A MULTI-CENTER RANDOMIZED, DOUBLE-BLIND STUDY TO ASSESS THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF LBP-EC01 IN PATIENTS WITH LOWER URINARY TRACT COLONIZATION CAUSED BY *E. COLI*

SHORT TITLE: "SAFETY, TOLERABILITY, AND PHARMACOKINETICS OF LBP-EC01 IN PATIENTS WITH LOWER URINARY TRACT COLONIZATION CAUSED BY E. COLI"

Investigational Product: LBP-EC01

Protocol Number: LBx-1001

Development Phase: 1b

National Clinical Trial (NCT) Identified Number: N/A

IND Sponsor: Locus Biosciences, Inc

523 Davis Drive, Suite 350

Morrisville, NC 27560

Version Number: 1.4

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Summary of Changes from Previous Version:

Affected Section(s)	**Summary of Revisions Made**	**Rationale**
2.1, 4.0, 10.4.1.	Updated to clarify that PD analysis will be conducted with urine samples across all timepoints collected: Baseline (Day 1), Day 2, Day 3, Day 5, Day 7 (EOT), Day 14, and Day 28.	Administrative clarification

Protocol Approval Page

Study Title	A Multi-Center Randomized, Double-Blind Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of LBP-EC01 in Patients With Lower Urinary Tract Colonization Caused by *E. Coli*
Protocol Number:	LBx-1001
Protocol Version:	Version 1.4
Date:	01 Oct 2020

Approved for the Sponsor by:

Name: Paul Kim, PhD
Title: Chief Development Officer
Locus Biosciences, Inc.

Signature

01 Oct 2020

Date (ddmmmyyyy)

Revision History

Version	Date	Comment
1.1	28 FEB 2019	For IRB/IND submission
1.2	22 MAR 2019	Clarifications
1.3	04 OCT 2019	Incorporation of FDA feedback
1.4	01 OCT 2020	Clarifications/administrative change

Study Contact Information

Any serious adverse event (SAE) must be reported within 24 hours. See Section 9.3.6 for detailed adverse event reporting procedures.

Study Contact Information

Role in Study	Name, Title, Address	Telephone / fax / email
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TABLE OF CONTENTS

LIST OF TABLES

1. STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), and applicable United States (US) Code of Federal Regulations (CFR). The PI will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) Sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the patients. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any patient is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from patients who provided consent, using a previously approved consent form.

2. PROTOCOL SUMMARY

2.1. Synopsis

Title:	A MULTI-CENTER RANDOMIZED, DOUBLE-BLIND STUDY TO ASSESS THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF LBP-EC01 IN PATIENTS WITH LOWER URINARY TRACT COLONIZATION CAUSED BY E. COLI
Study Description:	Study LBx-1001 is a multi-center randomized, double-blind study to assess the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of LBP-EC01 in patients with indwelling urinary catheters, or requiring intermittent catheterization, and/or patients with asymptomatic bacteriuria caused by *Escherichia coli (E. coli)*. This study population has been selected because LBP-EC01 is a phage cocktail where active bacterial host engagement is required to allow for amplification of the phage and evaluation of the safety and PK of the phage cocktail. Eligible patients will require confirmation of colonization with a urine sample taken within 10 days of randomization that cultures contain $\geq 10^3$ *E. coli* CFU/mL, without the patient having clinical signs or symptoms of an active urinary tract infection (UTI) requiring antibiotic treatment. Patients should have *E. coli* as the primary colonizing bacteria and must not have a secondary bacterial colonization at levels equal to or greater than that seen from *E. coli*. Patients may concomitantly receive standard of care treatment for a **newly** diagnosed symptomatic UTI caused by Gram-positive bacteria while on study with consultation of the medical monitor. Active or recent (within 14 days prior to randomization) antimicrobial therapy will not be allowed. Analgesic use is permitted. A complete or abbreviated physical examination will be performed at the timepoints indicated in the Schedule of Assessments (SoA) table. An ultrasound assessment of the spleen and a spleen exam will be performed at the time points indicated in the SoA table. Adverse events (AEs) and concomitant medications will be monitored throughout the entire study (screening through follow-up). Vital signs: body temperature, blood pressure (BP), pulse rate and respiratory rate will be collected at the time points indicated in the SoA table. Electrocardiography (ECG) will also be performed as indicated on the SoA table. <u>Special Safety Monitoring</u>: General immunogenicity will be evaluated by assessing elevations in IgA, IgE, IgG and IgM levels across pre-treatment, Day 3, End of Treatment (EOT) and on follow-up visit on Day 14 and Day 28. <u>Clinical Laboratory Tests</u>: Hematology, coagulation, blood chemistry and urinalysis will be collected at the timepoints indicated in the SoA (Table 1).
Objectives:	<u>Primary Objectives</u>: To evaluate the safety, tolerability and PK of LBP-EC01 in patients ≥18yrs of age with lower urinary tract colonization caused by *E. coli*. <u>Secondary Objective</u>:

	To evaluate the pharmacodynamics (PD) of LBP-EC01. Exploratory Objective: To explore the influence of LBP-EC01 on the urinary tract microbiota.
Endpoints:	Primary Endpoints: • Safety and tolerability analysis of adverse events • Pharmacokinetic analysis Secondary Endpoints: • Reduction in urinary *E. coli* burden at any of the following timepoints: Day 2, Day 3, Day 5, EOT, Day 14, and Day 28 • Time to 1 log reduction in urinary *E. coli* count • Recurrence of *E. coli* colonization or incidence of infection based on clinical signs and symptoms • Evaluation of possible immunogenicity by measuring changes in IgA, IgE, IgG, IgM levels
Study Population:	Approximately 30 patients 18 years of age or older with a history of urinary tract infection or colonization caused by *E. coli* who have indwelling urinary catheters, or who require intermittent catheterization, and/or patients with asymptomatic bacteriuria caused by *E. coli* colonization ($\geq 10^3$ CFU/mL) on microbiological diagnosis, without clinical signs or symptoms of infection requiring antibiotic treatment will be enrolled. Patients will be screened for presence of *E. coli* colonization ($\geq 10^3$ CFU/mL) prior to randomization and evaluated for potential bacterial susceptibility to LBP-EC01.
Phase:	1b
Sites/ Facilities Enrolling Patients:	Approximately 8 investigational sites in the US.
Description of Study Intervention:	Consenting adult male or female patients meeting entry criteria will be randomized 2:1 to receive either LBP-EC01 (approximately 1.5×10^7 – 1.5×10^{13} PFU/vial - the maximum feasible dose based on the manufacturing process) or placebo (Lactated Ringer's solution, injection, USP) twice-daily (BID) by urinary or indwelling catheter. LBP-EC01 is a mixture of 3 distinct obligate lytic bacteriophages, each carrying a universal CRISPR RNA expression cassette embedded in the wild-type phage genome.
Study Duration:	Estimated duration of enrollment, treatment period and follow-up will be approximately 8 months.
Patient Duration:	Study duration for patients will be up to 56 days, which includes up to 21 days for screening, 7 days of Investigational Medicinal Product (IMP) treatment, a Day 14 assessment, a Day 28 assessment (28 days after first dose), and at EOS (35 days after first dose). Patients will be in the clinic or hospital the evening prior to receiving the first dose of treatment and throughout the 7 days of treatment. Once the study is complete, patients should resume their regular medications and any catheters installed specifically for the conduct of the

	study should be replaced by new catheters at the discretion of their physician.

Table 1: Schedule of Assessments

Assessment	Screening		In Hospital / Clinic ——— Treatment Period												
			Doses 1 - 6							Doses 7 - 12		Dose 13			
	Day (-21 to -7)	Day (-6 to -1)	Day 1			Day 2		Day 3		Day 4 - 6		Day 7 (EOT)	Day 14 (± 3 days)	Day 28 (± 3 days)	Day 35[m]
			AM	+6hr	PM	AM	PM	AM	PM	AM	PM	AM only	Clinic Visit	Clinic Visit	Telephone Contact
Written Informed Consent	X														
Inclusion/ Exclusion Criteria	X	confirm													
Demographics	X	confirm													
Medical/ Medication History	X	confirm													
Physical Examination [a]	X	confirm						X[b]				X[b]	X	X	X[n]
Spleen Evaluation[c]		X	X			X		X		X		X	X	X	X[n]
Vital Signs [d, e]		X	X	X	X	X	X	X	X		X	X	X	X	X[n]
12-lead ECG		X											X	X	X[n]
Clinical Labs [f]	X	confirm	X		X				X		X (Day 5)	X	X	X	X[n]
Urine Culture and Sensitivity	X	confirm [m]	X			X		X		X (Day 5)		X	X	X	
Urinalysis [g]	X	confirm	X			X		X		X (Day 5)		X	X	X	X[n]
PK Sampling (Blood and Urine) [h]			X	X	X	X	X	X	X	X	X	X	X	X	
Immunogenicity Assay	X	confirm							X			X	X	X	
EPT Urine Pregnancy [i]		X	X[i]											X	
Randomization [j]		X													
Drug IMP or Placebo Administration [k]			X		X	X	X	X	X	X	X	X			
Unsolicted AE [l]	●														●
Solicited AE [l]			X	X	X	X	X	X	X	X	X	X	X	X	X

AE=adverse event; AM=morning administration; ECG=electrocardiogram; EOT=End of Treatment; EPT=early pregnancy test; IMP=investigational medicinal product; PK=pharmacokinetic; PM=evening administration; confirm=conduct assessment if not previously done or assessment is outside window (see footnote for assessment).

