criteria

1. Age: ≥12 years
2. Surgical confirmation of NSTI by attending surgeon (e.g. presence of necrotic tissue, thrombosed vessels in the subcutaneous tissue, lack of bleeding and "dishwater" (cloudy, thin, gray) fluid) due to presumed bacterial infection (necrotizing cellulitis (most commonly group A strep), necrotizing fasciitis, necrotizing myositis and myonecrosis, NSTI of the perineum, bacterial synergistic gangrene, Clostridial gas gangrene***) that may be supported by specific signs and symptoms (e.g. tense edema outside area of compromised skin, pain disproportionate to appearance, skin discoloration, ecchymosis, blisters/bullae, necrosis, tense edema, crepitus and/or subcutaneous gas).***
    - Patients with NSTI following intra-abdominal operation are eligible if adequate source control of intra-abdominal process has been established
3. mSOFA score ≥3 (in any one or combination of the 5 major components of SOFA score with any one organ component having a score of at least 2: cardiovascular, respiratory, renal, coagulation, CNS), measured as close as possible to the first debridement, (but before first debridement is performed).
4. IV drug administration within 6 hours from the clinical diagnosis and the decision at the study site, to have an urgent surgical exploration and debridement (drug should not be administered until surgical confirmation is established).
5. If a woman is of childbearing potential, she must consistently use an acceptable method of contraception from baseline through Day 28. Acceptable birth control methods include oral contraceptive medication, an intrauterine device (IUD), an injectable contraceptive (such as Depo-Provera®), a birth control patch, a barrier method (such as condom or diaphragm with spermicide) or abstinence.
    - Non-childbearing potential is defined as current tubal ligation, hysterectomy, or ovariectomy or post-menopause (1 year without menses with an appropriate clinical profile at the appropriate age e.g. >45 years).
6. If a male patient's sexual partner is of childbearing potential, the male patient must acknowledge that they will consistently use an acceptable method of contraception (defined above) from baseline through Day 28.
7. Signed and dated ICF as defined by the IRB and, if applicable, California Bill of Rights. By signing the ICF, the patient agrees to release any medical records pursuant to current HIPAA Guidelines. If patient is unable to comprehend or sign the ICF, patient's legally acceptable representative may sign the ICF.

3.2 Exclusion Criteria

1. BMI >51;
2. Patient who has been operated at least once for the current NSTI infection and had a curative deep tissue debridement (patients who underwent prior diagnostic minor surgery are allowed to enter into the study);
3. Patients with overt peripheral vascular disease in the involved area - associated with ischemic wounds/ulcers or gangrene, and /or other significant symptoms of inadequate vascular supply or where limb amputation is considered likely within 7 days due to the peripheral vascular disease;
4. Diabetic patients with peripheral vascular disease who present with below the ankle infection;
5. Patient with burn wounds;
6. Current condition of: (a) Inability to maintain a mean arterial pressure > 50 mmHg and/or systolic blood pressure > 70 mmHg for at least 1 hour prior to screening despite the presence of vasopressors and IV fluids or (b) a patient with respiratory failure such that an SaO_2 of 80% cannot be achieved or (c) a patient with refractory coagulopathy (INR >5) or thrombocytopenia (platelet count <20,000) that does not partially correct with administration of appropriate factors or blood products;
7. Chronic neurological impairment that leads to a neuro mSOFA component ≥2;
8. Recent cerebrovascular accident in the last 3 months;
9. Patients with cardiac arrest requiring cardiopulmonary resuscitation within the past 30 days;
10. Patient is not expected to survive throughout 28 days of study due to underlying medical condition, such as poorly controlled neoplasm (e.g. Stage III or IV cancer);
11. Patient or patient's family are not committed to aggressive management of the patient's condition, or the combination of necrotizing skin infection and underlying illness makes it unlikely that life support will be maintained;
12. Any concurrent medical condition, which in the opinion of the Investigator, may compromise the safety of the patient or the objectives of the study or the patient will not benefit from treatment such as:
    - CHF {NYHA class III-IV}
    - Severe COPD {GOLD stage III-IV. or chronic hypoxemia (PaO_2 <55 mmHg) on room air, or chronic use of home ventilation, or unable to climb stairs or perform household duties due to chronic obstructive disease resulting in severe exercise restriction, or use of continuous home oxygen prior to hospital admission (sleep apnoea treated with continuous positive airway pressure or biphasic positive airway pressure oxygen during sleep is acceptable)}
    - Liver dysfunction {Childs-Pugh class C}
    - Immunosuppression (see Appendix F, Section 15.6 for list of excluded immunosuppressive medications)
    - Neutropenia < 1,000 cells/mm^3not due to the underlying infection
    - Idiopathic Thrombocytopenic Purpura

- Receiving or about to receive chemotherapy or biologic anti-cancer treatment although hormonal manipulation therapies for breast and prostate malignancies are permitted
- Hematological and lymphatic malignancies in the last 5 years

13. Known HIV infection with CD4 count < 200 cells/mm3 or < 14% of all lymphocytes;
14. Patients with known chronic kidney disease (documented pre-illness creatinine value(s) ≥2.0) or patients receiving renal replacement therapy for chronic kidney disease: either hemodialysis, peritoneal dialysis, hemofiltration such as Continuous Veno-Venous Hemofiltration (CVVH) or hemodiafiltration
15. Patients that are treated with continuous hemofiltration (e.g. Continuous Veno-Venous Hemofiltration) for acute kidney dysfunction, not due to NSTI, starting prior to study drug administration.

    Exception: Patients with acute kidney dysfunction due to NSTI may be enrolled in the event that the patient is off the treatment from time of study drug administration and up to at least one hour post study drug administration.

16. Pregnant or lactating women; Women of childbearing potential must have a negative β-subunit hCG pregnancy test immediately prior to study entry.
17. Previous enrollment in a clinical trial involving investigational drug or a medical device within 30 days before provision of written informed consent for the study or within five half-lives of the investigational drug, whichever is longer.
18. Previous enrollment in this protocol, ATB-202 or the Phase 2 NSTI trial of AB103, ATB-201, or the current Phase 2 SA-AKI trial of AB103, ATB-203.

3.3 Withdrawal

Patients will be informed that they are free to withdraw from the study at any time and for any reason. The date the patient is withdrawn from the study and the reason for withdrawal will be recorded in the eCRF and in the source documentation.

3.3.1 Criteria for Premature Discontinuation from Study

In rare instances, the patient (or Investigator) may determine that he/she is not able to make all visits required by the protocol. Possible reasons for discontinuation from the study may be:

- Withdrawal of consent
- Lost to follow-up
- Other reasons such as administrative reasons

4. INVESTIGATIONAL PLAN

4.1 Study Rationale

4.1.1 Dose Selection Rationale

Selection of a dose regimen for the pivotal Phase 3 study was based on the safety results from the Phase 1 and 2 trials and efficacy results from Phase 2. Evidence for dose response in the Phase 2 study is based on consistency across multiple domains of outcome variables. In several efficacy parameters, a linear dose response emerged for individual endpoints. In addition, to adjust for group differences at baseline and for the distorted distribution of values, non-parametric comparisons between dose groups were used, which indicated that 0.5 mg/kg had an advantage over the 0.25 mg/kg, especially in cases of patients that stayed for a longer time in the ICU or required ventilation. Overall, based on the reproducibility of the dose response in the various parameters, and given that there was no change in the safety profile when increasing the dose from 0.25 mg/kg to 0.50 mg/kg, Atox Bio believes that the 0.5 mg/kg given as a single dose is the optimal dose for the proposed Phase 3 ATB-202 clinical trial.

The comparative effect of high and low dose groups is shown in Table 5.

Table 5: Comparative effect of high and low dose groups – Study ATB-201

Outcome	0.5 mg/kg	0.25 mg/kg
Total SOFA at Day 14	√	
Individual organ SOFA score at Day 14	√	
Changes in total score between days 1-14	√	
Percent of patients with organ failure at Day 14	√	
SOFA score in patients with established organ failure at Day 1	√	
Debridements to Day 7 or 28	√	
ICU stay	√	√
Ventilation days	√	√
Hospital stay	√	√
Inflammatory biomarkers (systemic)	√	
Inflammatory biomarkers (local infection site)	√	
Mortality	√	√

√= higher effect size compared to Placebo.

The determination of this dose has also been discussed with the FDA. Overall, the dose and dosing regimen for the pivotal study is based on: (i) the Phase 2 results demonstrated a strong and consistent effect of the 0.5 mg/kg; (ii) the pre-clinical data demonstrated that the 5 mg/kg dose (equivalent to human 0.5 mg/kg dose) consistently provided optimal efficacy; (iii) pre-clinical data demonstrated a bell shape dose response curve, common to many immunomodulatory and peptide therapies, in which a single dose of 5 mg/kg (mouse) is optimal, with higher doses being less efficacious.

Therefore, a single dose of 0.5 mg/kg was considered to provide the most favorable risk/benefit for this patient population with severe and potentially life-threatening necrotizing skin and skin structure infection.

4.2 Allocation of Treatment, Randomization and Study Drug Administration

4.2.1 Allocation of Treatment and Randomization

Once the patient has a clinical diagnosis of bacterial NSTI at the study site and a decision for urgent surgical wound exploration and debridement has been taken, all inclusion/exclusion criteria have to be verified and an ICF has to be obtained. The pharmacist should be notified. The pharmacist will get the treatment allocation via a web-based dedicated (Interactive Web Response, IWR) software available only to the study pharmacist. All other study-related personnel, hospital care givers, the Sponsor and the patient will remain blinded.

Following allocation of treatment and surgical confirmation of NSTI, the study pharmacist will prepare the study drug (sites may choose to have the study pharmacist prepare study drug after treatment allocation but before surgical confirmation of NSTI but study drug can only be administered upon surgical confirmation of NSTI). When the study drug is ready for infusion, last verification of inclusion/exclusion criteria will take place pertaining to the 6 hours' time limit for drug administration (and any significant change in patient condition that would warrant the patient's exclusion from the study).

4.2.2 Study Drug Administration

The drug can be administered at either one of 2 time points: Option A: during surgery and Option B: after surgery. In both instances, the study drug administration must be initiated within 6 hours of the time at which the patient was clinically diagnosed at the study site as having NSTI and the decision was made to undergo urgent surgical debridement. The NSTI diagnosis should also be confirmed surgically, and in case it is not confirmed surgically the patient will not receive the drug and will be considered as a screening failure.

In case study drug has been prepared but a patient did not receive it, the web-based dedicated software will be updated to ensure an overall 1:1 ratio between the AB103 and placebo treatment groups.

4.3 Schedule of Procedures/Visits

A schedule of study assessments is shown in Appendix A, Section 15.1 the protocol. Visiting nursing services, including Symphony Clinical Research, are allowed for outpatient visits. Symphony Clinical Research is contracted to evaluate subjects as outpatients as early as Day 10 through Month 3.

4.3.1 Visit 1: Screening

Evaluation starts once a clinical diagnosis of NSTI was entertained and a decision for urgent surgical wound exploration and debridement was made:

- Obtain ICF

- Verification of Inclusion/Exclusion criteria
- Randomization & study drug preparation (study drug should not be prepared until there is surgical confirmation of NSTI)
- Demographics, Medical history, Infected lesion history, Concomitant medications, Vital signs, Height, Weight, calculate BMI, PE
- Urine output recording to start from time of ICU admission and while Foley catheter is in place (captured in 6 hour intervals based on calendar day)
- Recording baseline signs and symptoms and AEs/SAEs (AEs of Day 0 are AEs/ SAEs starting after obtaining ICF until study drug administration)
- Safety baseline laboratory tests:
    - Full chemistry panel
    - Obtain complete blood count (CBC) with differential and platelet count (sample can be same as that required to be taken in conjunction with the RNA expression profile test sample)
- C-reactive protein (CRP)
- Arterial blood gases and FiO2 (if applicable) -may use SpO2 value if arterial blood gas is not clinically indicated
- Collect data on acute renal replacement therapy
- Collect data on plasma or platelet transfusion
- Blood culture for both aerobic and anaerobic bacteria
- Pregnancy test if applicable (blood or urine whichever is faster)
- Screening mSOFA score measurements must be performed prior to first surgery: should be evaluated any time after arrival at the hospital (may include referring hospital), although no more than 6 hours prior to anticipated first surgery. Sites are encouraged to re-evaluate mSOFA score as close to surgery as feasible for patients not meeting mSOFA ≥3 inclusion criteria during initial screening. Baseline mSOFA will include measurements of the score components in the following organ/systems (with any one organ having a score of at least 2 at screening): respiratory, cardiovascular, renal, coagulation and CNS
- APACHE II score, LRINEC and Anaya scores (measurement for these scores will be collected and scores will be calculated retrospectively). The clinical parameters of these scores (respiratory, cardiovascular, vital signs) should be taken as close as possible to the surgery, but prior to the surgical anesthesia or any other surgical related procedures e.g. mechanical ventilation. The worst laboratory values available within 24 hours of surgical intervention for NSTI should be recorded. Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU and hospital admission and discharge)
- Local lesion assessment and evidence of amputation (excision to a joint space)
- Surgery for NSTI and confirmation of diagnosis. Record time of surgery
- During surgery: Tissue samples for microbiology
- 12 Lead ECG (can be performed any time during screening period or just prior to study drug administration)

- Upon study drug administration patient is transited to visit 2 (either during or after the surgery)
- Collect blood for serum for storage (immunogenicity testing)
- Collect urine and blood for plasma for storage (AKI biomarker analysis)
- Collect whole blood for leukocyte transcriptome (RNA expression) profiling
- Perform randomization

4.3.2 Visit 2 (Day 1)

Starts ONLY upon study drug administration and terminates at the end of the calendar day. Study drug may be given either during surgery or immediately after the surgery.