Note: Urine Culture and Sensitivity at Screening should be collected and shipped to the central laboratories by Day -4 to allow time for results to be reported prior to patient randomization.

[a] A complete physical examination should include an evaluation of the head, eyes, ears, nose, throat, neck and lymph nodes, and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurological systems. No invasive examinations (e.g., rectal examination) will

be performed. A symptom-directed physical examination will be performed at all other time points specified. A targeted physical examination may be performed as needed in response to adverse events or changes in medical history.

[b] Physical exam will be performed prior to AM dose.

[c] Spleen evaluation will include ultrasound assessments for splenomegaly at Day -6 to -1, Day 3, and Day 7. Clinical examinations to assess for splenomegaly will be performed at all visits. Ultrasound assessment and clinical examinations may be performed at any time during the visit.

[d] Vital signs include single measurements of systolic and diastolic blood pressure, pulse rate, respiratory rate and oral body temperature. All measurements are to be taken after the patient has rested quietly for at least 5-10 minutes.

[e] During dosing, vital signs will be taken within 30 minutes prior to dosing and within 30 minutes after dosing (± 10 minutes) for the first 5 doses, and at 6 hours (± 30 minutes) after the first dose. Vital signs will be collected prior to the PM doses of Day 3 and Days 4-6, but do not need to be collected within 30 minutes of dosing. Vital signs can be taken at any time during the day for Day 7, Day 14, Day 28, and Day 35 (if a clinic visit).

[f] Clinical labs include assessment of hematology, clinical chemistry, lipids and coagulation. See Table 2 for timing of blood collection for these parameters. If patient screening is done on Day -10 to Day -7 then this will not need to be repeated during Day -6 to -1.

[g] Urinalysis samples will be collected via catheter during the Treatment Period, at Day 14 and Day 28. If patient screening is done on Day -10 to Day -7 then no need to be repeated during Day -6 to Day -1.

[h] Blood and urine samples will be collected for PK analysis at the time points described in Table 2.

[i] Only women of childbearing potential. Day 1 will be obtained prior to the first dose.

[j] Randomization will take place on Day -1 or Day 1; patients will be randomized to receive active or placebo treatment.

[k] AM dose is to be given at 8:00 AM ± 2 hr and PM dose is to be given 12 hr ± 3 hr after the AM dose. Concomitant medications should be given during study conduct as required/prescribed. Adverse Events will be monitored and reported from patient screening to end of study.

[l] Patient diary cards will be provided to the patient to document AEs that occur between visits. At Days 14, 28, and 35, the patient diary cards will be reviewed with the patient. AEs will be solicited at all visits and also once per day by telephone for the 7 days after the last dose. Unsolicited AEs will be collected at any time after the ICF is signed until the end of the study.

[m] Urine culture sample should be obtained within 10 days of randomization and *E. coli* should be present at $\geq 10^3$ CFU/mL. If patient screening is done on Day -10 to Day -7, then this collection will not need to be repeated during Day -6 to -1. Note: sample turnaround takes 48 -72 hours and therefore it is suggested to collect the sample no later than Day -4.

[n] Day 35 will be a telephone contact to solicit and assess AEs. At the discretion of the investigator, the patient may be asked to return to the clinic/hospital for a Day 35 (± 3 days) Visit to follow-up on any abnormal/clinically significant findings from the Day 28 Visit (e.g. abnormal clinical laboratory results). Assessments as deemed necessary by the investigator may be performed to ensure patient safety.

Table 2: Schedule of Blood and Urine Sampling

	Screen		DAY 1			DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7		DAY 14	DAY 28	DAY 35[f]
	(D-21 to D-7)	(D-6 to D-1)	AM	+6hr [c]	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	Clinic Visit	Clinic Visit	Telephone Contact
BLOOD:																				
PK [a]			Pre/ Post	X	Pre/ Post	Pre/ Post	Pre/ Post	Pre/ Post	Pre/ Post	Pre/ Post	Pre	Pre/ Post	Pre	Pre/ Post	Pre	Pre/ Post		X	X	
Labs [b]	X	confirm	Pre		Post				Post				Post			Post		X	X	X[f]
Immunogenicity	X	confirm							Pre							Pre		X	X	
Exploratory Biomarkers (blood)			Pre					Pre				Pre				Pre		X	X	
URINE:																				
PK [a]			Pre/ Post	X	Pre/ Post	Pre/ Post	Pre/ Post	Pre/ Post	Pre/ Post	Pre/ Post	Pre	Pre/ Post	Pre	Pre/ Post	Pre	Pre/ Post		X	X	
Culture & Sensitivity	X	confirm [e]	Pre			Pre		Pre				Pre				Pre		X	X	
Urinalysis & Micro [d]	X	confirm	Pre			Pre		Pre				Pre				Pre		X	X	X[f]
Exploratory Biomarkers (urine)			Pre					Pre				Pre				Pre		X	X	

AE=adverse event; AM=morning administration; PK=pharmacokinetic; PM=evening administration; confirm=conduct assessment if not previously done or assessment is outside window (see footnote for assessment).

Note: Sample turnaround takes 48 -72 hours and therefore it is suggested to collect the sample no later than Day -4 to allow time for results to be reported prior to patient randomization.

[a] Pre-dosing PK samples for urine and blood will be collected after catheterization (if there is no existing catheter), up to 30 minutes prior to dosing. Post-dosing PK samples will be taken within 60 minutes after the catheter is released and re-clamped for 20 minutes to take additional urine and blood samples. The times of collection will be recorded.

[b] Labs include hematology, clinical chemistry, lipids, and coagulation. If patient screening is done on Day -10 to Day -7 then this will not need to be repeated during Day -6 to -1.

[c] Additional collection 6 hours (± 1 hr) after AM dose.

[d] If patient screening is done on Day -10 to Day -7, then this collection will not need to be repeated during Day -6 to -1.

[e] Urine culture sample should be obtained within 10 days of randomization and *E. coli* should be present at $\geq 10^3$ CFU/mL. If patient screening is done on Day -10 to Day -7, then this collection will not need to be repeated during Day -6 to -1.

[f] Day 35 will be a telephone contact to solicit and assess AEs. At the discretion of the investigator, the patient may be asked to return to the clinic/hospital for a Day 35 (± 3 days) Visit to follow-up on any abnormal/clinically significant findings from the Day 28 Visit (e.g. abnormal clinical laboratory results). Assessments as deemed necessary by the investigator may be performed to ensure patient safety.

3. INTRODUCTION

3.1. Study Rationale

Antimicrobial resistance (AMR) is one of the greatest risks to human health today and one of the greatest challenges to our civilization. It is estimated that by 2050 ten million people may be dying annually and the economic burden may hit $100 trillion (O'Neill, 2016). The Center for Disease Control and Prevent estimates that in the US, more than two million people are sickened every year with antibiotic-resistant infections, with at least 23,000 dying as a result (Centers for Disease Control and Prevention, 2013).

Between 2010 and 2014, Extended Spectrum Beta-Lactamase (ESBL) rates among UTIs in the US increased from 7.8% to 18.3%, with the highest rates noted among hospital-associated infections (Lob et al, 2016). A recent epidemiological study showed that 14.9% of hospital-acquired infections were caused by carbapenem resistant *E. coli* (Guh et al, 2015). Urinary tract infections caused by susceptible Enterobacteriaceae, compared with those caused by ESBL-producing strains lead to greater median length of hospital stay, initial antibiotic failure and higher infection-related mortality (MacVane et al, 2015). The threat posed by these multi-drug and pan-resistant microorganisms are a dire warning to the world that we must increase our capacity to produce new modalities of antimicrobial therapy. There is particularly a great unmet need for novel compounds which are safe, effective and unaffected by existing resistance mechanisms (Payne et al, 2007; Talbot et al, 2006). The lack of antibiotic therapeutic options against multi- drug-resistant (MDR) bacteria has led to a revival in the investigative and rescue use of bacteriophage therapy in patients with difficult to treat life threatening infections where there are frequently no alternative antibiotic therapies (FDA-NIH Bacteriophage Workshop, 2017).

LBP-EC01 is a product containing a combination of 3 distinct obligate lytic bacteriophages (internally named as p0031-8, p004k-5, and p00ex-2) in equal amounts, each carrying a universal Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR) ribonucleic acid (RNA) expression cassette (hereafter termed "crRNA cassette") embedded in the wild-type phage genome. Each engineered CRISPR-enhanced bacteriophage (crPhage) is intended to retain wild-type lytic activity. Both the individual crPhages and the crPhage cocktail will retain broad host range, if not an expanded host range, by their ability to transduce lethal crRNA constructs in the absence of productive lytic infection, and improved lethality for each crPhage to the cognate wild-type bacteriophage. LBP-EC01 is being developed for intravesicular administration for treatment of UTIs caused by *E. coli* including MDR and carbapenem-resistant (CR) strains.

Phage-host PD are complex, and dependent on the replication rate of phages at the site of infection, as well as the density of susceptible bacterial hosts and the growth rate of that host in human tissues (Lavigne et al, 2013; Levin and Bull, 2004). Thus, for this first in human study (FIH) a population who has significant numbers of host bacteria has been chosen to evaluate the safety, tolerability and PK.