- Drug administration should be completed within 6 hours from the clinical diagnosis of NSTI and a decision at the study site for urgent surgical wound exploration and debridement
- Collect data on operative procedures(see Section 6.2.6.1 for description of adjudication of operative procedures for assignment of study defined debridement)
- Collect data on acute renal replacement therapy
- Collect data on plasma or platelet transfusion
- Collection of AEs/ SAEs
- Concomitant medications
- Vital signs (first set of vitals on that day)
- Symptom-driven interim PE
- 12-Lead ECG at the end of study drug infusion, at 4-6 hours and 12-24 hours post administration of study drug
- Urine output (should be recorded continuously from time of ICU admission to Day 7, if still in ICU and Foley catheter in place, using 6 hour intervals and by calendar day)
- Weight, only if not collected at screening
- Collect data on additional amputations
- Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU /hospital stay)
- Systemic response: mSOFA score. Measurements will be collected within 16-24 hours post study drug administration, unless the patient is scheduled to undergo additional debridement; in such case data collection for mSOFA parameters will be taken prior to the next debridement
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value (and record FiO2) if arterial blood gas is not clinically indicated
    - Obtain serum creatinine
    - Obtain CBC with differential and platelet count (sample can be same as that required to be taken in conjunction with the RNA expression profile test sample)
- Assess lesion status (healing/progression/significant change in appearance) if accessible for visible assessment at time of debridement or routine dressing change

- Collect whole blood for leukocyte transcriptome (RNA expression) profiling at 4-6 hours post study drug dose
- Survival

4.3.3 Visit 3 (Day 2)

- Collect data on operative procedures
- Urine output (should be recorded continuously from time of ICU admission to Day 7, if still in ICU and Foley catheter in place, using 6 hour intervals and by calendar day)
- Collection of AEs/ SAEs
- Collect data on additional amputations
- Collect data on acute renal replacement therapy
- Collect data on plasma or platelet transfusion
- Concomitant medications
- Vital signs (to be taken during the morning tests)
- Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU /hospital stay)
- Systemic response: mSOFA score (measurements will be collected and scores will be calculated retrospectively).
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value if arterial blood gas is not clinically indicated
    - Obtain serum creatinine
    - Obtain CBC with differential and platelet count (sample can be same as that required to be taken in conjunction with the RNA expression profile test sample but must be on the same calendar day as study Day 2)
- Assess lesion status (healing/progression/significant change in appearance) if accessible for visible assessment at time of debridement or routine dressing change
- Collect urine and blood for plasma for storage (AKI biomarker analysis) at 24±4 hours after study drug administration
- Collect whole blood for leukocyte transcriptome (RNA expression) profiling at 24±4 hours after study drug administration
    - CBC with differential required to be obtained in conjunction with sample for RNA expression profile best
- Record Survival

4.3.4 Visit 4 (Day 3)

- Collect data on operative procedures
- Obtain tissue for microbiology if debridement performed on Day 3
- Collect data on acute renal replacement therapy
- Collect data on plasma or platelet transfusion
- Urine output (should be recorded continuously from time of ICU admission to Day 7, if still in ICU and Foley catheter in place, using 6 hour intervals and by calendar

day). Collection of AEs/ SAEs including secondary infections (based on CDC definitions for healthcare associated infections)

- Collect data on additional amputations
- Concomitant medications
- Vital signs (to be taken during the morning tests)
- Symptom-driven interim PE
- Evaluation of adequacy of antimicrobial treatment, based on results from susceptibility testing once the data is available
- Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU /hospital stay)
- Systemic response: mSOFA score (measurements will be collected and scores will be calculated retrospectively)
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value (and record FiO2) if arterial blood gas is not clinically indicated
    - Obtain serum creatinine
    - Obtain CBC with differential and platelet count (sample can be same as that required to be taken in conjunction with the RNA expression profile test sample)
- Assess lesion status (healing/progression/significant change in appearance) if accessible for visible assessment at time of debridement or routine dressing change
- Collect whole blood for leukocyte transcriptome (RNA expression) profiling
    - CBC with differential required to be obtained in conjunction with sample for RNA expression profile best
- Record Survival

4.3.5 Visit 5 (Day7 [±1 day])

- Collect data on operative procedures
- Collect data on acute renal replacement therapy
- Collect data on plasma or platelet transfusion
- Urine output (should be recorded continuously from time of ICU admission to Day 7, if still in ICU and Foley catheter in place, using 6 hour intervals and by calendar day)
- Weight (if easily obtained)
- Collection of AEs/ SAEs including secondary infections (based on CDC definitions for healthcare associated infections)
- Collect data on additional amputations
- Concomitant medications
- Vital signs (to be taken during the morning tests)
- PE
- Safety laboratory tests (Chemistry, Hematology)

    - Sample for CBC with differential and platelet count can be same as that required to be taken in conjunction with the RNA expression profile test sample
- Assess lesion status (healing/progression/significant change in appearance) if accessible for visible assessment at time of debridement or routine dressing change
- Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU /hospital stay)
- Systemic response: collect data for mSOFA parameters
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value (and record FiO2) if arterial blood gas is not clinically indicated.
- Collect urine and blood for plasma for storage (AKI biomarker analysis)
- Collect whole blood for leukocyte transcriptome (RNA expression) profiling
    - CBC with differential required to be obtained in conjunction with sample for RNA expression profile best
- Record Survival

4.3.6 Visit 6 (Day 10 [±1 day]; perform only if patient is still hospitalized):

- Collect data on operative procedures
- Collection of AEs/SAEs, including secondary infections (based on CDC definitions for healthcare associated infections)
- Collect data on additional amputations
- Collect data on acute renal replacement therapy
- Collect data on plasma or platelet transfusion
- Selected concomitant medications (e.g. vasopressors, antimicrobials)
- Symptom-driven interim PE
- Vital signs
- Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU /hospital stay)
- Systemic response: collect data for mSOFA parameters
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value (and record FiO2) if arterial blood gas is not clinically indicated
    - Obtain serum creatinine
    - Obtain CBC and platelet count (differential not required for Day 10)
- Assess lesion status (healing/progression/significant change in appearance) if accessible for visible assessment at time of debridement or routine dressing change
- Record Survival

4.3.7 Visit 7 (Day 14 [±1 day])

- Collect data on operative procedures
- Weight

- Collection of AEs/SAEs, including secondary infections (based on CDC definitions for healthcare associated infections)
- Collect data on additional amputations
- Collect data on acute renal replacement therapy
- Collect data on plasma or platelet transfusion
- Selected concomitant medications (e.g. vasopressors, antimicrobials)
- Safety laboratory tests (Chemistry, Hematology)
- Collect blood for serum for storage (immunogenicity testing)
- PE
- Vital signs
- CRP
- Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU /hospital stay)
- Systemic response: collect data for mSOFA parameters
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value (and record FiO2) if arterial blood gas is not clinically indicated.
    - In case a patient is discharged from hospital prior to these days SOFA parameters should be obtained on the same day of discharge.
- Assess lesion status (healing/progression/significant change in appearance) if accessible for visible assessment at time of debridement or routine dressing change
- Record Survival

All efforts should be made to collect these data, but in case parameters cannot be collected, phone follow-up will be performed, and information that could be collected over the phone will apply.

4.3.8 Visit 8 (Day 21 [±1 day])

- Collection of AEs/SAEs, including secondary infections (based on CDC definitions for healthcare associated infections)
- Collect data on additional amputations
- Collect data on acute renal replacement therapy
- Collect data on plasma or platelet transfusion
- Selected concomitant medications (e.g. vasopressors, antimicrobials)
- Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU /hospital stay)
- Vital Signs
- Systemic response: collect data for mSOFA parameters
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value (and record FiO2) if arterial blood gas is not clinically indicated
    - Obtain serum creatinine
    - Obtain CBC and platelet count (differential not required for Day 21)

- Record Survival

4.3.9 Visit 9 (Day 29 [+2 days])

- Weight
- Collection of AEs/SAEs, including secondary infections
- Collect data on additional amputations
- Selected concomitant medications (e.g. vasopressors, antimicrobials)
- Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU /hospital stay)
- Systemic response: collect data for mSOFA parameters
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value (and record FiO2) if arterial blood gas is not clinically indicated
    - Obtain serum creatinine and albumin
    - Obtain CBC with differential and platelet count
- Collect blood for serum for storage (immunogenicity testing)
- Vital signs
- Collect data on acute renal replacement therapy
- Collect data on plasma or platelet transfusion
- Collection of health economic information: will include several parameters, such as (but not limited to): ICU charges, hospital stay (regular floor) charges, direct costs, indirect costs, reimbursement payments
- Record any hospital re-admissions (if previously discharged for original NSTI related hospitalization)
- Record Survival

All efforts should be made to collect these data, but in case parameters cannot be collected, phone follow-up will be performed, and information that could be collected over the phone will apply

4.3.10 Visit 10 (3 months [±5 days])

The 3-month visit is a follow-up visit to assess the long-term health condition of the patients including assessment of renal function in patients with documented persisting AKI at the Day 29 visit and ascertainment of readmission(s) within 30 days of discharge from original NSTI related hospitalization. Creatinine should be obtained at this visit given the requirement to also obtain serum for storage (for immunogenicity testing). The serum for storage (immunogenicity testing) has priority over creatinine in the order of blood draw if the blood drawn is limited.

4.3.11 Follow-up of Patients

Patients who received the study drug are required to complete the procedures outlined for this clinical study. Exceptions would only include the withdrawal of consent, lost to follow-up or death.

If a patient misses a scheduled visit, attempts to complete the patient data will be made via telephone or rescheduling the visit. After waiting one week for the response, a return-receipt, certified letter containing instructions to call for an appointment will be sent. If no response is obtained within one week after sending the return-receipt letter, the patient should then be considered as lost to follow-up. The returned receipt should be filed with the Investigator's copy of the patient's eCRF. A clinical evaluation should be made based on the last contact with the patient.

Serious Adverse Events (SAEs) occurring within 28 days of a patient receiving IMP will be recorded and processed as described in Section 8.4 of the protocol.

4.4 Sub-study mSOFA = 2

Projected number of patients 60. Note: These patients will not will not receive blinded study drug and are only considered to be an observational cohort.

4.4.1 Screening Visit for patients with mSOFA =2

Evaluation starts once a clinical diagnosis of NSTI was entertained and a decision for urgent surgical wound exploration and debridement was made:

- Obtain ICF
- Verification of Inclusion/Exclusion criteria
- Register in IWR
- Demographics, Medical history, Infected lesion history, Concomitant medications, Vital signs, Height, Weight, calculate BMI, PE
- Recording baseline signs and symptoms and AEs/SAEs (AEs of Day 0 are AEs/ SAEs starting after obtaining ICF until study drug administration)
- Pregnancy test if applicable (blood or urine whichever is faster)
- Baseline mSOFA per section 4.3.1. Baseline mSOFA (where 2 organ components score a 1 or one component scores a 2) will include measurements of the score components in the following organ/systems: respiratory, cardiovascular, renal, coagulation and CNS.
- Post-operative mSOFA score measurements to be performed after surgery and within 6 hours of decision for urgent surgical wound exploration and debridement
    - Repeat complete blood count (CBC)
    - Repeat creatinine
    - Arterial blood gases and FiO2 (if applicable) -may use SpO2 value if arterial blood gas is not clinically indicated
- Critical care parameters (dates and times of assisted ventilation, vasopressor use, ICU and hospital admission and discharge)
- Local lesion assessment and evidence of amputation (excision to a joint space)
- Surgery for NSTI and confirmation of diagnosis. Record time of surgery
- Record Survival

4.4.2 Day 7 or Day 10 Visit [±1 day]

- Collect data on operative procedures

- Collection of AEs/SAEs, including secondary infections (based on CDC definitions for healthcare associated infections)
- Collect data on additional amputations
- Collect CBC and creatinine
- Systemic response: collect data for mSOFA parameters
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value (and record FiO2) if arterial blood gas is not clinically indicated.
    - In case a patient is discharged from hospital prior to these days SOFA parameters should be obtained on the same day of discharge.
- Record Survival

4.4.3 Day 14 Visit [±1 day]

- Collect data on operative procedures
- Collection of AEs/SAEs, including secondary infections (based on CDC definitions for healthcare associated infections)
- Collect data on additional amputations
- Collect CBC and creatinine
- Systemic response: collect data for mSOFA parameters
    - All efforts should be made to obtain the actual measurements of SOFA parameters at these visits-may use SpO2 value (and record FiO2) if arterial blood gas is not clinically indicated.
    - In case a patient is discharged from hospital prior to these days SOFA parameters should be obtained on the same day of discharge.
- Record Survival

4.4.4 Day 29 Visit [+2 days] **(via phone if patient no longer hospitalized)**

- Collect data on additional amputations
- Record Survival

5. TREATMENT OF PATIENTS

5.1 Investigational Medicinal Product

This is a blinded randomized clinical study with 2 treatment groups consisting of 0.50 mg/kg AB103 and placebo. Patients will be randomized in a 1:1 ratio. Each patient will be administered a single intravenous infusion of AB103 based on actual body weight to be infused over 10 minutes. Volume of administration will depend on patients' actual weight.

The study drug can be administered during or after surgical exploration and debridement but no later than 6 hours from the time of clinical diagnosis of NSTI and a decision at the study site for urgent surgical wound exploration and debridement.

AB103 will be supplied in glass vials as a lyophilized powder to be reconstituted with sterile water for injection (WFI). Drug will be reconstituted on the day of its administration, in close proximity to infusion time, and several vials will be pooled together to compose the requested dose. Final volume for infusion will be calculated based on the patient's weight plus adequate priming volume of the IV line.

Placebo will be pyrogen-free, preservative-free sterile 0.9% saline, USP calculated based on the patient's weight, plus adequate priming volume of the IV line.

5.1.1 Presentation and Formulation

AB103 is the acetate salt of a 10 amino acid synthetic peptide. AB103 has homology to amino acid residues 8-15 of the T-lymphocyte molecule CD28, and has D-Ala residues abutted to N- and C-termini to render them more protease resistant. The final formulated drug product is a lyophilized powder containing 10.5 mg of peptide AB103 per vial, 300 mg of mannitol, and 36 mg of NaCl, to be reconstituted with 10.5 mL of sterile WFI. The reconstituted AB103 drug product contains 1 mg/mL of the AB103 peptide. The drug product is filled into clear, 20 mL Type I glass vials with a 20 mm neck, and is then lyophilized. Flurotec-coated rubber lyophilization stoppers are used and 20 mm blue aluminum overseals.

Each vial of the drug product to be used in clinical trials is reconstituted with 10.5 mL of WFI to deliver 10 mLof solution, or 10 mg of AB103. Each vial contains 10.5 mg of peptide AB103, 300 mg of mannitol, and 36 mg of NaCl to be reconstituted with 10.5 mL of WFI.

The lyophilized AB103 drug product is stored at -20° C ±5° C. Prior to use, each vial of drug (10.5 mg) is solubilized in 10.5 mL of water for injection to generate a drug concentration of 1 mg/mL.

The reconstituted drug product is administered directly and is not diluted prior to administration. To prepare the drug quantity needed for infusion, the contents of several vials need to be pooled together by the study pharmacist, under sterile conditions. The number of vials would depend on the patient's weight, in order to achieve a dose of 0.5 mg/kg. The AB103 drug product will be administered via a syringe infusion pump (may be manually pushed if approved by the medical monitor) in a separate catheter as a single intravenous infusion given over 10 minutes at a dose of 0.5 mg/kg.

The manufacturer of the clinical batches of the drug substance is Bachem Americas, Inc., Torrance CA. The manufacturer of the drug product is Pyramid Laboratories Inc., Costa Mesa, CA.

5.1.2 Storage

IMP will be stored in a limited access area, under appropriate environmental conditions. AB103 should be stored at -20°C ± 5°C in a secure, temperature-monitored freezer located in Investigational Drug Service. Reconstituted investigational agents may be stored at room temperature for up to 6 hours Study drug infusion should be performed as soon as possible but within the 6 hour window previously described. If reconstituted drug is not administered it should be discarded.