3.2. Background

3.2.1. Disease Background

Urinary tract infection is the most common infection in people with spinal cord injury (SCI), who need any form of bladder catheterization. Prolonged use of indwelling catheters increases the risk of UTI, the rate of hospital readmissions due to UTI, and long-term urological complications. Typical symptoms of UTI—dysuria, frequency, urgency, suprapubic discomfort and flank pain—are often absent in persons with SCI. Instead, non-specific symptoms of urinary leakage, change in voiding habits, worsening muscle spasm, increasing autonomic dysreflexia, sweating, malaise, and fever are often the presenting complaint for this population. Almost all persons with chronic indwelling catheters and about two-thirds of people using intermittent catheterization have bacteriuria. However, most cases of bacteriuria in this population represent bladder colonization, not UTI. In addition to the lack of specific symptoms, the presence of bacteriuria in this population results in the over-diagnosis of UTI in patients with SCI. Thus, the definition of UTI used in persons with SCI requires not only the presence of bacteriuria, but also evidence of significant pyuria, and subtle clinical manifestations that cannot be explained by another cause (Darouiche, 2010). The Infectious Disease Society of America defines catheter-associated asymptomatic bacteriuria as the presence of $\geq 10^5$ colony forming units (CFU)/mL of at least one bacterial species in the urine in catheterized or recently catheterized patients (preceding 48 hours) without symptoms suggestive of a UTI. A catheter-associated UTI is defined as the presence of $\geq 10^3$ CFU/mL of at least one bacterial species in the urine of catheterized or recently catheterized patients with symptoms suggestive of a UTI (Hooten et al, 2010).

3.2.2. Bacteriophage Therapy

Bacteriophages are viruses that infect and replicate within bacteria, and like other viruses, may have double-stranded or single-stranded deoxyribonucleic acid (DNA) or RNA genomes. Phages are generally specific to strains within a species due to their method of replication, which involves binding to a specific cell surface receptor on the host bacterial cell. The prototypical phage will attach to the relevant bacterial receptor, inject their genetic material, overtake the bacterial cellular machinery to instruct the cell to produce new phage virions, and then burst the host cell, releasing progeny. This mechanism of replication can be exploited to provide an advantageous antimicrobial effect in humans via phage therapy (Lavigne et al, 2013).

Bacteriophage therapy has been used extensively since the 1930s, particularly in Poland and Eastern Europe, where it has been shown to be safe and well tolerated, with no obvious class related AEs in the clinic (Wittebole et al, 2014). Recently in 2 controlled studies, 1 in 40 patients with chronic leg ulcers, and the other in 24 patients with chronic otitis media, phage therapy was effective with no related AEs detected (Wright, et al, 2009; Rhoads et al, 2009). Another recent review suggested that bacteriophages could also be an efficacious and safe therapeutic modality in immunocompromised patients (Borysowski and Gorski, 2008). This lack of toxicity is a consequence of bacteriophage composition, which is almost entirely protein and DNA which results in minimal interaction with the body's metabolism (Abedon and Thomas-Abedon, 2010). Several recent reports of phage therapy in individual patients have also confirmed the safety and efficacy of phage therapy used to treat MDR bacterial infections in critically ill patients (Schooley et al, 2017; LaVergne et al, 2018).

A 7-day non-Good Laboratory Practices (non-GLP) toxicology study was performed using a test article of the crPhage cocktail (crT37) containing crT7 and crT7M (derived from T7 and T3, respectively) phages. These phages are similar to the phages in LBP-EC01 in that they are engineered to carry a CRISPR-Cas3 construct and that they target *E. coli,* however, the crT37 phages contain a Type 1-E CRISPR-Cas system while LBP-EC01 contain Type 1-E and Type 1-F CRISPR-Cas systems. They also differ in that the crT37 cocktail was manufactured using a less stringent non-GMP manufacturing process while LBP-EC01 was manufactured according to GMP processes. The crT37 cocktail was administered either intravenously (IV) ($1.0x10^{11}$ PFU/dose/phage) or intraurethrally (IU) ($0.5x10^{11}$ PFU/dose/phage) once daily for seven consecutive days to female Crl:CD-1 mice. Groups 1 and 2 (9 female mice/group) were dosed IV with 0.1 mL of vehicle (1x Tris-buffered saline, pH 7.4) or test article. Groups 3 and 4 (9 female mice/group) were dosed with 0.05 mL of vehicle or test article into the bladder by intracatheter instillation.

The 7-day non-GLP toxicology study showed no crPhage-related mortality or moribundity, no effect on body weight, and no abnormal clinical observations for either route of administration. Possible test article-related effects in the Phage IV-treated group were limited to decreases in red blood cell mass-related parameters, increased reticulocyte counts, decreased eosinophils, and increased spleen, kidney, and decreased lung weights. Possible test article-related effects in the crPhage IU-treated group were limited to higher cholesterol and triglyceride levels. The clinical chemistry and organ weight results from this study should be viewed in the context of the small sample sizes used (3 for clinical chemistry and 6 for organ weights) and the possibility that alterations may be artifacts due to small sample size.

Also, of note is that the crPhage preparations in these preclinical experiments used research-grade non-GMP purification processes, which are not as robust as the GMP processes used to produce clinical trial material. Therefore, the test article production process did not effectively remove some of the residual impurities and endotoxin which has been known to cause tissue changes like splenomegaly (Seemann et al, 2017).

The tissue samples obtained from the 7-day non-GLP toxicology study were preserved by flash freezing in liquid nitrogen and then stored at -80°C. The tissues were sent to an independent outside pathology lab for processing and evaluation. Due to the duration of time between tissue harvest and tissue processing and evaluation (approximately 27 months) the samples had undergone significant degradation and were unevaluable by the pathology lab.

However, in conclusion, once daily IV or IU administration of crPhage was generally well tolerated (data on file).

Antimicrobial proof-of-concept studies in standard murine models of antimicrobial efficacy, including representative peritonitis models showed 80% survival with a single-dose treatment compared to 0% with no treatment, and thigh infection models demonstrated ~1.5–5.5-log CFU reduction in 60 minutes compared to no treatment.

A surrogate PK/PD and absorption, distribution, metabolism, and excretion study measured phages at high concentrations in the bladder (10^3 – 10^8 PFU/gram tissue up to 72 hours) and kidney (10^2 – 10^6 PFU/gram up to 12 hours) after a single intravesicular administration of 10^9 PFU in healthy mice.

3.2.3. Bacterial Host Range Analysis

Locus Biosciences has developed a product containing a combination of crPhages for the treatment of *E. coli* infection. The host range of each phage was measured via the change in area under the growth curve between untreated and phage-treated cultures across a diverse panel of clinical *E. coli* isolates. From a collection of over 150 phages, an optimized cocktail of 3 phages was selected that resulted in a cumulative host range of approximately 80% across a 153-strain panel of *E. coli* (phylogenetic distribution, A-10%; B1-15%; B2-48% D-27%; approximately 20% of strains were MDR by MIC testing against 25 distinct antibiotics). Selected phages exhibited significant activity against these isolates at concentrations of 0.01 – 10 phage particles per bacterium.

3.3. Risk/Benefit Assessment

3.3.1. Known Potential Risks

3.3.1.1. Immediate Potential Risks

A potential concern for safety is the possibility of endotoxin release associated with active bacterial lysis. An *in vitro* endotoxin release study following exposure of 2 strains of *E. coli* to either bacteriophage or antibiotics has shown that endotoxin production is similar with bacteriophage lysis to that seen with amikacin therapy, and less than that seen with a beta lactam antibiotic (Dufour et al, 2017). Whilst the Locus phage-drug compound will contain small amounts of endotoxin, the manufacturing process will provide strict limits to the amount of endotoxin in the final drug product. The endotoxin exposure will be similar to that seen with rapidly effective bactericidal antibiotic therapy.

A preclinical 7-day non-GLP toxicology study was performed where possible test article-related effects were observed including increased spleen weight in the Phage IV-treated group (see Section 3.2.2). As such, splenomegaly may be a potential risk and will be monitored during the conduct of this study.

Instrumentation or instillation of solutions into the bladder could induce autonomic dysreflexia in certain spinal injuries. Patients with severe autonomic dysreflexia will be excluded (see Section 6.2). Any instrumentation will be minimized and the intravesical dose volume will be approximately 60 mL and warmed to room temperature before instillation. All procedures related to drug preparation, instrumentation and catheter handling will be performed under sterile conditions to avoid introduction of infection.

3.3.1.2. Long Term Potential Risks

A potential safety concern with bacteriophage therapy (both wild type and CRISPR-enhanced bacteriophage), is the potential to transfer bacterial virulence factors or antibiotic resistance genes. The bacteriophages selected for LBP-EC01 drug cocktail production have been screened to exclude those with virulence genes or antibiotic resistance genes. There has been little information regarding any risk associated with phage cocktails, however, a recent bioinformatic analysis of a commercial Russian phage cocktail did not reveal undesired genes, and a small human volunteer trial did not associate adverse effects with oral bacteriophage exposure (McCallin et al, 2013).

In a Locus preclinical persistence and distribution study in mice treated with a cocktail of 2 bacteriophages (crT7 and crT7M) administered by intra-urethral instillation, all phages showed persistence in the bladder for a limited period without any amplification. In another study by oral administration using a polymerase chain reaction (PCR)-based method of quantification, all phages had been cleared by 72 hours (Data on file).

Bacteriophages as non-self antigens, may stimulate immunological responses resulting in detrimental effects for recipients, however, at present no evidence exists for this possibility. There is some concern that higher exposure to phages may result in the production of anti-bacteriophage antibodies that could potentially result in adverse reactions or neutralize therapeutic bacteriophage effects. A safety evaluation of bacteriophage therapy in 15 adult healthy volunteers using *E. coli* T4 phage administered orally indicated no increase in level of anti-bacteriophage immunoglobulin (Ig)G, IgM, and IgA in the serum after 1 month of dosing (Bruttin, 2005). In a cohort of patients treated with bacteriophage therapy for 15 to 60 days, by multiple routes of administration high anti-phage activity was found in 12.3% of patients within 15 to 60 days of bacteriophage therapy. Preliminary observations performed in 15 patients with high anti-bacteriophage activity of sera suggest that the induction of anti-bacteriophage activity of patients' sera during or after bacteriophage therapy does not exclude a good result for bacteriophage therapy (Łusiak-Szelachowska et al, 2014). The overall finding was of a generally low level of anti-bacteriophage antibodies in untreated subjects with only slight increase in anti-bacteriophage antibodies in bacteriophage treated patients which would be unlikely to result in major clinical concern. A further study by the same authors in 62 patients with various bacterial infections and 30 healthy volunteer controls was conducted to determine the association between the bacteriophage neutralization of patients' sera and the clinical outcome of bacteriophage therapy. The anti-bacteriophage activity of sera levels and clinical results indicated that the level of anti-bacteriophage activity was not correlated with outcome of bacteriophage therapy (Łusiak-Szelachowska et al, 2017).

3.3.2. Known Potential Benefits

Bacteriophage are bacteria-specific viruses that infect and replicate within bacteria. The prototypical phage will attach to the relevant bacterial receptor, inject their genetic material, overtake the bacterial cellular machinery to instruct the cell to produce new phage virions and then burst and destroy the host cell, releasing progeny. Phages are generally specific to strains within a bacterial species due to their method of replication, which involves binding to a specific cell surface receptor on the host bacterial cell. The antimicrobial effect of bacteriophages is therefore highly pathogen specific and given adequate exposure rapidly bactericidal. This bacteriophage activity is unrelated to any known mechanisms of antibiotic resistance and will therefore retain activity against MDR and extensive drug resistant (XDR) isolates. LBP-EC01 is a blend of 3distinct engineered bacteriophages ('crPhages') that each expresses an identical copy of a CRISPR-Cas3 construct targeting *E. coli*. The CRISPR construct is integrated into the phage genome without disrupting any genes necessary for the lytic lifestyle of the phage. This construct was optimized to leverage the Type I-E or Type I-F endogenous *E. coli* CRISPR-Cas3 system to simultaneously degrade 4 independent, highly conserved genes with functions related to cell division and maintenance (the 'CRISPR array'). Each CRISPR target alone can efficiently kill *E. coli* with an average CFU reduction of 3.6 to >5.3log (below limit of detection) in plasmid-based transformation experiments (data on file).

This novel bacteriophage technology could make a major difference to the treatment of patients with severe infections caused by MDR and XDR pathogens. This could have a marked impact on managing individual infections as well as social benefits in helping to decrease the deleterious effect of AMR on future advances in surgery and cancer therapy.

3.3.3. Assessment of Potential Risks and Benefits

Instrumentation or instillation of solutions into the bladder could induce autonomic dysreflexia in certain spinal injuries. Patients with severe autonomic dysreflexia will be excluded. Any instrumentation will be minimized, and solution volume will be limited and warmed to room temperature before instillation. All procedures related to drug preparation, instrumentation and catheter handling will be performed under sterile conditions to avoid introduction of infection.

Risks in this study are perceived to be low due to known lack of toxicity of phage therapy, whereas the benefits of establishing dosing and PK parameters as well as safety, are considered essential to progressing this type of therapy to the clinic.

4. OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary Pharmacokinetics, safety, tolerability	• Levels of crPhage will be assessed by quantitative plaquing assay to determine levels in urine and blood • AEs, hematology, chemistry	• Establish adequate dosing and exposure in urine and blood
Secondary Pharmacodynamics of LBP-EC01	• Reduction in urinary *E. coli* burden at any of the following timepoints: Day 2, Day 3, Day 5, EOT, Day 14, and Day 28 • Time to 1 log reduction in urinary *E. coli* count • Recurrence of *E. coli* colonization or incidence of infection based on clinical signs and symptoms • Changes in IgA, IgE, IgG, IgM levels	• Demonstrate reduction in level of *E. coli* colonization
Exploratory To explore the influence of LBP-EC01 on the urinary tract microbiota	• Urine culture and sensitivity (persistent, recurrent *E. coli*, new colonizing new infections will be documented) • Evaluation of bacterial biomarkers (e.g. phage sensitivity/resistance, bacterial antibiotic resistance, bacterial metagenomic analysis)	• Monitoring effect on microbiome • Evaluation of possible immunogenicity

5. STUDY DESIGN

5.1. Overall Design

This is a Phase 1b FIH study to assess safety, tolerability, and PK of LBP-EC01 in patients who have significant colonization of the urinary tract by *E. coli*. It is a multi-center randomized, double-blind study in patients with indwelling urinary catheters, or who require intermittent urinary catheterization and/or patients with asymptomatic bacteriuria caused by *E. coli*.

No formal hypothesis will be tested. Approximately 30 patients will be randomized 2:1 to receive either LBP-EC01 (at approximately 1.5×10^{7} – 1.5×10^{13} PFU/vial - the maximum feasible dose based on manufacturing process) or inert placebo given twice daily for 7 days by intravesical catheter. A new catheter will be installed at the beginning of the study and will be replaced at the discretion of the investigator. The study will be conducted in approximately 8 sites in the US. Study duration for patients will be up to 56 days, which includes up to 21 days for screening, 7 days of Investigational Medicinal Product (IMP) treatment, a Day 14 assessment (14 days after first dose), a Day 28 assessment (28 days after first dose), and at EOS (35 days after first dose). Patients will be in the clinic or hospital the evening prior to receiving the first dose of treatment and throughout the 7 days of treatment. Study enrollment will continue up to 30 evaluable patients.

The study will consist of a Screening Period of up to 21 days. On Day -1 patients will either be hospitalized or in a facility that can provide proper administration of study drug and then randomized to LBP-EC01 or placebo. The entire 7-day Treatment Period in which patients will receive 13 doses of IMP will be conducted with the patient in the clinic/hospital. In circumstances where the patient may not be able to be in clinic/hospital for the full 7 days of treatment, upon consultation between the treating physician and the study Medical Monitor, patients may be considered to be treated and monitored in clinic/hospital for Days 1-3 and then may be allowed to come back to the clinic/hospital for the PM dose on Day 3 and the remaining treatment from Days 4-7. The EOT will be after the 13^{th} dose on Day 7, after which the patient may be released from the clinic/hospital. The patient will return on Day 14 (±3 days) for the Day 14 Visit, and on Day 28 (±3 days) for the Day 28 Visit. AE data will be collected throughout the study, including Day 28 through Day 35. At Day 35, the patient will be contacted by telephone to assess any AEs or lab abnormalities since the Day 28 Visit. At the discretion of the investigator, the patient may be asked to return to the clinic/hospital for a Day 35 Visit (±3 days). Day 35 is the EOS. Assessments will be conducted as described in Table 1 and Table 2.

Safety will be assessed throughout the study. Spleen evaluations will include ultrasound assessments for splenomegaly at Day -6 to -1, Day 3, and Day 7 and clinical examinations to assess for splenomegaly will be performed at all visits. In addition, attention will be focused on identifying any progression of a urinary colonization to an active infection, including the signs and symptoms of local vesicular reactions (e.g. bladder pain, hematuria). Symptoms of active infection will be elicited during daily observations throughout the 7 days of treatment and to the end of study. Symptoms of note in this population include dysuria, urinary frequency, urinary urgency, suprapubic discomfort and flank pain in addition to non-specific symptoms of urinary leakage, change in voiding habits, worsening muscle spasm, increasing autonomic dysreflexia,

sweating, malaise, and fever or hypothermia. Urine microscopy and culture will be assessed frequently during the study (see Table 2).

5.2. Scientific Rationale for Study Design

Bacteriophages require host bacteria to engage and multiply thus healthy volunteer studies are unlikely to provide the environment to adequately explore dosing, PK, tolerability and multiplication of the active bacteriophage therapy in the infective disease setting. This planned population with significant urinary tract colonization with *E. coli* will provide an appropriate surrogate for evaluating these parameters in a relatively healthy population without the need for active antibiotic therapy.

There have been no previous clinical PK studies with intravesical phage therapy or any well controlled studies in humans to help plan this dosing regimen. It is widely believed that adequate exposure is important to get early effective bacterial killing, thus a maximal feasible dose which is dependent on manufacturing limitations will be used.

There is no formal hypothesis nor statistical analysis, however, 30 patients randomized 2:1 should provide sufficient information for an initial safety, tolerability, and PK analysis. This will also provide early proof of concept data with careful microbiological analysis of host range response and effectiveness of the bactericidal activity. The placebo group will provide a sufficient control for evaluating safety in this FIH study.