5.1.3 Administration

AB103 and placebo, formulation and/or preparation of each AB103 or placebo will be performed by the clinical site pharmacist. This process will be performed so that the clinical staff and Investigators will be blinded to each patient's treatment assignment and only the pharmacist will be unblinded. Administration will be by IV infusion using a syringe pump (may be manually pushed if approved by the medical monitor) over a 10 minute period.

5.1.4 Accountability and Destruction of Surplus Investigational Product

Authorization of the site to receive supplies of IMP will be given by Atox Bio/designee upon receipt of relevant essential documentation (for details refer to the study procedures manual). A designated individual at each site will maintain a log of IMP dispensed for each patient. In addition, the Investigator or designated individual at each site will maintain an IMP Accountability Log. This will include the description and quantity of IMP received at the study site, as well as a record of when and to whom it was dispensed.

All used and unused supplies will be retained at the investigational site until the study monitor gives instructions for their return or disposal. The IMP supplied for this study is investigational and under no circumstances may be used for purposes other than those described in this protocol.

The Investigator agrees not to supply the IMP to any person other than the patients participating in the study. IMP may not be relabeled or reassigned for use by other patients except under special circumstances previously approved by Atox Bio.

The Investigator will retain and store all original vials until these vials are inventoried by the unblinded study site monitor. Unless otherwise instructed by the Atox Bio medical monitor, the Investigator agrees to return all original vials at the end of the study, whether empty or containing IMP, to Atox Bio or designee. The Investigator agrees neither to dispense the IMP from, nor store it at any site other than the study sites agreed upon.

A separate unblinded study monitor will ensure proper disposition of original vials whether empty or full with returned or unused IMP. Appropriate IMP disposition documentation will be maintained as part of the source documentation. Permission may be granted for local disposal with supporting documentation.

5.2 Concomitant Medications

5.2.1 Disallowed Medications

Study participants should not receive immunosuppressant agents or chemotherapy and should preferably not be given high doses of steroids (defined as >40 mg or 0.5 mg/kg prednisone (or steroid with equivalent activity)/day for ≥2 weeks (see Appendix F – Section 15.6).

NOTE: Adrenal replacement therapy (corticosteroids) is permitted in patients with septic shock at the discretion of the Investigator balancing the potential benefits and risks in this situation. The optimal treatment regimen in this setting is not resolved. Investigators are referred to current Surviving Sepsis Campaign guidelines (www.survivingsepsis.org) as a reasonable source of guidance for steroid replacement therapy in septic shock patients.

5.2.2 Allowed Medications

5.2.2.1 Antimicrobial agents

All patients will receive one or more antimicrobial drugs used as SoC to treat the primary or subsequent infections. Antimicrobial agents include antibacterial, anti-fungal, anti-viral and anti-parasitic drugs. Topically applied antimicrobial agents should be included. Any other medications whether drugs or biologics used to treat the patient should be identified as non-antimicrobial medications.

Each antimicrobial agent will be identified by its generic or trade name and reported in the eCRF. Information should include the dose, dosing frequency, route of administration, duration (start and stop times and dates), and reason for administration (to treat primary or secondary infection or for surgical prophylaxis).

5.2.2.2 Non-antimicrobial medications

All non-antimicrobial (ancillary) drugs administered from the time of patient screening until end of study will be recorded in the eCRF. Information to be collected should include generic or trade name, route of administration, duration (start and stop dates) and reason for administration. A list of medications that are not required to be captured in the ancillary medication eCRF is provided in the Study Procedure Manual.

5.3 Study Medication Compliance

Compliance will be monitored by recording the total volume of each investigational agent actually delivered to each study patient.

5.4 Precautions and Overdose

5.4.1 Study Drug - AB103

5.4.1.1 Precautions

Currently available safety information does not require any special precautions to be exercised during treatment with AB103. Analysis of safety data collected in AB103 Phase 1 and 2 studies, did not reveal AEs that were determined as related to AB103 (including

possibly, probably and definitely related to study drug). In animal toxicology studies no target organs were identified.

5.4.1.2 Over dosage

To date, throughout the AB103 clinical development program, a single patient (study ATB-201) received an accidental overdose of approximately 30% (received 0.67 mg/kg instead of 0.50 mg/kg). This patient had no AEs determined as related to study drug. He had an SAE of acute renal failure that is expected in NSTI patients and was related to the patient's underlying NSTI. All the patient's AEs were resolved. In case of an overdose (defined as a 100% increase over the 0.5 mg/kg intended dose of AB103), the site staff should contact the medical monitor. Additionally, the patient should be monitored and followed-up according to the best medical practice.

5.4.2 Placebo Comparator

No known or expected adverse reactions are associated with pyrogen-free, preservative-free, physiologic saline.

5.4.2.1 Precautions

None for placebo comparator.

6. STUDY PROCEDURES

6.1 Informed Consent

The ICF must be in compliance with ICH GCP Guidelines, and in accordance with the Federal Regulations as detailed in 21 CFR §50.25 and the Declaration of Helsinki.

Prior to entering the study, the Investigator/designee must explain the nature of the study to each patient or legal representative, its purpose, expected duration, alternative forms of therapy available and the benefits and risks of study participation. After this explanation and before entering the study, the patient or legal representative must read and understand the ICF and voluntarily sign a consent form in the presence of the Investigator/designee.

The patient will be given a copy of the signed/dated ICF.

6.2 Clinical and Laboratory Procedures

6.2.1 Start of Drug Administration Clock

The IV drug administration clock of maximal 6 hours starts once a clinical diagnosis has been made in the study site and a documented decision is made to take the patient into the operating room for a surgical exploration and debridement and confirmation of the diagnosis. The exact time of decision to operate should be recorded.

6.2.2 Inclusion/Exclusion Criteria

The inclusion and exclusion criteria are specified in Sections 3.1 and 3.2. These criteria determine patient eligibility for recruitment. All patients must be evaluated for the presence of all inclusion criteria and the absence of all exclusion criteria before they are eligible to receive study drug.

6.2.3 Medical and Surgical History

A complete medical history identifying clinically relevant past medical and surgical conditions and active medical or surgical conditions will be reported on the Medical History eCRF. All chronic medications will be identified and characterized by trade or generic name, route of administration as well as reason for use.

Baseline signs and symptoms should be captured on the Adverse Event eCRF with attempts to capture dates and times that would identify them as pre-treatment emergent events.

Details of the NSTI will be recorded on the eCRF and include: “clinical diagnosis” (as a specific subset of NSTI) at the time of enrollment; location of primary infection; date of onset of symptoms, identified predisposing factors (trauma, surgery, IV drug use, and co-morbidities such as diabetes or vascular insufficiency; prior surgical procedures related to the etiology of NSTI; prior surgical procedures for the treatment of the primary infection site; antimicrobial use from the date of onset of current illness (may predate diagnosis of NSTI);. description of the NSTI lesion; date and time of surgical intervention for NSTI and surface area of NSTI debridement (when available).

6.2.4 Physical Examinations

Physical exam will include both the primary site of the NSTI and a clinically appropriate examination of vital organ systems. These should include cardiovascular, respiratory, abdomen, extremities, and neurologic body systems.

6.2.4.1 Interim physical examinations (symptom-driven)

Interim physical exam will be targeted to include clinically appropriate examination of specific organ systems based on patient related signs and symptoms.

6.2.5 Concomitant Medication

All concomitant medications antimicrobial & non-antimicrobial (with the exception of over the counter medications, vitamins, laxatives, intravenous fluid and electrolyte replacement, parenteral nutrition, topical medications and other selected medications as described in the Study Procedure Manual) will be entered into the eCRF and identified by their generic or trade name. Information about antimicrobial medications should include the dose, dosing frequency, route of administration, duration (start and stop times and dates), and reason for administration (to treat primary or secondary infection or for surgical prophylaxis). Information to be collected about non-antimicrobial medications should include generic or trade name, route of administration, duration (start and stop dates) and reason for administration.

Timing and adequacy of antibiotic therapy (based on institutional guidelines, baseline pathogens and antimicrobial sensitivity) will be reviewed by the Principle Investigator.

6.2.6 Primary Infection Site

The primary infection site will be described in detail. Any visible wound or drainage will be characterized as to amount, and cellular composition (purulent, sero-sanguineous, sanguineous, other). Tissue edema, focal pain and presence of subcutaneous crepitus, the presence of tissue discoloration, blisters, bullae or necrosis will be identified. The extent of progression of necrosis as well as the need for repeated surgical procedures will be documented by the Investigator.

6.2.6.1 Debridement

A debridement is defined as a surgical intervention, performed in the operating room to eliminate a substantial amount of necrotic tissue, based on the description by the surgeon in the operative note. Irrigation of the wound or dressing change in the operating room without tissue removal will not be considered a debridement. Also, minimal procedures to trim margins are not considered a true debridement, nor are bedside procedures, such as a dressing change or Vacuum Assisted Closure (VAC) change.

All operative procedure reports (following appropriate de-identification) will be collected up through Day 14 and any operative reports related to amputations will be collected through Day 28. An adjudication panel of 3 surgeons (investigators involved in the clinical trial) will evaluate the operative reports, in a blinded manner, to assign whether an operative procedure meets the debridement definition as noted above.

6.2.7 Vital Signs

Vital signs include heart rate, systolic and diastolic blood pressure, respiratory rate (spontaneous, assisted or controlled) and temperature. While these may be determined many times during the course of the patient's hospitalization, recording vital signs (first set of vitals on that day) in the eCRF will be based on once daily readings for specified time sequences according to time and events, Appendix A, Section 15.1.

6.2.8 Clinical Scores

Clinical scores/criteria components will be collected in the study. APACHE II, LRINEC and Anaya score criteria only at screening while mSOFA score throughout the study (see Appendix E, Section 15.5). APACHE II, LRINEC and Anaya criteria will be used to determine disease severity while mSOFA score will be used as a clinical endpoint. Individual parameters of the mSOFA score will be collected and will also be correlated to response. Clinical site personnel will calculate the mSOFA at screening to determine patient eligibility, otherwise all other clinical scores will be calculated retrospectively by the blinded statisticians. (See scores details in Appendix E, Section 15.5). Data will be recorded in the eCRF at specified time points according to time and events Section 15.1.

6.2.8.1 SOFA score definition

Screening mSOFA: includes measurements of 5 organ systems (cardiovascular, respiratory, renal, coagulation, CNS but not liver/hepatic component). Screening mSOFA score should be evaluated any time after arrival at the study site hospital, although no more than 6 hours prior to anticipated first debridement. Screening mSOFA score must be ≥3, with any one organ component having a score of at least 2, to be considered eligible for enrollment. Sites are encouraged to re-evaluate mSOFA score as close to surgery as feasible for patients not meeting mSOFA ≥3 inclusion criteria during initial screening.

mSOFA score: measurements of a 5 organ systems, including CNS. To be measured on Days 1, 2, 3, 7, 10 14, 21 and 29 and calculated retrospectively by the blinded statisticians.
mSOFA respiratory parameter:

In case it is not possible to take arterial blood gases to determine the mSOFA respiratory parameter, SpO2/FiO2 ratio can be imputed for PaO2/FiO2 ratio.

6.2.9 Electrocardiogram

12-lead electrocardiogram will be collected at visits 1 (prior to study drug administration) and 2 [at the end of study drug administration (when site personnel can gain access to the patient), within 4-6 hours and 12-24 hours post study drug administration], according to time and events (Section 15.0). The ECG will be evaluated for any abnormality and the following parameters will be recorded for each ECG: PR interval, RR interval, QRS complex time, QT interval and Corrected QT interval (QTc). Two copies of the ECG tracing will be produced. One copy is to be saved at the study site and the other copy to be sent to the independent central ECG reviewer (ACI Clinical, Bala Cynwyd, PA).

6.2.10 Clinical Laboratory Assessments

Safety laboratory investigations will include hematology (complete blood count including platelets and white cell differential), chemistry (Glucose; Electrolytes (Sodium, Potassium, Chloride, Bicarbonate); Renal function tests (Urea/BUN, Creatinine); Liver function tests (Albumin, Bilirubin Total, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP); Protein Total); Calcium; Phosphorus; CRP will be obtained during screening and at Day 14. Pregnancy testing will be done on all women of childbearing potential participating in the study, (using blood test or urine test whichever is faster). Those tests will be collected at intervals according to time and events Section 15.1

6.2.11 Immunogenicity

Blood (6 mL) for serum will be collected at screening, Days 14 and 29, and Month 3 visits for immunogenicity testing. The specific assay to test for immunogenicity against AB103 will be determined at the end of the trial. The study blood samples will be processed then aliquoted into three cryovials, flash frozen, stored in -70ºC freezer prior to transport to the Central Lab. The study sites shall send all blood (serum) samples collected under this protocol to the Central Lab (see Laboratory Manual). The results of any analyses of the samples will be blinded to the study staff during the study and will not impact the medical management of the patient.

6.2.12 Biomarkers

Urine (5 mL) and blood (5 mL) for plasma will be collected for evaluations of biomarkers to assess AKI. The specific AKI biomarkers will be determined at the end of the trial. Numerous novel candidate protein AKI biomarker targets may be analyzed by immunoassay in the samples from the preliminary studies. Analyzed biomarkers may include novel targets not previously described in the literature as well as existing biomarkers, which will be measured for comparison. The study blood and urine samples will be processed, flash frozen, stored in -70 freezer prior to analysis. The results of any analyses of the samples will be blinded to the study staff during the study and will not impact the medical management of the patient. The study sites shall send all blood (plasma) and urine samples collected under this protocol to the Central Lab (see Laboratory Manual). The samples may be stored up to 5 years but will be used by the investigators only to identify and validate biomarker targets for assessment of at-risk patients and will not be used for any other purpose. Samples will be stored at the Central Lab. Any excess sample will not be destroyed but will be maintained for additional study as described above. Each sample will not be individually identifiable.

6.2.13 RNA Expression

The purpose of the whole blood leukocyte transcriptome (RNA expression) profile testing is to obtain insight in the effect of AB103 on the host response to NSTI by detailed and unbiased analysis of the blood leukocyte response. For this, 5 mL of whole blood will be collected in 2 Paxgene tubes (PreAnalytix, Hombrechtikon, Switzerland; 2.5 mL per tube) before initiation of AB103 administration and at 4-6 hours, 24 hours, 72-96 hours and 7

days thereafter. At each of these time points whole blood leukocyte counts and differentials will be determined by the local laboratory. Genome-wide whole blood RNA expression profiling will be performed on the Affymetrix GeneTitan instrument using the U219 96-array microarray chip or equivalent. Samples may be stored for up to 5 years but will only be used for the evaluation of the effect of AB103 on host response to NSTI. Results will not be reported to the treating physician or the subjects (or their relatives). A detailed description of the collection, processing and storage of the samples can be found in a separate Laboratory Manual for the study.

6.2.14 Total blood volume collected

The total maximal volume of blood to be collected in the study is composed of study specific tests as well as tests that are part of the SoC. The list below represents the combined blood quantities. Less than a unit of blood (12.5 tablespoons) will be collected for each patient.

Table 6: Maximal Blood Drawing by Visit

Visit	Type	Maximal Volume (cc)
1 SCR	CRP	3
	Chemistry	4
	Hematology	4
	Arterial Blood Gas*	2
	Pregnancy test	2
	Blood Culture	20
	AKI Biomarker	5
	Blood leukocyte transcriptome profile	5
	Immunogenicity sample	6
	Total Volume	**52**
2 Day 1	Hematology (CBC with differential and platelets)	4
	Arterial Blood Gas*	2
	Creatinine & Hematology (For SOFA calculation 16-24h post study drug administration)	7
	Blood leukocyte transcriptome profile (4-6 hour post study drug administration sample)	5
	Total Volume	**18**
3 Day 2	AKI biomarker	5
	Blood leukocyte transcriptome profile	5
	Arterial Blood Gas*	2
	Chemistry (*only Creatinine*)	3
	Hematology (CBC with differential and platelets-)	4
	Hematology (only platelet count)-obtain if full CBC to be done with transcriptome sample is not performed on same calendar day as study Day 2	4
	Total Volume	**23**
4 Day 3	Chemistry (*only Creatinine*)	3
	Hematology (CBC with differential and platelets-can be used for mSOFA data and for the transcriptome)	4
	Arterial Blood Gas*	2

Visit	Type	Maximal Volume (cc)
	Blood leukocyte transcriptome profile	5
	Total Volume	**14**
5 Day 7	Chemistry	4
	Hematology (CBC with differential and platelets)	4
	Arterial Blood Gas*	2
	AKI biomarker	5
	Blood leukocyte transcriptome profile	5
	Total Volume	**20**
6 Day 10	Arterial Blood Gas*	2
	Chemistry (*only Creatinine)*	3
	Hematology (Platelets only)	4
	Total Volume	**9**
7 Day 14	Arterial Blood Gas*	2
	CRP	3
	Chemistry	4
	Hematology (CBC with differential and platelets)	4
	Immunogenicity sample	6
	Total Volume	**19**
8 Day 21	Arterial Blood Gas*	2
	Chemistry (*only Creatinine)*	3
	Hematology (Platelets only)	4
	Total Volume	**9**
9 Day 29	Arterial Blood Gas*	**2**
	Chemistry (*only Creatinine & Albumin*)	3
	Hematology (CBC with differential and platelets)	4
	Immunogenicity sample	6
	Total Volume	**15**
10 Month 3	Chemistry (*only Creatinine*)	3
	Immunogenicity sample	6
	Total Volume	**9**
Total Study		**188**

* Obtain ABG if clinically indicated. Otherwise record SpO2 value and also FiO2.

6.2.15 Microbiology

Microbiological testing will follow the SoC of treatment of NSTI patients. At the time of initial surgical exploration, and if surgical debridement is performed on study Day 3, a tissue sample will be cultured by the local microbiology laboratory.

The deep tissue specimens will be obtained at the time of the first debridement at the operating room at the study site medical center. Antibiotics received prior to the primary surgical debridement will be identified as well as antibiotics received during the study evaluation window of Days 1-28.

A specimen of infected tissue will be taken from the infected area, will be placed in a sterile container and transported by hand to the local designated clinical microbiology laboratory at the institution and according to the approved methods of each medical center. The specimens will then be processed as soon as possible by homogenization, the homogenate will be Gram stained, and cultured under 2 different conditions for testing of aerobic and anaerobic pathogens

Cultures of the NSTI wound will be obtained according to the schedule of events on Section 15.1. Bacterial isolates obtained from the patients in the study, and considered to be pathogens will be identified by genus and species using the methods established in the *Manual of Clinical Microbiology* [40]. Results will be used to categorize patients according to microbiology organism group (Gram-positive, Gram-negative, anaerobic, mixed infection).

Susceptibility testing will be performed on all aerobic pathogens according to clinical laboratory standards institute (CLSI) criteria.

Blood culture for both aerobic and anaerobic bacteria will be taken during screening process. Repeat blood cultures for microbiology will also be obtained according to physician discretion, if the patient is suspected to have a new or progressive infection (if deterioration in the hospitalized patient's condition is observed (e.g. spiking a fever).

Bacterial isolates identified as *Streptococcus pyogenes* or *Staphylococcus aureus* will be stored frozen at the site and shipped to the Central Microbiology Laboratory (IHMA, Schaumburg, IL) for future testing. Instructions for freezing, storage and shipping of the bacterial isolates can be found in a separate Laboratory Manual for the study.

6.2.16 Use of additional rescue therapy

The following measures of medical rescue therapies will be recorded:

- Hyperbaric oxygen
- Corticosteroid use
- IVIG use
- Plasmapheresis

7. EVALUATION MEASURES FOR EFFICACY

7.1 Primary Efficacy Measures

To demonstrate the efficacy of AB103 as compared to placebo, in patients diagnosed with NSTI, using a clinical composite success endpoint:

The NSTI clinical composite score, NICCE will be defined as the primary end point which will be composed of the following patient outcomes (i) Alive at Day 28, (ii) Day 14 debridements ≤3 (iii) No amputation done after the first debridement (iv) Day 14 modified SOFA (mSOFA) score ≤1 (mSOFA includes respiratory, cardiovascular, CNS, renal, and hematologic organ components but not hepatic/liver component) (v) Reduction of ≥3 score points between Baseline and Day 14 mSOFA score.

Conditional Co-Primary Efficacy Measure:

Modified clinical composite endpoint: Success is defined as meeting all 3 components of the composite score: Alive until Day 28, (ii) Day 14 debridements ≤3 (iii) No amputation done after the first debridement (See Section 2.1 and Table 4).

7.2 Secondary Measures

- 5 component clinical composite endpoint but with mSOFA ≤1 at Days 21 and 28
- Distribution of mSOFA scores at Days 14 and 21
- Kaplan-Meier analysis of the time to resolution of mSOFA score to ≤1 with censoring at Day 14
- Single components of the composite end point
    - Alive at Day 28
    - Number of patients with debridements by Day 14 ≤3
    - Number of amputations (excision to a joint space) (done after first debridement)
    - mSOFA score on Day 14 ≤1
    - Reduction of ≥3 score points between Baseline and Day 14 mSOFA score.
- Critical care and hospital stay parameters, to be measured until Day 28
    - ICU-free days
    - Days in ICU
    - Days on ventilator
    - Ventilator free days
    - Vasopressors days/ Vasopressors free days
    - Hospital length of stay (days)
- Clinical local parameters:
    - Number of debridement to days 7, 10, 14 and 28
    - Proportion of patients needing (up to Day 14):
        1. only one debridement to control the infection
        2. ≥2 debridements to control the infection
        3. ≥3 debridements to control the infection

- Clinical systemic parameters:
    - Evaluation of organ function over time, using mSOFA score
    - Recovery from AKI

7.3 Exploratory Measures:

- Evaluation of blood leukocyte transcriptome (RNA expression) profiling in patients with NSTI and compare genomic profile in patients treated with AB103 versus placebo
- Evaluation of the clinical response by baseline pathogen
- Evaluation of the microbiological outcome in the microbiological evaluable population
    - *By-Pathogen Bacteriological Response:* The by-pathogen bacteriological response for each causative organism identified at baseline (BL) will be defined as follows:
        - Eradication: BL causative organism cannot be isolated from any culture(s) obtained from a debridement performed on or after study Day 3.
        - Persistence: The BL causative pathogen is isolated from a debridement performed on or after study Day 3.
    - *By-Patient Bacteriological Response:* The by-patient bacteriological response will be determined according to the following definitions:
        - Eradication: All BL causative organism(s) have a response of Eradication.
        - Persistence: All or some BL causative organism(s) have a response of Persistence.
- Evaluation of plasma and urinary biomarkers in patients with AKI
- Data (including financially related data) will be gathered to evaluate possible health economic outcomes from the study, including but not limited to:
    - Length of stay (ICU and overall hospital)
    - Number of re-admissions
    - Reduction in ICU admission rates (depending on patient population being selected – i.e. severity of patients
    - Related to ICU admission – the Sequential Organ Failure Assessment (SOFA) score is an important outcomes measure that should be incorporated into any health economics and outcomes research (HEOR) analysis
    - Relative reduction in any physical outcome (including in-hospital deaths)
- To evaluate NICCE outcome in screen failure patients with baseline mSOFA = 2 and post-operative mSOFA ≥ 3
- Evaluation of the immunogenicity of AB103

8. EVALUATION CRITERIA FOR SAFETY

8.1 Primary Safety Measures

Measures throughout to Day 28: AEs (includes SAEs), clinical safety laboratory (through Day 14). Secondary infections and determination of survival. Specific times of these parameters are described in Schedule of Procedures, Appendix A, Section 15.1.

Timely, accurate, and complete reporting and analysis of safety information from clinical studies are crucial for the protection of patients, Investigators, and the Sponsor, and is mandated by regulatory agencies worldwide. The Sponsor or its affiliate has established SOP in conformity with worldwide regulatory requirements to ensure appropriate reporting of safety information; all clinical studies sponsored by Sponsor or its affiliates will be conducted in accordance with these procedures.

Patients (or their designee, if appropriate) must be provided with information indicating the name of the IMP, the study number, the Investigator's name and a 24-hour emergency contact number, and, if applicable, excluded concomitant medications.

8.2 Adverse Event Definitions and Classifications

The following definitions of terms are guided by the ICH and the United States Code of Federal Regulations [21 CFR § 312.32].

8.2.1 Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE could therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP. This definition includes illnesses or injuries, and exacerbations of pre-existing conditions.

8.2.2 Serious Adverse Event

A serious adverse event (SAE) is an AE occurring during any study phase (i.e., treatment, follow-up), and at any dose of the IMP or placebo, that fulfils one or more of the following criteria:

- Results in death (for purposes of this trial deaths will be captured as clinical outcomes and only recorded as an SAE if deemed related to IMP)
- Is life-threatening (i.e., the patient was, in the opinion of the Investigator, at immediate risk of death from the event as it occurred)
- Requires or prolongs hospitalization
- Results in persistent or significant disability or incapacity (i.e., the event causes a substantial disruption of a person’s ability to conduct normal life functions)
- Is a congenital anomaly or birth defect, or
- Is an important and significant medical event (e.g., allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or

convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse) that, based upon appropriate medical judgment, may jeopardize the patient and/or may require medical or surgical intervention to prevent one of the other outcomes defining SAE.

8.2.3 Adverse Reaction

Any AE for which there is a reasonable possibility that the IMP caused the AE. "Reasonable possibility" means there is evidence to suggest a causal relationship between the IMP and the AE.

8.2.4 Expectedness of the Adverse Event

Expected adverse events are adverse events consistent with the applicable product information provided by the Sponsor (the Investigator's brochure for an investigational product). The Sponsor, in consultation with the medical monitor, determines expectedness. If a SAE is **expected** no further action is required. If the Sponsor and medical monitor determine that SAE is **unexpected,** then the event may meet criteria for expedited SAE notification to the regulatory agencies.

8.2.5 Serious and Unexpected Suspected Adverse Reaction (SUSAR)

An adverse reaction that is both unexpected (not consistent with the observed or expected risk information applicable to the IMP) and also meets the definition of "serious" described above. All 3 of the definitions contained in the FDA requirement for expedited reporting must be met to qualify for expedited reporting to FDA: 1) Serious, 2) Unexpected, and 3) Suspected AR. AEs that do not meet the requirements for expedited reporting will be reported to FDA in the IND Annual Report. SUSARs that are fatal or life-threatening will be reported to FDA as soon as possible, but no later than 7 calendar days after Sponsor's initial receipt of the information (21 CFR 312.32(c)(2)). Other SUSARs will be reported to FDA within 15 calendar days of initial receipt or after determining that the information qualifies for reporting under 21 CFR 312.32(c)(1).

8.2.6 Adverse Events Based on Examinations and Tests

Deterioration as compared to baseline in protocol mandated laboratory values, vital signs and other safety variables should only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the IMP. However, the Investigator may record such findings as an AE at his/her discretion in addition to completing an unscheduled laboratory/vital signs page with the information on the clinically significant test abnormality. If a deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign/symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Any new or aggravated clinically relevant abnormal medical finding at a PE, dermal examination or lung auscultation as compared with the baseline assessment will be reported as an AE. Clinically relevant deterioration in unscheduled assessments of laboratory/vital signs/ECG parameters should be reported on additional eCRF pages.

Wherever possible, the reporting Investigator uses the higher level medical concept, rather than the laboratory term (e.g., anemia versus low hemoglobin value).

8.2.7 Pregnancies

If pregnancy is detected after the administration of study drug, the patient will be followed-up until resolution. The Investigator must immediately record the pregnancy on the pregnancy reporting form and notify Sponsor/designee Pharmacovigilance by telephone and fax form within 24 hours of becoming aware of the event. In addition, the Investigator must report follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome.

If pregnancy is reported in a female partner of a male patient, the female partner will be asked to sign a separate consent form to allow the site personnel to capture data about the pregnancy and to follow the pregnancy until completion. No information about such pregnancies will be captured without informed consent from the female partner.

8.2.8 Relationship to Investigational Medicinal Product

The relationship of an AE to the administration of IMP (unrelated, probable or definite) is a clinical decision based on all available information at the time of the completion of the eCRF.

IMP relationship will be evaluated according to the following definitions:

Unrelated: includes the existence of a clear alternative explanation (e.g., mechanical bleeding at surgical site) or non-plausibility (e.g., the patient is struck by an automobile, at least where there is no indication that the IMP caused disorientation that may have led to the event; cancer developing a few days after IMP administration).

Probable: The AE, including clinical laboratory abnormality, follows a reasonable sequence from the time of IMP administration, follows a known response pattern of the IMP class, is unlikely to be attributed to the patient's clinical state, and is confirmed by improvement on stopping the IMP (de-challenge).

Definite: The AE, including clinical laboratory abnormality, follows a plausible sequence from the time of IMP (or placebo) administration; follows a known or expected response pattern to the IMP class; cannot be explained by disease or it disappears or decreases on cessation or reduction in IMP dose; and/or it reappears or worsens when the IMP is re-administered.

8.2.9 Severity

The Investigator will determine the intensity of each AE using the following terms and definitions:

Mild: An AE that was usually transient required no intervention or special treatment and did not interfere with usual/normal activities.

Moderate: An AE that interfered with usual/normal activities but was ameliorated by therapeutic measures.

Severe: An AE that was intense or debilitating and which interfered with usual/normal activities. Recovery was usually aided by therapeutic measures. Discontinuation of IMP might have been required.

8.3 Reporting of Adverse Events

All AEs that occur between randomization through Day 28 visit will be reported. AEs that start prior to study drug administration will also be captured. Worsening signs and symptoms of the underlying disease will be reported as AEs.