5.3. Justification for Dose

Twice daily dosing with the maximal feasible dose, based on the manufacturing process ($1x10^6$ – $1x10^{12}$ PFU/mL which is approximately 1.5×10^7 – 1.5×10^{13} PFU/vial), of LBP-EC01 is planned. As bacteriophage therapy has been shown to be safe and effective and bactericidal activity is dependent on the number of bacteria infected by bacteriophages, early adequate exposure at the site of infection is important. Exposure of bacteria to sufficient numbers of bacteriophages without relying on multiplication within the host is considered to be the most efficient approach. Wild-type bacteriophage efficacy is dependent on host engagement, multiplication and lysis of the bacteria whereas with crPhage additional bactericidal activity is achieved by using the CRISPR-Cas3 system to simultaneously degrade independently, highly conserved genes with functions related to cell division and maintenance. As bacteriophage activity has been shown to be dependent on host exposure, we have selected the maximum feasible dose to be administered twice daily.

5.4. End of Study Definition

A patient is considered to have completed the study if he or she has completed all portions of the study including the last visit or the last scheduled procedure shown in the SoA (Table 1)

The end of the study (EOS) is defined as the last date that patient safety data is collected (i.e., Day 35). Once patients have completed Day 28, they may resume their regular medications and have any new intermittent catheters re-established as directed by their physician.

6. STUDY POPULATION

6.1. Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form.
2. Stated willingness to comply with all study procedures and availability for the duration of the study.
3. Males or females 18 years of age or older.
4. Patients with a lower urinary tract colonization caused by *E. coli* ($\geq 10^3$ CFU/mL) and who meet <u>at least one</u> of the following criteria:
    - Has an indwelling urinary catheter and medical documentation of a urinary tract infection by *E. coli* within the past 12 months
    - Requires intermittent catheterization and medical documentation of a urinary tract infection by *E. coli* within the past 12 months
    - Has medical documentation of a history of asymptomatic bacteriuria (i.e., lower urinary tract colonization) with *E. coli* at least once in the past 12 months
5. Patients must have experience with urinary catheterization or have Medical Monitor approval if the patient does not have prior experience with catheterization.
6. In good general health as evidenced by medical history and physical examination.
7. Women of childbearing potential and men with female partners of childbearing potential must use two forms of effective contraception, at least 1 of which is a physical barrier method, during the study and which is recommended to continue for 2 weeks after completing the study.

6.2. Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Patients with clinical signs of active UTI or other infection requiring antimicrobial treatment. These may include dysuria, urinary frequency, urinary urgency, suprapubic discomfort and flank pain in addition to non-specific symptoms of urinary leakage, change in voiding habits, worsening muscle spasm, increasing autonomic dysreflexia, sweating, malaise, and fever or hypothermia. Analgesic use is permitted.
2. Patients who have received Gram-negative bacteria antimicrobials within 14 days of randomization.

    Note: Patients who are currently only receiving antibiotics with only Gram-positive activity (e.g., vancomycin, daptomycin, linezolid) to treat active infections against Gram-positive non-UTIs can be included in the trial.
3. Presence of a surgically-modified bladder, except for a repaired ruptured bladder.

4. History of severe autonomic dysreflexia, which is defined as those patients who have a spinal cord injury and who have had a documented sudden increase in systolic blood pressure of greater than 40 mm Hg due to an irritation or stimulation (including bladder or bowel irritation) below the level of the spinal cord injury. Autonomic dysreflexia can include findings of hypertensive crisis or emergency, clinically significant bradycardia/tachycardia, severe headache or other severe reaction requiring an acute intervention, so consultation with the Medical Monitor should take place if a history of severe autonomic dysreflexia is suspected but not clearly identified.
5. Active severe, progressive or uncontrolled renal, hepatic, hematologic, gastrointestinal, endocrine, pulmonary, cardiac, or neurologic disease per the investigator's discretion.
6. Any malignancies within the past 5 years (except those in remission).
7. Unless deemed acceptable by the Investigator, prescription drugs, over-the-counter (OTC) medications and supplements that acidify the urine are excluded.
8. Patients who have had allergic reactions to similar compounds, or any excipients.
9. Participation in an investigational drug or device study within 1 month (or 7 half-lives of drug, whichever is longer) prior to randomization.
10. Patients who are pregnant or expecting to conceive, are breast feeding or are planning to breast feed, within 1 month of completion of the study.

6.3. Randomization Criteria

Randomization criteria is expected to be met by Day -1. An individual who continues to meet all of the Inclusion and none of the Exclusion Criteria may proceed to randomization if the below criteria are met. There is no minimum time required between Screening and Randomization as long as patients meets all Inclusion criteria and none of the exclusion criteria for the study, including required lab testing.

1. *E. coli* has been identified in a urinary sample obtained within 10 days of randomization and is present at $\geq 10^3$ CFU/mL
2. Patients with mixed colonization with additional Gram-negative or Gram-positive bacteria isolated will be eligible if the other bacteria are present with a CFU/mL that is less than the CFU/mL for *E. coli* within 10 days of randomization.

6.4. Lifestyle Considerations

During this study, patients will be admitted to a clinic or hospital for 7 days while investigational product or placebo is being administered. Outside of the days within clinic/hospital, patients will be asked to maintain normal activities.

6.5. Screening and Screen Failures

During the screening period, patients will complete the screening procedures outlined in Table 1. A urine sample will be taken within 10 days of randomization as part of the initial screening to test for colonization of *E. coli* at $\geq 10^3$ CFU/mL. Patient urine samples will also be assessed for the presence of *E. coli* to be greater than any other bacteria. Patients meeting the urine culture

criteria at the initial screening will then be asked to complete the remaining screening procedures per Table 1. Patients continuing to meet all screening criteria can then be randomized into a treatment group. Baseline urine samples will be tested to provide a baseline assessment of the response to LBP-EC01 by bacteriophage plaquing technique at the Central Lab, but these results are not necessary to randomize the subject.

Screen failures are defined as patients who consent to participate in the clinical trial but are not subsequently randomized to the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients, to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demographic information, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a recent antibiotic or insufficient urinary colonization may be re-screened. Re-screened patients will be assigned a new patient number from the initial screening.

6.6. Strategies for Recruitment and Retention

The following will be considered to recruit study patients:

- There will be competitive enrollment across sites.
- Source of patients will include but is not limited to inpatient or outpatient including patients with SCI or other patients that may require long-term or intermittent urinary catheterization; patients will be in hospital, or urology or special clinic settings.
- Recruitment venues will be in-hospital or regular follow-up clinic visits.
- Potential patients will be identified from medical records and previous history of urinary tract infection and cultures and will be approached either at clinic/hospital visits or by phone or mail contact.
- Public media and other external types of recruitment strategies are anticipated.
- No specific strategies that will be used to recruit and retain historically under-represented populations. The majority of patients may have had SCI and will include all population groups. Women may be underrepresented because of their prevalence in SCI in in VA populations.
- A patient stipend will be considered. Any travel and time required for the study may be compensated, according to approval from the site IRB.

7. STUDY TREATMENT

7.1. Treatment Administration

7.1.1. Investigational Medicinal Product Description

LBP-EC01 is a clear to opalescent, colorless to slightly yellow, aqueous solution. LBP-EC01 is a mixture of 3 crPhages internally named as p0031-8, p00ex-2, p004k-5, which are derived from wild-type myroviridae phages that target *E. coli.* These phages have been engineered to contain an identical CRISPR-Cas3 construct and have been combined in equal amounts at 1×10^{6} – 1×10^{12} PFU/mL (equivalent to approximately 1.5×10^{7} – 1.5×10^{13} PFU/vial). One vial of LBP-EC01 containing approximately 15 mL of solution will be sterilely mixed with 45 mL of Lactated Ringer's solution (injection, USP) by the site pharmacy prior to each dose administered (details will be included in the pharmacy manual). LBP-EC01 and placebo solutions will be indistinguishable.

7.1.2. Dosing and Administration

LBP-EC01 will be administered by bladder instillation via a newly placed urinary catheter BID at the maximum feasible dose, based on the manufacturing process (approximately 1.5×10^{7} – 1.5×10^{13} PFU/vial), totaling approximately 60 mL of solution via sterile catheter tipped syringe (details will be included in the pharmacy manual). The urinary catheter will be clamped and the solution left in situ for a minimum of 30 minutes (to a maximum of 40 minutes) following instillation and then it will be placed on free drainage (duration of clamp should be documented).

LBP-EC01 will be provided in a 20 mL vial containing approximately 15 mL of the crPhage cocktail. This vial will be brought to room temperature prior to mixing with 45 mL of Lactated Ringer's solution (injection, USP) under sterile conditions and placed into a sterile syringe with a catheter tip. Handling and mixing of the vials making up LBP-EC01 will be done by the unblinded study pharmacist (details to be provided in the pharmacy manual). Placebo solution Lactated Ringer's solution (injection, USP) will be brought to room temperature and will also be prepared by the pharmacist under aseptic technique and placed into a sterile catheter tipped syringe. The unblinded pharmacist will use the interactive voice/web-based response system (IVRS) system to determine the randomization allocation.

The mixed blinded solutions will be provided to the investigator/nursing staff for administration. If not used immediately, the IMP combined with Lactated Ringer's solution should be stored under refrigerated conditions and must be administered within 24 hours of preparation. Doses may be given at 8 AM within +/- a 2-hour window for the AM dose and 12 hours after the AM dose +/- a 3-hour window for the PM dose. The clinical site staff should consult with the Medical Monitor about any missed dose outside the allowable dosing window. Typically, these missed doses should be given and documented following standard procedure.

All patients will receive a total of 13 doses of drug product or placebo across 7 days of treatment (a single morning dose will be given on Day 7) (see Table 1). All doses should be given using strict aseptic techniques and may be administered by clinic/hospital staff provided they have been given adequate training. Upon completion of the study, patients requiring subsequent catheterization should have their study catheter replaced. The catheter used in the study should be discarded per normal site policy.