The course of each event should be followed until resolution. All unresolved AEs should be followed-up by the Investigator until the events are resolved, or the AE is stabilized and otherwise explained.

AEs should be recorded according to findings and abnormalities detected by the Investigator. Specific capturing of potential secondary infections will take place. In addition, patients should report AEs voluntarily and in response to general, non-directed questioning. For each AE reported by the patient, or observed by the Investigator, the Investigator should obtain all the information required to complete the AE page of the eCRF, in accordance with the guidelines that accompany it.

Atox Bio or designee assumes responsibility for appropriate reporting of SAEs to the regulatory authorities. Atox Bio or designee will also report to the Investigators all SAEs that are unexpected and associated with the use of the drug.

The Investigator must report these events to the appropriate IRB/IEC in accordance with local regulations.

8.4 Reporting of Serious Adverse Events

All SAEs occurring with any patient participating in this clinical study must be recorded. All SAEs must be reported within 24 hours of becoming aware of the event to the Sponsor or designee, with the exception of SAEs that are specifically attributed to underlying disease (see section 8.5). All SAEs beginning within 28 calendar days after the last exposure to IMP must be recorded.

8.4.1 Initial Report

Once an Investigator becomes aware that a SAE has occurred in a study subject, the Investigator (or designate) must complete the information in the SAE screens of the eCRF WITHIN 24 HOURS. The SAE screens will always be completed as thoroughly as possible with all available details of the event. Even if the Investigator does not have all information regarding a SAE, the SAE screens should still be completed within 24 hours. The report should contain, at a minimum, the following information:

- Subject identifiers (i.e., subject number)
- Suspected medicinal product (or if related to IMP, note “AB103/Placebo”)
- Adverse event term (must be listed as serious)
- Contact information for person reporting event

Once additional relevant information is received, the SAE screens in the eCRF should be updated WITHIN 24 HOURS.

The medical monitor is available on a 24-hour basis if clinical personnel wish to discuss any safety related events.

8.4.1.1 Electronic SAE reporting system back-up

If the electronic SAE reporting system does not work, the Investigator (or designate) must complete, then date and sign a SAE Report Form and fax it to Atox Bio at 919 765 6693 within 24 hours.

This back-up system should only be used if the electronic SAE reporting system is not working and NOT if the system is slow. As soon as the electronic SAE reporting system is working again, the Investigator (or designate) must complete the SAE screens in the eCRF within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

8.4.2 Follow-up and Final Reports

After the initial AE/SAE report, the Investigator is required to proactively follow each subject and provide further information on the subject's condition to CRO.

All SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the end of the study.

All AEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until study end.

Follow-up information to an initial SAE reports should be reported in the SAE screens of the eCRF within 3 business days. At the time of resolution of a SAE, a final report must be provided to the Sponsor by completing the appropriate SAE Report Form.

Any AE, regardless of severity, and whether or not ascribed to the IMP administration, will be recorded as such using medical terminology in the source documentation and on the appropriate eCRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g. nausea and vomiting should be reported as "gastroenteritis" if gastreoenteritis is the etiology of both). However, worsening sign(s) and symptom(s) of a diagnosis should be recorded separately. Patients withdrawn from the study due to AEs will be followed by the Investigator until the outcome is determined and, when appropriate, additional written reports and documentation will be provided.

All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the patient's participation in the study, must be followed-up until either:

- the event resolves
- the event stabilizes
- the event returns to baseline, if a baseline value is available
- the event can be attributed to agents other than the study drug, or
- the event can be attributed to factors unrelated to study conduct

8.5 Disease Related Adverse Events

It is recognized that the patient population with NSTIs who require critical care support will experience a number of common aberrations in laboratory values, signs, and symptoms due to the severity of the underlying disease and the impact of standard therapies. These will not necessarily constitute an AE unless they require significant intervention, lead to discontinuation of blinded study medication, felt to be related to blinded study medication or are considered to be of concern, in the Investigator's clinical judgment. Examples of the type of AEs that may be associated with the initial NSTI event are listed below.

8.5.1 List of Expected Adverse Events

Acute respiratory failure; respiratory distress; acute lung injury; acute respiratory distress syndrome (ARDS); acute renal failure; coagulation dysfunction; decreased platelet count; neutropenia; hypothermia; fever, bacteremia; sepsis, septic shock; hypotension; metabolic acidosis, pleural effusion; abdominal compartment syndrome; repeated surgeries/debridement; amputation.

For purposes of this study, deaths will be captured as clinical outcomes and do not need to be reported as SAEs unless felt related to study drug administration.

8.6 Safety Monitoring

The study medical monitor will assess all SAEs on an ongoing basis. Attention will be given to SAEs assessed as drug-related. The medical monitor may request unblinding of the treatment allocation of a patient for regulatory reporting purposes. The medical monitor will convene the DMC on ad-hoc basis if safety concerns arise from the medical monitor review.

8.7 Independent Data Monitoring Committee (iDMC)

An iDMC will be established to evaluate the safety of the study.

An iDMC review of the safety data is planned after 50, 100 and 200 patients have completed 28 days of the study. Futility analyses will also be performed after 100 patients have completed 28 days. After completion of the study the iDMC will meet again to evaluate the full study safety data and provide their evaluation to the Sponsor.

For each planned iDMC review, all safety data on all patients who received one dose of AB103 will be made available to the iDMC. The data reviewed by the iDMC will be unblinded and presented by group to facilitate recommendations to the Sponsor. Data submitted to iDMC review will be unaudited. A designated unblinded statistician will provide unblinded safety data to the DMC using programs constructed and validated by the blinded Study Statistician and Statistical Programming Team. Further details on data management will be provided in the statistical analysis plan (SAP).

The iDMC may also meet on an ad-hoc basis when immediate safety concerns arise. In case of urgency, the chair of the committee could address any questions or concerns regarding the safety of the patients.

9. STATISTICS

9.1 Synopsis of Key Statistical Approaches

9.1.1 Primary Efficacy Comparison

The primary efficacy comparison involves testing the following superiority hypotheses: **Ho: $\pi_{0.50} - \pi_{Placebo} = 0$ vs Ha: $\pi_{0.50} - \pi_{Placebo} > 0$**; where $\pi_{0.50}$ and $\pi_{Placebo}$ represent the true probability that a patient achieves specific composite clinical success criteria, NICCE, designed to be sensitive to both local and systemic drug effects. Each probability represents the proportion of subjects on each arm expected to respond according NICCE. Success according to NICCE requires meeting all 5 components of the composite endpoint: (i) Alive until Day 28; (ii) Day 14 debridements ≤3; (iii) No amputation done after the first debridement; (iv) Day 14 mSOFA score ≤1; and (v) Reduction of ≥3 point in mSOFA between Baseline and Day 14. A two-sided α=0.01 will be used to test superiority of AB103 relative to placebo for the primary endpoint (NICCE). A conditional co-primary end point will be tested for superiority if the primary endpoint is significant at a two-sided α=0.01 significance level in order to account for multiple comparisons. A two-sided α=0.05 will be used to test superiority of AB103 relative to placebo for the co-primary endpoint which is defined as (i) Alive at Day 28; (ii) Day 14 debridements ≤3; and (iii) No amputation done after the first debridement. To be considered a success for the co-primary endpoint, patients must meet all 3 components.

9.1.2 Sample Size Justification

This trial will enroll 290 subjects that will be randomized in a ratio of 1:1 to either AB103 0.50 mg/kg (n=145) or placebo (n=145), each in addition to SoC. Sample size analysis was performed assuming that all patients will be evaluable for the primary endpoint. This assumption is justified since all patients in the Phase 2a trial and in the retrospective study were evaluable for NICCE. The primary efficacy hypothesis will be tested using an unadjusted χ^2 statistic with a 0.01 two-sided significance level. Statistical power was computed for a range of expected treatment group differences supported by the results of preliminary studies. For treatment group differences equal to 0.30, 0.25, and 0.20, statistical power will be equal to 99%, 95.9%, and 80.2%, respectively. These estimates were determined conservatively assuming an average success rate of 0.5. In this way these power estimates are applicable across the range of expected response rates but may be conservative in some cases. The use of NICCE and this range of expected treatment effects is supported by preliminary studies. In the Phase 2a trial, 71.4% (5/7) patients with baseline mSOFA≥3 treated with AB103 0.50 mg/kg achieved NICCE. In contrast, 40% (2/5) placebo patients with baseline mSOFA≥3 achieved NICCE, a difference of 0.314. The retrospective study provided further support for the untreated response rate. Among 69 patients with baseline mSOFA≥3, 33.3% (23/69) achieved NICCE, difference of 0.381 relative to Phase 2a treated group. If the observed differences are equal to 0.35, 0.25, and 0.20 (centered about 0.5), then the corresponding 2-sided p-values will be $p<0.0001$, $p<0.0001$, and $p=0.0007$, respectively. The observed difference will need to be larger than about 0.16 (e.g., 0.58 vs 0.42) for $p\leq0.01$. Based on a similar evaluation

of the co-primary endpoint, the study is designed to demonstrate statistical significance at two-sided α=0.05 for co-primary success rates of 0.80 vs 0.60 in the AB103 and placebo groups, respectively. If these are the true success rates, then power will 96.4%. However, if the true rates are 0.78 vs 0.62 (difference = 0.16), then power is 84.8%. The sample size has also been selected to obtain sufficient enrollment on the treatment arm for relevant secondary effectiveness endpoints and for safety endpoints.

9.1.3 Futility Analysis

A futility analysis will be performed based on the results of the first 100 patients (50 per group). The futility decision will be based on the predictive probability of eventual study success, conditioned on the data available at interim analysis. Independent, non-informative prior distributions will be used for each parameter: $\pi_{0.50} \sim$ Beta(1,1), $\pi_{Placebo} \sim$ Beta(1,1). The Bayesian predictive probability of study success (i.e., two-sided $p \leq 0.01$) when the remaining patients are finally observed will be determined. The trial will stop enrollment for futility if the predictive probability of study success is below a lower bound threshold of 10%. The following table summarizes what the observed predictive probabilities will be as a function size of the treatment group differences and assuming an observed control success rate of 0.40. If there are 20 success and 30 failures among placebo controls (40% success rate), then the futility boundary will be crossed if the number of active success is 22 (44%) or less. The futility bound is 'non-binding' in the sense that no effort was made to 'recover' alpha to increase power.

Diff	0.000	0.020	0.040	0.060	0.080	0.100	0.120	0.140	0.160	0.180	0.200	0.220	0.240	0.260	0.280	0.300
PP	0.029	0.050	0.082	0.127	0.187	0.261	0.348	0.444	0.543	0.639	0.728	0.804	0.866	0.913	0.946	0.969

9.1.4 Control Of Blinding And IDMC

This study will remain blinded as to treatment allocation. The actual randomized treatment allocations will be kept by a contracted third-party responsible only for managing the randomization process. A variable indicating the blinded treatment allocations will not be part of the clinical study data to be managed by the data management CRO. Therefore, only blinded study data will be available to the primary study statistician and responsible primary programming staff.

An Independent Data Monitoring Committee (iDMC) will be utilized in this study. A detailed iDMC charter will be provided to clarify all relevant issues relating to the conduct of the futility analysis including specific details regarding the operational procedures with fire-walls to protect against potential operational biases. The charter will provide decision rules, composition of the iDMC members and their conflict of interest statements.

A second, unblinded statistician will work with a iDMC to evaluate the unblinded data necessary to perform the futility analysis and to provide to the iDMC other unblinded data such as unblinded safety comparisons as requested by thei DMC. The primary statistical programmer under the direction of the primary study statistician will develop SAS based programs that processes clinical data provided by the blinded data management CRO to populate planned analysis tables and listings. This programming and other relevant

instructions will be made accessible (one-way direction on information flow) to a second statistician. This second statistician will serve as the unblinded statistician. The unblinded statistician will maintain a secured version of the project analysis system not accessible to the primary study statistician or anyone else. The unblinded statistician will obtain the unblinding codes from directly from the randomization vendor. No one will have access to the project analysis system containing the true randomized treatment codes except for the unblinded statistician. The unblinded statistician will therefore be able to efficiently provide unblinded tables and listings developed by the primary study statistician and team directly to the iDMC. The unblinded study statistician will be capable of providing additional unblinded data upon request. The unblinded statistician will perform the necessary computations involving the Bayesian predictive power to be used in the planned futility assessment at the planned interim analysis based on the results from the first 100 evaluable patients. These computations will be performed in accordance to prescribed plans and results provided to the iDMC for the futility assessment. Relevant details will be provided in a separate SAP and in the separate iDMC charter.

9.2 Summary of Other Elements of Analysis Plan

9.2.1 Randomization

290 patients will be recruited into the study and randomized to either 0.5 mg/kg AB103 or placebo in a 1:1 ratio. Randomization will be within site stratified by the diagnosis of Fournier's Gangrene and magnitude of mSOFA score at screening categorized as 3-4 versus >4. In the retrospective study analysis set with baseline mSOFA ≥ 3 (N=69), the median value was 4 and 46.6% had values larger than 4. Four computer generated, blocked randomization lists will be provided for each site corresponding to the cross-tabulation of Fournier's Gangrene and baseline mSOFA category status. Within each block, half of the assignments will be to active drug and half to placebo, in random order. Block sizes will be varied.

The following will be done to insure balance through randomization. Every initial randomization code will end in '-1'. If randomization code is used but the patient does not receive drug due to no confirmatory NSTI diagnosis, then the randomization code is not reused. Instead, the next patient from that center meeting the same strata criteria as the non-dosed patient, will receive the same allocation and the same randomization code except that the code will end in '-2'. In this way, randomization codes are not reassigned which can cause confusion but the same allocation is used preserving balance.

9.2.2 Patient Populations

The following analysis sets are defined:

- Intent-to-Treat (**ITT**): The ITT analysis set will include all randomized patients.
- As-Treated (**AT**): The AT analysis set will include all randomized patients who were exposed to study medication (active or placebo). The AT analysis

set will be used in primary safety analyses with patients assigned to actual treatment received.

- Modified Intent-to-Treat (**mITT**): The mITT analysis set will include patients who were exposed to study medication and who had a definitive diagnosis of NSTI based on surgical verification. The mITT analysis set will be used in primary effectiveness analyses with patients assigned to their intended randomized assignment.
- Per Protocol (**PP**): Optionally, a PP analysis set may be used in secondary effectiveness analyses. The PP analysis would include patients in the mITT analysis set assigned according to actual treatment received and excluding patients with either 1) significant violations of inclusion or exclusion criteria with potential to confound estimates of drug effects or 2) post randomization protocol violations with potential to confound estimates of treatment effects. Exclusions from the PP analysis set will be determined based on blinded clinical data. The PP analysis may be further restricted to include patients that survive at 3 least days when evaluation critical care variables.