7.2. Preparation/Handling/Storage/Accountability

7.2.1. Acquisition and Accountability

All IMPs required for completion of this study (LBP-EC01 and placebo) will be provided by the Sponsor. The investigational site will acknowledge receipt of IMPs, to confirm the shipment condition and content. Any damaged shipments will be replaced by the Sponsor.

The Investigator/study pharmacist is responsible for the control of biologics under investigation. Adequate records of the receipt (e.g., IMP Receipt Record) and disposition (e.g., IMP Dispensing Log) of the IMP must be maintained. The IMP Dispensing Log must be kept current and should contain the following information:

- The identification of the patient to whom the IMP was dispensed (for example patient initials and date of birth).
- The date(s), quantity of the IMP dispensed to the patient**.**
- The date(s) and quantity of any IMP sent to the clinical site and replaced**.**
- All records and drug supplies must be available for inspection by the Medpace Monitor at every monitoring visit.

The IMP will either be disposed of at the study site according to the study site's institutional standard operating procedure (SOP) or returned to the Sponsor with the appropriate documentation. The site's method of IMP destruction must be agreed upon by the Sponsor. Local or institutional regulations may require immediate destruction of used IMP for safety reasons. In these cases, it may be acceptable for investigational study site staff to destroy dispensed IMP before a monitoring inspection provided that source document verification is performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, returned, destroyed and provided that adequate storage and integrity of drug has been confirmed.

The site must obtain written authorization from the Sponsor/Medpace before any IMP is destroyed, and IMP destruction must be documented on the appropriate form.

Written documentation of destruction must contain the following:

- Identity medication number of IMP(s) destroyed.
- Quantity of IMP(s) destroyed.
- Date of destruction.
- Method of destruction.
- Name and signature of responsible person (or company) who destroyed IMP(s).

Accurate records of all IMPs received at, dispensed from, returned to, and disposed of by the study site should be recorded on the IMP Inventory Log.

7.2.2. Formulation, Appearance, Packaging, and Labeling

A full course of LBP-EC01 treatment will be contained in thirteen 20 mL vials filled with approximately 15 mL of drug product crPhage cocktail contained in a cardboard outer box. Each vial will contain approximately 15 mL of clear, to opalescent, colorless to slightly yellow, solution which will be individually labeled with the study identifier. Each box will contain the vials for a single patient dose which should be stored refrigerated as described in Section 7.2.3.

7.2.3. Product Storage and Stability

Both LBP-EC01 and placebo (sterile Lactated Ringer's solution, injection, USP) will be provided by the Sponsor via a Contract Manufacturing Organization. A single patient's full 7-day treatment of LBP-EC01 (13 vials) will be packaged in a cardboard outer box. Each vial of LBP-EC01 will contain approximately 15 mL of clear, to opalescent, colorless to slightly yellow, solution. The outer box secondary packaging will be labelled with the appropriate coding for the site pharmacy.

Boxes of drug product vials should be stored at 4°C in the refrigerator at all times unless being prepared for use. Short exposures (less than 4 hrs) of the vials to room temperatures (25°C) are acceptable. The IMP drug cocktail mixed with Lactated Ringer's solution (injection, USP) should be administered immediately but can be stored under refrigerated conditions for up to 24 hours. If the IMP is exposed to conditions beyond these limits, the IMP should not be used and should be replaced by existing IMP at the site (using corresponding LBP-EC01 or placebo material dispensed by the site pharmacist) and the site should follow instructions contained in the Pharmacy Manual, to arrange for replacement of the material.

7.2.4. Preparation

Instruction for preparation of IMP will be included in a Pharmacy Manual supplied to the clinical sites.

7.3. Measures to Minimize Bias: Randomization and Blinding

7.3.1. Randomization

Study patients will be randomly assigned to LBP-EC01 or placebo treatment groups in a 2:1 ratio using IVRS.

7.3.2. Blinding

This is a double-blind study. Blinding to study treatment allocation will be achieved through use of matching placebo solution and administration sets. Neither the patient, nor the Investigator, nor Medpace/Sponsor will be aware of the treatment allocation. Key pharmacy staff involved in the preparation of the IMP or placebo will need to be unblinded.

7.3.3. Emergency Unblinding

In the event of an emergency, the treatment code for an individual patient will be readily available to the Investigator and Sponsor/Medpace through the IVRS. If unblinding is necessary for patient management (in the case of an SAE), the Investigator will be able to break the treatment code by contacting the IVRS. Treatment codes should not be broken except in emergency situations. If the Investigator wishes to know the identity of the IMP for any other reason, he or she should contact the Medical Monitor directly. The Investigator should document and provide an explanation for any premature unblinding (e.g., accidental unblinding, unblinding due to an SAE).

As per Health Authority reporting requirements, the Sponsor/Medpace will break the treatment code for all unexpected SAEs (see Section 9.3) that are considered by the Investigator to be related to IMP.

Whenever disclosure of the identity of the IMP is necessary, adequate procedures will be in place to ensure integrity of the data. Any unblinding, at the investigating site end, will be documented in the study report with date, reason for identifying the drug and the name of all the person(s) who required unblinding.

7.4. Treatment Compliance

The Investigator/study pharmacist is responsible for the control of IMPs under investigation. Adequate records of the receipt (e.g., IMP Receipt Record) and disposition (e.g., IMP Dispensing Log) of the IMP must be maintained. The IMP Dispensing Log must be kept current and should contain the following information:

- The identification of the patient to whom the IMP was dispensed (for example patient initials and date of birth).
- The date(s), quantity of the IMP dispensed to the patient.
- The date(s) and quantity of the IMP returned by the investigator/clinical site.
- All records and drug supplies must be available for inspection by the Medpace Monitor at every monitoring visit.

The pharmacy dispensing log will be monitored by study monitors at study visits and dosing compliance will be monitored on an ongoing process by the EDC system.

7.5. Concomitant Therapy

The following concomitant therapies will be allowed/excluded during the study:

- Patients may receive standard of care for treatment of urinary symptoms.
- Analgesics are permitted.
- Antibiotics to treat newly active Gram positive non-urinary tract infections are permitted. All other antibiotic use will not be permitted. Note: Antibiotics with Gram-positive coverage include, but are not limited to, vancomycin, daptomycin, and linezolid.

- Urinary antiseptics will not be allowed.
- Topical lidocaine and lubricants for catheter care are allowed.
- Unless deemed acceptable by the Investigator, prescription drugs, over-the-counter (OTC) medications and supplements that acidify the urine are excluded.
- Patients may continue to receive concomitant prescription medications (excluding Gram-negative antimicrobials), OTC medications and supplements for pre-existing conditions.
- For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the electronic Case Report Form (eCRF) are concomitant prescription medications, OTC medications and supplements. Medications will be reported in the eCRF from Screening through to the EOS Visit.

7.5.1. Rescue Medicine

Any AE will be treated fully by appropriate medical and supportive treatment. There is no specific antidote or rescue medication.

8. TREATMENT DISCONTINUATION, STUDY PAUSING/SUSPENDING OF DOSING AND ENROLLMENT, AND PATIENT DISCONTINUATION/ WITHDRAWAL FROM STUDY

Patients may withdraw voluntarily from the study or the PI may discontinue a patient from the study. There are no known AEs which have been specifically related to this product or this type of therapy which would result in discontinuation of study intervention or patient discontinuation/withdrawal. In addition, patients may discontinue the study intervention, but should, if at all possible, remain in the study for follow-up, especially for safety and pharmacodynamic study endpoints. Documentation for study intervention discontinuation and patient discontinuation/withdrawal from the study will be captured in the eCRF. This will capture the date and the specific underlying reason for discontinuation of study intervention or patient discontinuation/withdrawal. Study enrollment will continue up to the planned 30 evaluable patients unless a decision is made by, or in conjunction with, the Sponsor to stop the study prior to meeting the target enrollment.

Evaluation of safety signals will be conducted by a drug safety management committee (DSMC). The committee will review all potential safety signals including all adverse event (AE) and serious adverse event (SAE) reporting across the study to make a recommendation to the Sponsor on actions to take related to study-wide dosing, enrollment and stopping decisions. The DSMC will include 3 suitably qualified individuals from the CRO and Sponsor (also see Section 11.1.5).

8.1. Treatment Discontinuation

There are no known or theoretical reasons or AEs that should require LBP-EC01 to be discontinued, however, if in the Investigators opinion it should be discontinued prior to planned EOT, it should be discussed with the Medical Monitor and study Sponsor. Discontinuation from LBP-EC01 or placebo does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the Investigator or qualified designee will determine if any change in patient management is needed. Any new clinically relevant finding will be reported as an AE.

A patient will be discontinued from therapy if they exhibit:

- a Grade 3 or 4 IMP-related adverse event (AE) based on Division of AIDS (DAIDS) Table for Grading Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, (https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf),
- an IMP-related serious adverse event (SAE),
- evidence of splenomegaly defined as a doubling of the largest dimension of the spleen from baseline (i.e. Day -6 to -1 assessment) or have hematologic evidence of splenic sequestration defined as a hematologic abnormality (i.e. anemia, leukopenia, thrombocytopenia) of Grade 3 or greater based on DAIDS criteria, or
- any other conditions as deemed medically appropriate based on the Investigator's judgement.