9.2.3 Preliminary And Descriptive Analyses

Before proceeding with between-group comparisons of primary and secondary clinical endpoints, data quality will be assessed by descriptive summaries and graphical methods (e.g., histograms and scatterplots) in order to examine assumptions such as normality that underlie statistical models. Transformations will be used, if needed, to produce variables that conform to the distributional assumptions underlying the analytic techniques employed. For instance, some variables (e.g., physiologic measurements) may be transformed to log scales[40] to reduce any marked positive skew. Descriptive analyses will be performed in order to characterize the treatment groups and to confirm that the randomization resulted in no clinically significant group differences at baseline. Although emphasis will be on clinical significance, baseline comparisons will include t-tests or Wilcoxon rank sum tests as appropriate for interval variables and chi-square or Fisher's exact tests as appropriate for nominal variables to aid in the screening for baseline differences. Similarly, changes in clinical endpoints overtime will be summarized within each treatment group using summary statistics including mean and median change scores, standard deviations and ranges. Pearson or Spearman rank correlation coefficients will be used to characterize associations among variables within treatment group. When summarizing across groups, partial correlations may be computed controlling for treatment group. For time to event outcomes (i.e., event-free survival), descriptive analyses will include construction of group specific Kaplan-Meier survival curves[41] as appropriate.

9.2.4 Secondary Endpoints

Secondary endpoints have been specified from several domains including a similar composite endpoint but defined on the basis of Day 21 mSOFA, individual components of the primary composite endpoint, time to resolution of organ dysfunction, critical care and hospital stay parameters (ICU and ICU-free days, ventilator days and –free days,

vasopressor days and –free days, hospital LOS), clinical local parameters (debridement history variables), and clinical systemic parameters (mSOFA over time, incidence and recovery from AKI). Analyses for these endpoint will generally be descriptive, with emphasis on characterizing clinical effect sizes. Nominal p-values will be presented. Categorical outcomes will be described using counts and percentages with nominal p-values determined through chi-square or exact methods. Critical care and hospital stay endpoints will be described using non-parametric approaches including using concordance statistics to characterize clinical effect size and Wilcoxon rank sum tests to determine nominal statistical significance. Methods appropriate for time-to-event endpoints including survival and life-table methods will be used for time-to-organ dysfunction resolution endpoints. Where appropriate, for continuous measures, statistical testing and estimation will be based on the results from a Mixed Model Repeated Measures (MMRM) analysis of covariance (ANCOVA) model. AKI endpoints will be assessed in several ways include incidence of Stage 2/3 AKI and among incident cases, resolution of AKI. A similar analysis will be performed based on any stage AKI. Treatment groups will be compared using counts and percentages with nominal p-values determined through chi-statistics.

9.2.5 Exploratory Analyses

Exploratory subgroup analysis will be performed. For these exploratory analyses, active dose versus placebo differences in NICCE will be evaluated in several subgroups defined by (i) severity of disease at baseline (3-4 vs >4), (ii) presence of non-Fournier's gangrene (iii) involvement of suspected superantigen-producing bacteria and (iv) type of bacteria (Gram-positive vs. Gram-negative). Other similar comparisons may be performed.

9.2.6 Assessment Of Poolability Among Sites

Site poolability will be evaluated using a random effects meta-analysis approach using the R package ***metafor*** to implement the analysis[42]. True effects are assumed to be normally distributed with mean μ and variance τ^2. By imposing a specified distribution on the site-to-site variability, i.e. a normal distribution with mean μ and variance τ^2, sensitivity to small sample sizes in individual sites is reduced and the parameters reflecting the magnitude of site-to-site variability are naturally derived. The quantitative measure of the magnitude of heterogeneity is I^2 [43]. I^2 is the fraction of $\boldsymbol{\tau^2}$ that is due to effect size heterogeneity, as opposed to sampling variance. Fractions 25% and less are considered small. If there is significant site to site variability, the impact on this variability will be evaluated using a random effects logistic regression to test the null hypothesis that the likelihood of achieving NICCE is the same for treated and placebo patients accounting for a random site effect. Poolability according to baseline demographic, disease severity status, and number of levels treated will be evaluated using descriptive stratified analyses.

9.2.7 Analysis Of Other Covariate Effects

Covariates will be assessed for potential confounding (due to lack of perfect randomization balance) or effect modification (subgroup efficacy heterogeneity) using multiple logistic regression. Covariates will include but are not limited to age, race, sex, site, and clinical severity scores at baseline. Other baseline variables in which randomization failed to

produce balance between groups will be examined in supporting analyses. This will be done through stratified analyses and multiple logistic regression. Covariate effects on estimates and interactions will be assessed to see if there is evidence of efficacy heterogeneity. Results from all subgroups analyses will be considered hypothesis-generating.

9.2.8 Multiplicity

There is a single primary hypothesis test utilizing a composite clinical success endpoint, NICCE and a conditional co-primary endpoint consisting of 3 of the 5 NICCE components. The use of a composite endpoint serves to address the multiplicity issue with regard to the key outcomes of mortality, local activity, and systemic activity[44]. The co-primary endpoint will only be test if two-sided $p \leq 0.01$ in order to avoid the need for multiplicity adjustment and will be tested using a two-side $\alpha=0.05$. The 5 components of NICCE will be individually evaluated in supporting analyses will no adjustment for multiplicity.

9.2.9 Longitudinal Data

A number of secondary efficacy endpoints will be assessed over time including mSOFA total score.

Where possible for continuous measures, statistical testing and estimation will be based on the results from a MMRM analysis of covariance (ANCOVA) model. This model will include the baseline value of the outcome variable if appropriate. Other clinically relevant baseline variables predictive of outcome will be considered in cases of where there is missing outcome data over time. Inclusion of such covariates helps with the implicit imputation of missing values inherent in the MMRM approach. MMRM is a direct likelihood approach requiring specialized statistical software for optimizing the likelihood function. For this study, all MMRM parameters will be estimated using SAS PROC MIXED [SAS Institute[a]]. The MMRM model is notable for its ability to include all available data from all eligible subjects and does not require their exclusion as in complete case analysis or arbitrary assignment of values as in last observation carried forward (LOCF). The MMRM model generally includes a factor for group by time interaction in order to allow group difference in mean value to vary over time. Inclusion of outcome data from all time points informs the implicit imputation of values missing at specific time points through the outcome covariance matrix. Inclusion of baseline covariates has potential for further reduction of potential bias due from missing values.

Specifically, MMRM will be used to compare mean values over time between the 0.50 mg/kg dose and placebo. Model parameters will be estimated using Restricted Maximum Likelihood[45,46] (REML) as implemented Proc Mixed [SAS Institute[b]]. A generalized Satterthwaite approximation will be used to determine accurate estimates of denominator degrees of freedom for statistical tests. Analyses will characterize changes over time as functions of the baseline value, treatment group, time, and treatment by time interaction. Between-group differences at each time point will be evaluated using contrasts derived

Capture data on any readm
issions within 30 days of original hospital disc

from mixed model parameter estimates. This approach produces inferences that are valid under the assumption of missing at random (MAR) which produces valid inference under broader assumptions than complete case analysis or analyses utilizing last observation carried forward[47].

9.2.10 Analysis Of Event-time

Time to event endpoints will be assessed in descriptive analyses using survival and life-table methods as appropriate. Results will be displayed in terms of morbidity/mortality or event rate ratios (i.e., incidence density ratios [IDR]) along with 95% confidence intervals. Analyses will include time to symptom resolution.

9.2.11 Handling Of Missing Data

9.2.11.1 Intent-to-Treat

The purpose of intent-to-Treat comparisons is to ensure that randomization is protected (i.e., all groups have comparable baseline characteristics and that any differences besides therapy are due to chance) and to preclude the possibility of bias due to selectively excluding subjects from therapy groups. This is intended to avoid systematic differences among the groups attributable to factors other than therapy assignment[48]. Therefore, we will attempt to include all randomized patients, regardless of intervention or length of follow-up in the primary efficacy comparison.

9.2.11.2 mSOFA Scores

mSOFA scores will be assessed as 1) observed cases, 2) LOCF, 3) using MMRM, and 4) categorized and combined with mortality and local activity as part of the primary composite clinical success endpoint, NICCE.

Individually missing mSOFA component values due to non-measurement are conventionally assumed as normal and this convention will be followed for this study.

LOCF will not be applied to missing mSOFA scores after patient death in analyses that employ LOCF to describe mean values of time. Therefore, analysis of SOFA mean values using LOCF will focus on morbidity rather than mortality.

Mixed model repeated measures analyses (MRMM) will utilize implicit imputed values for all missing values including those subsequent to patient death.

In categorical analyses including formulation of the primary efficacy endpoint, an mSOFA value that is greater than 1 at Day 14 is considered a treatment failure in the same fashion as patient death. LOCF for SOFA is necessary to guarantee that every patient has a value at Day 14 for use in determining NICCE among patients that are alive at the Day 28 and who have not otherwise failed due to other NICCE components including more than 3 debridements, or amputation beyond the first debridement.

9.2.11.3 Tipping Point Analysis for Primary Endpoint

If there are any patients with missing primary endpoints, a tipping point sensitivity analysis will be conducted. In this analysis, missing values in each group are separately assumed to be either successes or failures. Treatment group differences are computed based on all possible combinations of assigning success or failure to NICCE to the patients in the 2 groups. For example, one scenario will be that all missing AB103 observations are failures and all missing placebo observations are successes. The next scenario would have one success and the remaining missing values as failure for AB103 and all missing placebo outcomes as successes. For each scenario, the 1-sided p-value for testing the primary effectiveness hypothesis will be determined. These results will be plotted using a dot plot with the number of missings assumed as failures for AB103 on the x-axis and the number of missing assumed as failures for placebo on the Y-axis. The dots will be color coded to indicate whether or not the primary statistical conclusion changes under each individual scenario. If the fraction of scenarios in which the statistical conclusion changes is small, the primary results will have been shown to be robust against assumptions concerning missingness. If there are more than a very small amount of missing data, multiple imputation of missing endpoints may be performed in additional supporting analyses using the SAS procedures, Proc MI and Proc MI Analyze.

9.2.11.4 Mixed Model Repeated Measures

As describe above, continuous measure observed over time will be assessed using MMRM where possible. MMRM uses all available data and results in implicit imputations for missing data which are valid under MAR, a more general set of assumptions than ‘missing completely at random (MCAR)’.

9.3 Safety Analysis

The primary safety measures are AEs (including SAEs), clinical safety laboratory, physical exam, vital signs through Day 28 and ECG (days 0 and 1) and including determination of survival through Day 28.

The safety profiles will be compared between active and placebo groups using descriptive statistics as appropriate for continuous and categorical safety variables. Changes in continuous safety measures such as laboratory values will be summarized by mean changes over time using descriptive statistics (mean, SD, median, minimum and maximum). The presence of clinically significant safety findings will be summarized by shift tables separately for each group using counts and percentages. AEs will be classified according to system organ class and preferred term and summarized by counts and percentages separately for those recorded on Day 0 (prior to drug administration) and those with onset on Day 1 or later. AEs will also be summarized by relationship to study drug, severity, and whether they are serious. Specific summaries will involve AEs and SAEs in the Infection/Infestation system organ class (SOC). Electrocardiogram results from Day 0 and Day 1, and change from Day 0 to Day 1 will be summarized using mean, median, standard deviation, minimum, and maximum values. Results from physical exams will be tabulated

for the Day 0, Day 7, and Day 14. For each test, the number of patients evaluated, and the numbers and percentages of patients with Normal and Abnormal results will be tabulated. Vital signs including weight, temperature, systolic BP, diastolic BP, respiration rate, and heart rate will be summarized across time (Day 0, Day 1, Day 2, Day 3, Day 7, and Day 14 separately by treatment group by N, Mean, and SD.

10. SOURCE DATA ACCESS, DATA MANAGEMENT AND DATA HANDLING

10.1 Data Quality Assurance

The Sponsor or Sponsor's designee will conduct a site visit to verify the qualifications of each Investigator, inspect the site facilities, and inform the Investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. All information recorded on the eCRF/EDC system for this study must be consistent with the patients' source documentation (ie, medical records).

10.2 Database Management

Data Management services will be provided by the Sponsor or designee. The data management system will be specified in the Data Management Plan.

After the data have been entered and verified, various edit checks will be performed for the purpose of ensuring the accuracy, integrity and validity of the database. These edit checks may include:

- Range checks
- Consistency checks
- Sequence checks
- Protocol adherence checks

Queries generated from these checks will be sent to the investigational site for resolution, and the database will be updated to reflect query resolutions as appropriate.

Adverse events will be coded using the latest version of MedDRA (currently version 18.0). Prior and concomitant medications will be coded according to the World Health Organization (WHO) Drug Dictionary.

10.3 Case Report Forms and Source Documentation

All data obtained during this study should be entered in the EDC system promptly. All source documents from which eCRF/EDC system entries are derived should be placed in the patient's medical records. Measurements for which source documents are usually available include laboratory assessments, ECG, and microbiological outcome.

Data that will be entered directly into the eCRF/EDC system are considered to be source data.

The original eCRF/EDC system entries for each patient may be checked against source documents at the study site by the Criterium site monitor.

After review by the site monitor, completed eCRF/EDC system entries will be uploaded and forwarded to Criterium. Instances of missing or uninterpretable data will be discussed with the Investigator for resolution.

The specific procedures to be used for data entry and query resolution using the EDC system/eCRF will be provided to study sites in a study manual. In addition, site personnel will receive training on the EDC system/eCRF.

10.4 Data Collection

The Investigators (and appropriately authorized staff) will be given access to an online web-based EDC system which is compliant with 21 CFR Part 11. This system is specifically designed for the collection of the clinical data in electronic format. Access and right to the EDC system will be carefully controlled and configured according to each individual's role throughout the study. In general, only the Investigator and authorized staff will be able to enter data and make corrections in the eCRF/EDC system.

The EDC system/eCRF should be completed for each patient included in the study and should reflect the latest observations on the patients participating in the study. Therefore, the EDC system/eCRFs are to be completed as soon as possible (within 5 working days) after the patient's visit or assessment. The Investigator must verify that all data entries in the EDC system/eCRFs are accurate and correct. If some assessments cannot be done, or if certain information is unavailable, not applicable or unknown, the Investigator should indicate this in the EDC system/eCRFs.

Computerized data-check programs and manual checks will identify any clinical data discrepancies for resolution. Corresponding queries will be loaded into the system and the site will be informed about new issues to be resolved online. All discrepancies will be solved online directly by the Investigator or by authorized staff. Off-line edit checks will be done to examine relationships over time and across panels to facilitate quality data.

After completion, the Investigator will be required to electronically sign off the clinical data.

Data about all study drug dispensed to the patient will be tracked on the EDC system/eCRFs.

10.5 Access to Source Data

During the study, a monitor will make site visits to review protocol compliance, compare EDC system/eCRFs entries and individual patient's medical records, assess drug accountability, and ensure that the study is being conducted according to pertinent regulatory requirements. EDC system/eCRFs entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that patient confidentiality is maintained and the study blind is respected.

Checking of the EDC system/eCRFs entries for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the study. Moreover, Regulatory Authorities of certain countries, IRBs, IECs, and/or the Sponsor's Clinical Quality Assurance Group may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical

confidentiality. The Investigator assures Criterium and the Sponsor of the necessary support at all times.

10.6 Archiving Study Records

According to ICH guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational Product. However, these documents should be retained for a longer period if required by the applicable legal requirements.

11. QUALITY CONTROL AND QUALITY ASSURANCE

To comply with ICH GCP Guidelines, an independent audit at the study site may take place at any time during or after the study. The independent audit can be conducted by the Quality Assurance Department of the CRO, Sponsor or a regulatory authority. ICH GCP Guidelines state that investigational sites and all data, including source data, must be available for inspection by competent authorities. The Investigator shall inform patients that their medical records will be reviewed during these audits, however, pursuant to current HIPAA regulations; the patients' privacy must be respected. Sufficient prior notice will be provided to allow the Investigator to properly prepare for the audit.

12. GENERAL STUDY ADMINISTRATION

12.1 Ethical Aspects

12.1.1 Good Clinical Practices / Local Regulations

This study will be carried out according to the Declaration of Helsinki, the Notes for Guidance on Good Clinical Practice (2000) (CPMP/ICH/135/95), with ICH GCP, and any local applicable regulations.

The Sponsor will be responsible for reporting all serious, life-threatening or fatal adverse Investigational Product events with a causal relationship to the Investigational Product to appropriate regulatory agencies within their required timelines.

The Principal Investigator will ensure that this study is conducted in full compliance with the protocol, the Declaration of Helsinki, the ICH guideline for GCP, and all other applicable local laws and regulations. Compliance with these standards provides assurance that the rights, safety, and well-being of patients are protected.

In agreeing to the provisions of the protocol, these responsibilities are accepted by the Investigator.

12.2 Informed Consent

Prior to initiation of the study, the Investigator will provide the Sponsor with a copy of the investigational site's IRB/EC approved study ICF. The ICF must contain all elements required by ICH GCP (E6). The ICF must also adhere to local privacy requirements as well as any other elements required by state, local and institutional policies.

All prospective patients or the patient's legally authorized representative (LAR) will be given a copy of the approved study ICF to read.

Before being admitted to the clinical study, the patient must have given written consent or LAR must have given phone/fax/email/e-consent or written consent to participate in the study, after the nature, scope, and possible consequences of the study (including insurance and other procedures for compensation in case of injury) have been explained in an understandable form by the Investigator or nominee.

It will be pointed out that patients/LARs can refuse to participate in the study, or withdraw from the study without prejudice to further care and treatment.

Ample time and opportunity will be allowed for each patient/LAR to inquire about details of the study and to decide whether or not to participate in the study.

Both the patient/LAR and the person who conducts the informed consent discussion will sign and personally date the document. The acquisition of informed consent should be documented in the patients' medical records.

Where consent has been obtained from a LAR, if the patient is deemed by the Investigator able to provide informed consent on their own behalf any time during the study period,

informed consent from the patient will be obtained. The patient has the ability to withdraw from the study at any time, for any reason.

Patients/LARs will be informed of any significant new finding which arises during the course of the research that may affect their decision to continue participation.

12.3 Institutional Review Board / Ethics Committee

The protocol will be submitted for approval to the appropriate IRB/EC. Prior to initiation of the study, the Investigator must provide the Sponsor with a copy of the written IRB/EC approval of the protocol and study ICF. This approval letter will identify the study ICF by date or version number, and the study protocol by protocol number, title, and date. The Investigators will receive all the documentation needed for submitting the present protocol to the IRB/EC. The composition of the IRB/EC will also be provided to the Sponsor. If approval is suspended or terminated by the IRB/EC, the Investigator will notify the Sponsor immediately.

It is the responsibility of the Investigator to report study progress to the IRB/EC as required or at intervals not greater than one year.

The Principal Investigator or his/her nominee will be responsible for reporting any SAEs to the IRB/EC as soon as possible, and in accordance with the guidelines of the IRB/EC.

12.3.1 Conditions for Modifying the Protocol

No changes (amendments) to the protocol may be implemented without prior approval from the Sponsor and the appropriate IRB/EC, except where necessary to eliminate an immediate hazard to patients, or when the change involves only logistical or administrative aspects of the study.

Once the final protocol has been issued and signed by the Investigator and the authorized signatories, it shall not be informally altered. Protocol amendments are alterations to a legal document and have the same legal status. Therefore, they must pass through appropriate steps before being implemented. In general, any important change that theoretically increases risk to patients constitutes an amendment. Protocol modifications that impact on patients' safety or the validity of the study will be approved by the IRB/EC. Minor changes such as administrative changes may be documented without approval, if permissible by the IRB/EC.

The Investigator will not modify the protocol without first obtaining the concurrence of the Sponsor in writing. Should the Sponsor modify the protocol, they will provide the Investigator with a written amendment. It is the responsibility of the Investigator to submit the amendment to the IRB/EC for their approval; written approval should be obtained and a copy provided to the Sponsor. The Sponsor is responsible for determining whether or not the local regulatory authority must be notified of the protocol change. Completed and signed protocol amendments will be circulated to all those who were on the circulation list for the original protocol.

The original signed copy of amendments will be kept in the Study File with the original protocol. It should be noted that where an amendment to the protocol substantially alters

the study design or the potential risks to the patient, each patient's consent to continue participation should be obtained.

If a protocol amendment requires changes to the ICF, the revised ICF, prepared by the Investigator, must be approved by the IRB/EC.

12.4 Patient Confidentiality

The anonymity of participating patients must be maintained. Patients will be identified on eCRF/EDC system and other documents submitted to Criterium by their patient number, initials and/or birth date, not by name. Documents not to be submitted to Criterium that identify the patient (e.g., the signed informed consent) must be maintained in confidence by the Investigator.

12.5 Investigator Responsibilities

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, the Declaration of Helsinki, current ICH GCP Guidelines, and applicable regulatory requirements. These documents set forth that the informed consent of the patients is an essential precondition for participation in the clinical study.

12.6 Laboratory Accreditation

Any laboratory facility to be used for analysis of routine clinical laboratory samples required by this protocol should provide reference values and/or normal ranges for the test results used in conducting this protocol.

12.7 Study Initiation

All personnel expected to be involved in the conduct of the study will undergo an orientation to include review of the study protocol, instructions for record completion, and overall responsibilities.

12.7.1 Study Completion

The Investigator will complete the study and complete all patients' eCRFs in satisfactory compliance with the protocol within 2 weeks after study completion. Continuation of this study beyond this time must be agreed upon in writing by both the Investigator and Sponsor and may be implemented without amendment to the protocol.

12.7.2 Study Termination

An initiative for center closure or study termination can be taken at any time either by the Sponsor/designee or by the Investigator, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Reasons for such action taken by the Sponsor/designee include, but are not limited to:

- Successful completion of the study at the center
- The required number of patients for the study has been recruited
- Failure of the Investigator to comply with the protocol, the Sponsor's/designee's procedures, or ICH GCP Guidelines
- Safety concerns

- Sufficient data suggesting lack of efficacy
- Inadequate recruitment of patients by the Investigator

13. CONFIDENTIAL INFORMATION

13.1 Confidential Information

All information obtained as a result of this study or during the conduct of this study concerning Atox Bio operations, patent application, formulas, manufacturing processes, basic scientific data, and formulation information, supplied by the Atox Bio to the Investigator and not previously published, is considered confidential and remains the sole property of the Atox Bio. The Investigator agrees to use this information only to conduct this study and will not use it for other purposes without Atox Bio prior written consent.

13.2 ClinicalTrials.gov Study Registration

This clinical study, ABT-202, will be registered on www.ClincalTrials.gov prior to enrollment of the first patient. Patients are to be made aware of this study registration through the informed consent process prior to study participation. Final study results will be posted on ClinicalTrial.gov according to US regulatory guidelines.

13.3 Publication Policy

By signing the study protocol, the Investigator agrees with the use of results of the study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, Regulatory Authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement.

An Investigator shall not publish any data (poster, abstract, paper, etc.) without having consulted with the Sponsor in advance. Details are provided in a separate document.

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15. APPENDICES

15.1 Appendix A - Study visits and procedures

Visit	1 Screening	2 Day 1	3 Day 2	4 Day 3	5 Day 7 ±1 day	6 Day 10 ±1 day	7 Day 14 ±1 days	8 Day 21 ±1 days	9 Day 29 +2 days	10 3 months ±5 days
Informed Consent	X									
Demographics	X									
Medical history	X									
Lesion history	X									
Concomitant Medications[a]	X	X	X	X	X	X	X	X	X	
Weight & Height[b]	X				X		X		X	
Urine output[c]	→	→	→	→	→					
Vital Signs[d]	X	X	X	X	X	X	X	X	X	
Physical Examination (PE)	X				X		X			
Interim PE (symptom-driven)		X		X		X				
Baseline Signs & Symptoms[e]	X									

[a] See Section 5.2 for instruction on level of detailed information required for recording concomitant medications
[b] Height will be taken only at screening
[c] Urine output is required for Days 1-7 (while the patient is in the ICU and has a Foley catheter in place) to assess for development or resolution of acute kidney injury.
[d] Systolic & Diastolic blood pressure, Heart rate, Respiration rate, Temperature; (first set of vitals on that visit day)
[e] Adverse events reported from ICF signature to AB103 administration

Visit	1 Screening	2 Day 1	3 Day 2	4 Day 3	5 Day 7 ±1 day	6 Day 10 ±1 day	7 Day 14 ±1 days	8 Day 21 ±1 days	9 Day 29 +2 days	10 3 months ±5 days
12-Lead ECG	X	X[f]								
Blood Chemistry[g]	X	X[h]	Xh	Xh	X	Xh	X	Xh	X[i]	Xh
C-reactive protein	X						X			
Blood Hematology[j]	X	X	X	X	X	X	X	X	X	
Pregnancy test[k] (if applicable)	X									
Blood for leukocyte transcriptome (RNA expression) profile[l]	X	X	X	X[l]	X					
Urine and Plasma for Storage[m]	X		X		X					
Arterial blood gases (if applicable)	X	X	X	X	X	X	X	X	X	

[f] 12 lead ECG will be performed at the end of the study drug infusion, then within 4-6 hours and 12-24 hours of study drug administration, and the time of its performance should be recorded.
[g] Glucose; Electrolytes - Sodium, Potassium, Calcium; Phosphorus, Chloride, Bicarbonate; Renal function tests – Urea/BUN, Creatinine; Liver function tests – Albumin, Bilirubin Total, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP); Protein Total. In case of abnormal results at the end of the study the results should be followed by the investigator until the abnormalities are resolved or determined as stabilized.
[h] Only Creatinine. At Visit 10 (3 months), creatinine obtained only if day 29 creatinine is abnormal.
[i] Creatinine and Albumin only
[j] Complete blood count including platelet count and white blood cell differential
[k] Preferably the fastest pregnancy test method (urine or blood);
[l] 5 mL (2 x 2.5 mL) of whole blood to be collected at screening, Day 1 at 4-6 hours post dose, Day 2 at 24±4 hours post dose, 72-84 hours post-dose and Day 7. Complete blood count with white blood cell differential to be obtained at same time of whole blood for genomic profile sample (Can be coordinated with CBC sample already requested at screening, Day 2, 3 and 7). Sample at 72-84 hours post-dose may require CBC sample to be obtained on Day 4.
[m] Obtain 5 ml urine and 5 ml blood for plasma for storage for AKI biomarker analysis. Second sample to be collected 24±6 hours (Day 2) after study medication administration. Final sample to be obtained at Day 7.

Visit	1 Screening	2 Day 1	3 Day 2	4 Day 3	5 Day 7 ±1 day	6 Day 10 ±1 day	7 Day 14 ±1 days	8 Day 21 ±1 days	9 Day 29 +2 days	10 3 months ±5 days
SpO2 and FiO2 (if unable to obtain arterial blood gases)	X	X	X	X	X	X	X	X	X	
Inclusion & Exclusion[n]	X									
Randomization	X									
AB103/Placebo Administration		X								
Debridement[o]	X	→	→	→	→	→	→			
Blood culture[p]	X									
Tissue for microbiology[q]	X			Xq						
Adverse Events / Serious Adverse Events[r]	X[s]	X	X	X	X	X	X	X	X	
Lesion assessment[t]	X	X	X	X	X	X	X			
Collect data on additional amputations		→	→	→	→	→	→	→	→	

[n] Should be verified twice: a full list of criteria will be verified once before instructing the pharmacist to prepare study drug and a second time immediately before study drug administration according to a partial list to include verification that 6 hours has not elapsed from clinical diagnosis and evaluation of organ function/ patient status;
[o] NSTI should be confirmed surgically, patients with non-NSTI should not receive drug; Subsequent surgical procedures through to Day 14 should be recorded in the eCRF log as they occur (see Section 6.2.6.1 for definition of debridement). Surgical procedures involving amputation(s) should be recorded in the eCRF log through Day 28.
[p] Blood culture for both aerobic and anaerobic bacteria should be repeated during the study in case of new systemic infection suspicion, as clinically indicated.
[q] Tissue for microbiology will be taken at the 1st debridement (performed at visit 1), to be used for identification of pathogen(s), susceptibility to antimicrobial drugs and Gram stain. Obtain tissue for microbiological culture if debridement performed on Day 3 (Visit 4). If culture from debridement on Day 3 is positive, then repeat culture if subsequent debridement(s) performed.
[r] Adverse events including SAEs will be collected from obtaining ICF through day 28; AEs that are not resolved should be followed-up until resolution or until determined as stable and due to known cause.
[s] AEs of day 0 are baseline AEs (not TEAE) starting after obtaining ICF until study drug administration.
[t] Assess lesion status (healing/progression/significant change in appearance) if accessible for visible assessment at time of debridement or routine dressing change

Visit	1 Screening	2 Day 1	3 Day 2	4 Day 3	5 Day 7 ±1 day	6 Day 10 ±1 day	7 Day 14 ±1 days	8 Day 21 ±1 days	9 Day 29 +2 days	10 3 months ±5 days
Collect data on acute renal replacement therapy, plasma and/or platelet transfusion		→	→	→	→	→	→	→	→	
Evaluation of adequacy of antimicrobial treatment				X						
Critical care and hospital stay parameters[u]	→	→	→	→	→	→	→	→	→	
mSOFA score[v]	X	X[w]	X	X	X	X	X	X	X	
APACHE II score[x]	X									
LRINEC and Anaya[y] scores	X									
Survival	→	→	→	→	→	→	→	→	→	→
Hospital readmissions[z]									X	X
Serum for immunogenicity[aa]	X						X		X	X

[u] Hospital length of stay (days), ICU stay (days), ICU free days, Mechanical ventilation days/mechanical ventilation free days;
[v] Screening mSOFA: To be calculated prospectively from time patient presents at the hospital (may include referring hospital) but within 6 hours prior to first debridement. Screening mSOFA may be reassessed up to actual time of surgery if initial mSOFA evaluation had score <3. To include measurements of 5 organ systems to achieve score of ≥3, in any one or more organs, with any one organ having a score of at least 2: cardiovascular, respiratory, renal, coagulation and CNS (To include evaluation of oxygenation either directly by arterial blood gas test or by calculation of PaO_2 from SpO_2, in case it is not possible to obtain arterial blood to determine the SOFA respiratory parameter),
[w] Subsequent mSOFA data collection (Days 1, 2, 3, 7, 10, 14, 21 and 29): taken once a day, in the morning (between 6AM until 12 noon) adjusted to the time of normal routine assessment activities. However, if surgery is performed during the usual time of the assessments, these assessments should then be collected at a minimum of 2 hours post-surgery. The following blood samples must be taken: platelets, and creatinine. In addition, clinical parameters should be taken (see Section 15.5). For SpO2 ≤ 95%, check for the most consistent result and record that value.
[x] For the required laboratory and clinical parameters (see Section 15.5)
[y] For the required laboratory and clinical parameters (see Section 15.1).
[z] Capture data on any readmissions within 30 days of original hospital discharge
[aa] 6 mL blood for serum for storage (for immunogenicity testing) at Screening, Days 14 and 29, and Month 3

15.2 Appendix B - APACHE II Score

APACHE II Score:

- For each variable used in the APACHE II Score, the value from the previous 24 hours yielding the most points should be used
- Since it is not possible to assess the Glasgow Coma Scale (GCS) in patients who are sedated and paralyzed, by convention it is regarded as normal, unless it is known that there was a brain injury prior to sedation, as in the case of head injury (but not confusion associated with tiredness, hypoxia, etc.). This will result in a GCS of 15 and a neurologic score of 0 (zero).
- Chronic Health Evaluation

If any of these conditions are marked "Y", assign points based on the patient's condition at the time of ICU admission (or in the 24-hour period prior to screening) - See Section C on last page.