If an antibiotic should become necessary for the treatment of a newly diagnosed UTI or a newly diagnosed Gram-negative non-urinary tract infection, the patient should be discontinued from treatment. All remaining study assessments will be performed as planned.

The data to be collected at the time of study discontinuation will include all activities required at the EOS visit per SoA (Table 1).

8.2. Study Pausing/Suspending of Dosing and Enrollment

Dosing for all patients and enrollment will be paused/suspended until a full cumulative data review is completed if any of the following occur:

- Two or more subjects have the same or similar IMP-related Grade 3 or 4 AEs, based on DAIDS Table for Grading Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, (https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf)
- Any one subject has an IMP-related SAE,
- Any observation of splenomegaly defined as a doubling of the largest dimension of the spleen from baseline (i.e. Day -6 to -1 assessment) or hematologic evidence of splenic sequestration defined as a hematologic abnormality (i.e. anemia, leukopenia, thrombocytopenia) of Grade 3 or greater based on DAIDS criteria, or
- Any other conditions as deemed medically appropriate based on the Investigator's judgement.

The Sponsor and/or DSMC may also pause/suspend dosing for any other reason based on emerging data from this or other ongoing studies. Should dosing be temporarily suspended, the IRB's of the studies sites involved in the study and the FDA will be notified of the dosing suspension (See Section 9.4 – Unanticipated Problems) and the DSMC may review and discuss all available safety data from all patients participating in the study up until the time of the event. The DSMC will decide whether knowledge of the treatment assignment of any patient(s) is(are) necessary to make an appropriate decision about continuation of the study. If so, a request to unblind will be made and relevant data will be reviewed in an unblinded manner. All unblinding Standard Operating Procedures will be followed and documented as appropriate.

Upon completion of the cumulative data review (whether fully blinded or partially unblinded), the DSMC may recommend to the Sponsor terminating the study or resuming study conduct. If study conduct is resumed, the DSMC may recommend, but is not limited to, one of the following actions to the Sponsor:

- Continue dosing with the same number of patients originally planned
- Change the frequency and/or duration of dosing at the current dose level (if there is thought to be a safety event after a certain number of doses)
- De-escalate to a lower dose level

Once an appropriate course of action has been determined the study IRBs and the FDA will be notified (see Section 9.4 - Unanticipated Problems).

8.3. Patient Discontinuation/Withdrawal from the Study

Patients are free to withdraw from participation in the study at any time upon request, without prejudice to their continued care.

An Investigator may discontinue or withdraw a patient from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- If a prohibited concomitant medication is required
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs, including splenomegaly, such that continued participation in the study would not be in the best interest of the patient
- Disease progression to active infection which requires discontinuation of the study intervention
- If the patient meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Termination of the study by the Sponsor or the regulatory authority.

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

The reason for patient discontinuation or withdrawal from the study will be recorded on source documentation and in the eCRF. Patients who sign the informed consent form and are randomized but do not receive the study intervention may be replaced.

Note: patients may withdraw voluntarily from the study or discontinue the study intervention at any time. Investigators should, however, seek to minimize patient discontinuation/withdrawal from study except for safety reasons.

8.4. Lost to Follow-Up

Patients are considered lost to follow-up when they stop reporting to scheduled study visits and cannot be reached to complete all protocol-required study procedures.

The following actions must be taken if a patient fails to return to the clinic for a required study visit:

- The site will attempt to contact the patient and reschedule the missed visit or return for the next scheduled visit and counsel the patient on the importance of maintaining the assigned visit schedule and ascertain if the patient wishes to and/or should continue in the study.
- Before a patient is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the patient (where possible, 3 telephone calls and, if necessary, a certified letter to the patient’s last known mailing address or local

equivalent methods). These contact attempts should be documented in the patient's medical record or study file.

- Should the patient continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

Study procedures and their timing are summarized in the SoA (Table 1). Blood and urine collection for assessment of PK, clinical laboratory values, urinalysis, urine cultures and sensitivity and immunogenicity are detailed in Table 2.

9.1. Study Assessments

9.1.1. Medical History and Demographic Data

Medical history includes full urological history including urinary catheter management, previous UTIs with dates (Month/Year), any comorbid diseases, all surgeries, reproductive status, smoking history, use of alcohol and drugs of abuse and all concomitant medications (e.g., prescription drugs, OTCs, herbal or homeopathic remedies, nutritional supplements) used by the patient within 30 days prior to the Screening Visit. Demographic data will include date of birth (DOB), sex, and self-reported race/ethnicity.

A full medical history will be taken at the Screening Visit, however, other medical history time points indicated in the SOA (Table 1) may be targeted to identify any changes and potential AEs.

9.1.2. Physical Examination

Any abnormality identified at baseline should be recorded on the General Medical History and Baseline Conditions eCRF. A complete or abbreviated physical examination will be performed at the time points indicated in the SOA (Table 1).

A complete physical examination should include an evaluation of the head, eyes, ears, nose, throat, neck and lymph nodes, and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurological systems. No invasive examinations (e.g., rectal examination) will be performed.

At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed. Changes from baseline abnormalities should be recorded in study patient's notes/source documents. New or worsened clinically significant abnormalities should be recorded as AEs on the Adverse Event eCRF.

9.1.3. Spleen Evaluation

Spleen evaluations will include ultrasound assessments for splenomegaly at Day -6 to -1 (baseline), Day 3, and Day 7 and clinical examinations to assess for splenomegaly will be performed at all visits. A clinical examination may also be performed at a clinic visit on Day 35 at the discretion of the study investigator if there are abnormal or clinically significant findings at Day 28.

9.1.4. Adverse Event Solicitation

Adverse Events will be solicited before and after each dose on Days 1 – 7, once per day for the 7 days after the last dose by telephone, at the follow-up visits on Days 14 and 28, and the telephone contact (or clinic visit at the discretion of the study investigator if there are abnormal or clinically significant findings at Day 28) on Day 35. If the Day 14 visit occurs earlier or later than 14 days after initiating treatment the clinical staff will solicit AEs until Day 14 or the

Day 14 visit, whichever is the longer duration. In addition to solicitation of general AEs, specific signs and symptoms of a UTI (e.g. dysuria, urinary frequency, urinary urgency, suprapubic discomfort and flank pain in addition to non-specific symptoms of urinary leakage, change in voiding habits, worsening muscle spasm, increasing autonomic dysreflexia, sweating, malaise, and fever or hypothermia) and local vesicular reaction (e.g., bladder pain, hematuria) to the study treatment will be solicited during Days 1-7 and for 7 days after the last dose of IMP or the Day 14 visit, whichever is longer. All AEs will be recorded on the Adverse Event eCRF.

Patient diary cards will be provided to the patient to document AEs that occur between visits. At Days 14, 28, and 35, the patient diary cards will be reviewed with the patient. All AEs will be recorded on the Adverse Event eCRF as described in Section 9.3 (Adverse Events and Serious Adverse Events).

9.1.5. Pharmacokinetic Assessments

Blood and urine samples for measurement of concentrations of LBP-EC01 will be collected at the timepoints in Table 1 and Table 2. Blood for PK will be drawn immediately before urine sampling both pre- and post-dosing. The date and time of each urine and blood sample collection will be recorded in the eCRF

Pre-dose PK urine and blood samples should be collected within 30 minutes prior to dosing; post-dose PK samples should be collected within 60 minutes after dosing. A post-dose urine and blood PK sample will be collected on Day 1, 6 hours after the first dose.

9.1.5.1. Urine Pharmacokinetics

At the start of the treatment period prior to the first dose a new catheter should be installed. Frequency of catheter changes during the treatment period will be at the discretion of the investigator.

Urine will be collected as clean catheter specimens using the following procedure:

Pre-dose: Bladder will be drained and then the catheter clamped for approximately 30 minutes; a clean catheter sample will be collected immediately before dosing (within 10 minutes) and the bladder will be fully evacuated prior to dosing.

Post-dose: After administration of the IMP or placebo the catheter will be clamped for no less than 30 minutes (no longer than 40 minutes) post-dosing, then the catheter will be released, and the bladder contents drained. The catheter will be re-clamped and then sampled after 20 minutes. This same procedure will be used for each PK sampling time point.

Urine concentrations of LBP-EC01 will be measured by a specific and validated quantitative bacterial plaquing assay. Samples collected for PK assessment will be separated from samples collected for microbiologic assessment and will be placed on ice immediately upon collection, stored refrigerated, and shipped immediately (overnight) at between 2°C and 8°C to the Central Lab for PK analysis as described in the laboratory manual.

9.1.5.2. Blood Pharmacokinetics

The PK samples at the EOT, Day 14 and Day 28 Visits will be obtained during the safety blood draws.

Blood concentrations of LBP-EC01 will be measured by a specific and validated quantitative bacterial plaquing assay. Samples collected for PK assessment will be separated from samples collected for microbiologic assessment and will be placed on ice immediately upon collection, stored refrigerated, and shipped immediately (overnight) at between 2°C and 8°C to the Central Lab for analysis as described in the laboratory manual.

9.1.6. Pharmacodynamic Assessments

9.1.6.1. Urine Microbiology

Urine samples for culture and sensitivity will be collected at the timepoints specified in Table 2. Urine samples that are obtained outside of this schedule may be requested if available.

Urine samples will be collected as clean catheter specimens (unless taken during screening from a non-catheterized patient then no catheter installation required) and will be sent to the Central Laboratory for quantitative culture and sensitivity of *E. coli* as outlined in Table 1 and Table 2. Quantitative culturing will be done for all *E. coli* isolates at the Central Lab.