Organ insufficiency or immuno-compromised state must have been evident prior to this hospital admission and conform to the following criteria.	
LIVER: Biopsy-proven cirrhosis and documented portal hypertension. or episodes of past upper GI bleeding attributed to portal hypertension, or prior episodes of hepatic failure/encephalopathy/coma.	Y N
CARDIOVASCULAR: New York Heart Association Class IV.	Y N
RESPIRATORY: Chronic restrictive, obstructive, or vascular disease resulting in severe exercise restriction, (i.e., unable to climb stairs or perform household duties); or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension (>40 mm Hg), or respirator dependency.	Y N
RENAL: Receiving chronic hemo- or peritoneal dialysis.	Y N
IMMUNO-COMPROMISED:	
(1) The patient has received therapy that suppresses resistance to infection, eg immuno-suppressive agents, chemotherapy, radiation, long term low dose steroids, 10 mg/day prednisone for >1 month prior to hospitalization) or recent high dose steroids (> 15 mg/kg/day of hydrocortisone or >3 mg/kg/day of methylprednisolone for >5 days).	Y N
(2) The patient has a disease that is sufficiently advanced to suppress resistance to infection, eg leukemia, lymphoma, AIDS, documented diffuse metastatic cancer.	Y N

Physiologic Variable		HIGH ABNORMAL RANGE (Check one range per variable and write the severity score in the column to the right. Note: use the worst possible score for all variables, except for the GCS score.)					LOW ABNORMAL RANGE				Severity Score
	Severity Points	+4	+3	+2	+1	0	+1	+2	+3	+4	
1	Temperature – rectal (°C)	☐ ≥41°	☐ 39-40.9°		☐ 38.5°-38.9°	☐ 36°-38.4°	☐ 34°-35.9°	☐ 32°-33.9°	☐ 30°-31.9°	☐ ≤29.9°	
2	Mean Arterial Pressume (mmHg)	☐ ≥160	☐ 130-159	☐ 110-129		☐ 70-109		☐ 50-69		☐ ≤49	
3	Heart Rate (Ventricular Response)	☐ ≥180	☐ 140-179	☐ 110-139		☐ 70-109		☐ 55-69	☐ 40-54	☐ ≤39	
4	Resp. Rate (non-ventilated or ventilated)	☐ ≥50	☐ 35-49		☐ 25-34	☐ 12-24	☐ 10-11	☐ 6-9		☐ ≤5	
5	Oxygenation: a. $FIO_2 \geq 0.5$ record $A{\cdot}aDO_2$*	☐ ≥500	☐ 350-499	☐ 200-349		☐ <200					
	b. $FIO_2 < 0.5$ record only PaO_2					☐ PaO_2>70	☐ PaO_2 61-70		☐ PaO_2 55-60	☐ PaO_2 <55	
6	Arterial pH	☐ ≥7.7	☐ 7.6-7.69		☐ 7.5-7.59	☐ 7.33-7.49		☐ 7.25-7.32	☐ 7.15-7.24	☐ <7.15	
7	Serum Sodium (mmol/L)	☐ ≥180	☐ 160-179	☐ 155-159	☐ 150-154	☐ 130-149		☐ 120-129	☐ 111-119	☐ ≤110	
8	Serum Potassium (mmol/L)	☐ ≥7	☐ 6-6.9		☐ 5.5-5.9	☐ 3.5-5.4	☐ 3-3.4	☐ 2.5-2.9		☐ <2.5	
9	Serum Creatinine (µmol/L) (double point score for acute renal failure)	☐ ≥309.4	☐ 176.8-309.3	☐ 132-177		☐ 53-133		☐ <53			
10	Hematocrit (%)	☐ ≥60		☐ 50-59.9	☐ 46-49.9	☐ 30-45.9		☐ 20-29.9		☐ <20	
11	White Blood Count (total/mm^3) (in 1000s)	☐ ≥40		☐ 20-39.9	☐ 15-19.9	☐ 3-14.9		☐ 1-2.9		☐ <1	
12	Glasgow Coma Score (GCS) Score=15 minus actual GCS	Eye	Verbal	Motor	GCS Total (= Eye + Verbal + Motor)					(Note: The best GCS used for the 1st 24 hours)	(15 - GCS Total)
A=Total ACUTE PHYSIOLOGY SCORE (APS): Total severity points indicated for Variables 1-12 in the column to the right.											
	Serum HCO_3 (venous-mmol/L) (Use in place of variable 5 if no ABGs)	☐ ≥52	☐ 41-51.9		☐ 32-40.9	☐ 22-31.9		☐ 18-21.9	☐ 15-17.9	☐ <15	

* $A{\cdot}aDO_2 = [(FiO_2\ (713)-(PaCO_2/0.8)]-PaO_2$

Glasgow Coma Scale		**Age Points = B**	**Chronic health Points = C**	**Apache-II Score** Sum of A + B + C
Circle appropriate response			If the patient has a history of severe organ systems insufficiency or is immuno compromised, assign points as follows	
Eyes open:	**verbal - nonintubated:**	**Age Points**		APS points **A =** ☐☐
4 = spontaneously	5 = oriented and Controverses	≤ 44 0		
3 = to verbal	4 = disoriented and talks	45 54 2	• 5 points for nonoperative or emergency postopera tive patients	
2 = to painful stimuli	3 = inappropriate words	55 64 3		Age points **B =** ☐
1 = no response	2 = incomprehensible sounds	65 74 5	• 2 points for elective postoperative patients	
	1 = non response	≥ 75 6		
			Otherwise zero (0) points	
Motor response	**verbal - intubated:**			Chronic health points **C =** ☐☐
6 = to verbal command	5 = seems able to talk			
5 = localizes to pain	3 = questionable ability to talk	**Age Points =**		
4 = withdraws to pain	1 = generally unresponsive			
3 = decorticate				
2 = decerebrate		☐☐	**Chronic Health Points =** ☐☐	**Total Apache II =** ☐☐

15.3 Appendix C - modified SOFA

Value	0	1	2	3	4
Respiratory PaO_2/FiO_2[1]	>400	≤400	≤300	≤200 with respiratory support	≤100 with respiratory support
Coagulation Platelets	>150	≤150	≤100	≤50	≤20
Cardiovascular[2]	No hypo-tension	MAP <70	Dopa ≤5 PE <100	Dopa > 5 Epi ≤ 0.1 NE ≤ 0.1 PE 100-300	Dopa>15 Epi>0.1 NE>0.1 PE>300 VP>0.01
Neuro GCS	15	13-14	10-12	6-9	<6
Serum Creatinine OR Urine Output	<1.2	1.2-1.9	2.0-3.4	3.5-4.9 <500cc/ day	≥5.0 <200cc/ day

1. For patients on supplemental oxygen via nasal cannula, nasopharyngeal catheter or mask and for converting SpO2 values to PaO2 use the attached oxygen conversion tables.

2. Doses of dopamine (Dopa), epinephrine (Epi), norepinephrine (NE) are in micrograms/kg/min; phenylephrine (PE) is micrograms/min; vasopressin (VP) is U/min. Vasopressors must have been administered for at least one hour.

Oxygen Conversion tables

Estimating PaO_2 from a given SpO_2

SpO_2 (%)	PaO_2 (mmHg)
80	44
81	45
82	46
83	47
84	49
85	50
86	52
87	53
88	55
89	57
90	60
91	62
92	65
93	69
94	73
95	79
96	86
97	96
98	112
99	145

Estimating FiO_2

Method	O_2 flow	Estimated FiO_2 (%)
Nasal cannula	1	24
	2	27
	3	30
	4	33
	5	36
	6	40
Nasopharyngeal catheter	4	40
	5	50
	6	60
Face mask	5	40
	6-7	50
	7-8	60
Face mask with reservoir	6	60
	7	70
	8	80
	9	90
	10	95

15.4 Appendix D - Glascow Coma Scale

Enter one score for each response (Eyes, Motor and Verbal).			
Eyes Open	Motor Responses	Verbal - Nonintubated	Verbal - Intubated
4 = spontaneously 3 = to verbal 2 = to painful stimuli 1 = no response	6 = to verbal command 5 = localized to pain 4 = withdraws to pain 3 = decorticate 2 = decerebrate 1 = no response	5 = oriented and converses 4 = disoriented and talks 3 = inappropriate words 2 = incomprehensible words 1 = no response	5 = seems able to talk 3 = questionable ability to talk 1 = generally unresponsive
Total Glasgow Coma Score (Eyes + Motor + Verbal)			

15.5 Appendix E - Clinical Scores

Table 7: Clinical Scores Parameters

Parameter	LRINEC	Anaya	mSOFA	APACHE II
Clinical				
Cardiovascular system (Mean Arterial Pressure)			+	+
Heart Rate		+		+
Nervous system (Glasgow Coma Score)			+	+
Oxygenation *(Calculation)*			+ (PaO_2/FiO_2)*	+ (Pao2 or A-a difference)
Respiratory rate				+
Temperature		+		+ (rectal)
Demographics				
Age		+		+
Chronic health evaluation				+
Laboratory				
Arterial pH				+
Coagulation Function (Platelets)			+	
C-Reactive Protein	+			
Renal system / Creatinine	+	+	+	+
Glucose	+			
Hemoglobin/Hematocrit	+	+		+
Sodium	+			+
Potassium				+
Total white cell count	+	+		+

*In case it is not possible to obtain arterial blood gases to determine the SOFA respiratory parameter, SpO2/FioO2 ratio can be imputed for PaO2/FiO2 ratio

15.6 Appendix F - Recommendations for EXCLUSION OF Immunosuppressive Agents

Immunosuppressive Agent[a]	Upper limit dosage use
1. Corticosteroid	> 40 mg/day of prednisone or its equivalent daily for > 2 weeks.
Equivalent Dose (mg)	
a) Prednisone	40 mg
b) Hydrocortisone	160 mg
c) Methylprednisolone	32 mg
d) Dexamethasone	6 mg
e) Cortisone	200 mg
f) Betamethasone	4.8 mg
2. Methotrexate (Rheumatrex, Trexall)	Excluded at any dose.
3. Leflunomide (Arava)/Teriflunomide (Aubagio)	Acceptable if being used as monotherapy.
4. Cyclophosphamide (Cytoxan)	Excluded at any dose.
5. Cyclosporine A	Excluded at any dose. Ophthalmic formulation (Restasis) is permitted
6. FK506 (Tacrolimus)	Excluded at any dose Topical formulation (Protopic) is permitted**.**
7. Azathioprine	Excluded at any dose.
8. Cancer Chemotherapy	Patients having received cancer chemotherapy in the previous 4 weeks are excluded.

Immunosuppressive Agent[a]	Upper limit dosage use
9. Mycophenolate Mofetil (MMF) (CellCept)	Solid organ transplant and bone marrow transplant patients are excluded.
10. Sirolimus (Rapamycin, rapamune)	Excluded at any dose.
11. Everolimus (Certican)	Excluded at any dose.
12. Temisirolimus (Torisel)	Excluded at any dose.
13. Thalidomide	Patients receiving this drug within the last 72 hours are excluded.
Biologics	
(a) Anti-tumor necrosis factor (TNF) agents	Patients receiving anti-TNF agents within the past 8 weeks are excluded.
– Entanercept (Enbrel)	
– Adalimumab (Humira)	
– Infliximab (Remicade)	
– Certolizumab (Cimzia)	
– Golimumab (Simponi)	
(b) Interleukin-1 Receptor Antagonist (IL-1 RA) (Kineret)	Patients receiving IL-1 RA within the last 8 weeks are excluded.

Immunosuppressive Agent[a]		Upper limit dosage use
(c)	**CTLA-4 Fusion Protein** – Atapacept (Orencia) – Belatacept (Nulojix)	Patients receiving CTLA-4 Fusion protein within the past 8 weeks are excluded.
(d)	**Anti-C20** – Rituximab (Rituxan/MabThera) – Obintuzumab (Gazyva)	Patients receiving this drug within the last 2 years are excluded**.**
(e)	**Anti-CD52** – Alemtuzumab (Campath)	Patients receiving this drug within the last 2 years are excluded.
(f)	**Anti-IL2** – Daclizumab or Anti-Tac (Zenapax) – Basiliximab (Simulect)	Patients receiving any of these drugs within the last 2 years are excluded.

Immunosuppressive Agent[a]		Upper limit dosage use
(g)	**Anti-IL6** – Tocilizumab (Actemra/RoActemra)	Patients receiving any of these drugs in the last 2 years are excluded.
(h)	**Anti-IL12/13** – Ustekinumab (Stelara)	Patients receiving any of these drugs within the last 2 years are excluded.
(i)	**Anti-BAFF (B-cell activating factor)** – Belimumab (Benlysta)	Patients receiving this drug within the last 8 weeks are excluded.
(j)	**Integrin Inhibitor** – Natalimumab (Tysarbi)	Patients receiving this drugs within the last 2 years are excluded.

[a] For agents not listed, patients should be off such therapies for a time sufficient to restore immune function.