Pre-dose and post-dose samples of urine collected on Day 1, Day 2, Day 3, Day 5 and samples collected at EOT (Day 7) and the single samples taken at Day 14 and Day 28 Visits, will be aliquoted for microbiology from PK analysis.

9.1.7. Laboratory Assessments

Laboratory safety tests will be collected at timepoints specified in the SOA (Table 1) and the schedule of blood and urine sampling (Table 2).

Samples for the following laboratory tests will be sent to the central laboratory for analysis:

- **Hematology:** Hemoglobin, hematocrit, platelet count, red blood cell (RBC) count, mean corpuscular volume, absolute reticulocyte count, total and differential leucocyte (WBC) absolute count (neutrophils, eosinophils, lymphocytes, monocytes and basophils).
- **Serum chemistry:** Albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, C-reactive protein, calcium, chloride, creatinine phosphokinase, gamma-glutamyl transferase, glucose, phosphate, potassium, creatinine, sodium, total bilirubin, total protein, troponin, urea, IL-6 and an immunoglobulin panel (IgA, IgE, IgG and IgM).
- **Coagulation:** activated partial thromboplastin time, prothrombin time
- **Lipids:** High density lipoprotein-cholesterol, low density lipoprotein cholesterol, triglycerides, total cholesterol.
- **Urinalysis and Microscopy** A pre-dose catheter urine specimen will be collected for dipstick analysis of protein, blood, white blood cells, glucose, and pH and microscopy.
- **Pregnancy test:** All women of childbearing potential (including those who have had a tubal ligation) will have an EPT urine pregnancy test at Screening, Day 1 prior to the first dose, and repeated at the Day 28 Visit (see Table 1). Results of the Day 1

pregnancy test will be reviewed to ensure that the patient is not pregnant prior to the first dose of study drug. The results will be documented in the eCRF.

Additional blood or urine samples may be taken at the discretion of the Investigator if the results of any test fall outside the reference ranges, or clinical symptoms necessitate additional testing to monitor a patient's safety. Where the clinical significance of abnormal lab results is considered uncertain, screening lab tests may be repeated before randomization to confirm eligibility.

In the event of unexplained abnormal clinically significant laboratory test values, the tests should be repeated immediately and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found. Results of central laboratory testing will be recorded on the eCRF or will be received as electronically-produced laboratory reports. Situations may arise when local laboratories are utilized such as stat safety labs. In these cases, reference ranges must be submitted where applicable.

9.1.8. Screening Assessments

Written informed consent for participation in the study must be obtained before performing any study-specific screening tests or evaluations. Informed Consent Forms for enrolled patients and for patients who are not subsequently enrolled will be maintained at the study site. All screening and pre-treatment assessments must be completed and reviewed to confirm that patients meet all eligibility criteria. The Investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure. An Eligibility Screening Form documenting the Investigator's assessment of each screened patient with regard to the protocol's inclusion and exclusion criteria is to be completed by the Investigator and kept at the investigational site. Screening assessments will be performed within 21 days prior to enrollment. For assessments, and timing of assessments at Screening, please see Table 1.

9.1.9. Biological Specimen Collection and Laboratory Evaluations

All laboratory evaluations will be performed at central laboratories, in compliance with Clinical Laboratory Improvement Amendments of 1988.

- Clinical laboratory evaluations (hematology, clinical chemistry, coagulation, lipids, and urinalysis) will be performed by the Central Laboratory and affiliated labs using routine methodology (see Section 9.1.7). Approximately 40 mL of blood will be drawn at each of 7 timepoints (Screening, Day 1, Day 3, Day 5, Day 7 (EOT), Day 14, and Day 28). Repeat lab testing may be performed at Day 35 if there are abnormal or clinically significant findings at Day 28.
- Screening microbiology evaluations: Standard quantitative urine culture will be performed at Screening to identify *E. coli* colonization of $\geq 10^3$ CFU/mL. Quantitation of non-*E. coli* bacteria will be done to confirm the predominance of *E. coli* as the primary bacteria colonizing the bladder. This will be performed at the central microbiology laboratory (IHMA).
- Pharmacodynamic microbiology: quantitative urine culture and sensitivity will be performed at the central microbiology laboratory for study visits between screening to Day 28 (see Table 2). The quantitative culture will include identification of genus and species and quantitation of *E. coli*. Quantitation of non-*E. coli* bacteria will be done

at time of screening to confirm the predominance of *E. coli* as the primary bacteria colonizing the bladder and will be evaluated at Day 1, Day 2, Day 3, Day 5, Day 7 (EOT), Day 14 and Day 28, to evaluate the presence and quantities of *E. coli* and non-*E. coli* bacteria before and after treatment with LBP-EC01. Antibiotic sensitivity of *E. coli* will be tested by the Central Lab and will include colistin and fosfomycin.

- Urine culture: All *E. coli* isolates collected will be tested for phage sensitivity by the Central Lab using standard methodology developed at Locus.
- Pharmacokinetic analysis will be performed by a quantitative bacterial plaquing assay to identify components of LBP-EC01. Methodology will be developed at the Central Lab. Instructions will be outlined in the laboratory procedures manual.
- Immunogenicity assay to measure increases levels of antibodies (IgA, IgE, IgG, IgM) during treatment will be tested at specified visits (see Table 1 and Table 2).
- Both blood and urine samples will be collected for general biomarker assessment. Biomarkers will include inflammatory biomarkers (e.g., interleukin [IL]-6), bacterial sensitivity to phage and bacterial metagenomic analysis which will be conducted on samples at the end of study.

Special instructions for the preparation, handling, storage, and shipment of specimens will be detailed in the study's Manual of Procedures (MOP).

9.2. Safety and Other Assessments

The following procedures/evaluations will be performed for safety evaluation:

- Physical examination

  A targeted physical examination will be performed at the specified timepoints (or as clinically indicated). This will be a limited, symptom-directed physical examination only. Changes from baseline abnormalities should be recorded in patient's notes. New or worsened clinically significant abnormalities should be recorded as AEs on the Adverse Event eCRF.

- Spleen examination

  Spleen evaluations will include ultrasound assessments for splenomegaly at Day -6 to -1, Day 3, and Day 7 and clinical examinations to assess for splenomegaly will be performed at all visits. A clinical examination may also be performed at a clinic visit on Day 35 at the discretion of the study investigator if there are abnormal or clinically significant findings at Day 28. Splenomegaly defined as a doubling of the largest dimension of the spleen from baseline (i.e. Day -6 to -1 assessment) or hematologic evidence of splenic sequestration defined as a hematologic abnormality (i.e. anemia, leukopenia, thrombocytopenia) of Grade 3 or greater based on DAIDS criteria.

- Adverse event solicitation

  Adverse events will be solicited before and after each dose on Days 1 - 7, once per day for the 7 days after the last dose by telephone, at the follow-up visits on Day 14 (the clinical staff will solicit AEs until Day 14 or the Day 14 visit, whichever is the

longer duration, if the Day 14 visit does not occur on Day 14) and Day 28, and at the telephone contact (or clinic visit at the discretion of the study investigator if there are abnormal or clinically significant findings on Day 28) on Day 35. In addition to solicitation of general AEs specific signs and symptoms of a UTI (e.g. dysuria, urinary frequency, urinary urgency, suprapubic discomfort and flank pain in addition to non-specific symptoms of urinary leakage, change in voiding habits, worsening muscle spasm, increasing autonomic dysreflexia, sweating, malaise, and fever or hypothermia) and local vesicular reaction (e.g., bladder pain, hematuria) to the study treatment will be solicited during Days 1-7 and for 7 days after the last dose of IMP or the Day 14 visit, whichever is longer.

Patient diary cards will be provided to the patient to document AEs that occur between visits. At Days 14, 28, and 35, the patient diary cards will be reviewed with the patient. All AEs will be recorded on the Adverse Event eCRF.

- Vital signs

  Vital signs will include resting BP, heart rate, respiratory rate and oral body temperature and will be recorded at the timepoints specified in the SoA (Table 1). Additional vital signs pre- and post-dosing and 6 hours (on Day 1) post-dose will be recorded on the first day of treatment (see Table 1).

- ECGs

  Triplicate 12-lead ECG recordings (i.e., 3 useful ECGs without artifacts) will be obtained within approximately 2-5 minutes at each specified timepoint for screening and safety. The average of the 3 readings will be used to determine ECG intervals (e.g., PR, QRS, QT). Additional unscheduled ECGs should be performed in case of abnormalities and if clinical symptoms occur. Whenever possible, the same brand/model of a standard high quality, high-fidelity electrocardiograph machine equipped with computer-based interval measurements should be used for each patient. The conditions should be as close as possible to pre-dose timepoints; this includes, but is not limited to, food intake, activity level, stressors and room temperature. To minimize variability, it is important that patients be in a resting position (semi-recumbent) for ≥10 minutes prior to each ECG evaluation. Body position should be consistently maintained for each ECG evaluation to prevent changes in heart rate.

  Environmental distractions (e.g., television, radio, conversation) should be avoided during the pre-ECG resting period and during ECG recording. In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality. For safety monitoring purposes, the Investigator or designee must review, sign, and date all ECG tracings. Paper or electronic copies will be kept as part of the patient's permanent study file at the site. If considered appropriate by Sponsor, ECGs may be analyzed retrospectively at a central laboratory.

  The ECG characteristics, including heart rate, QRS duration, and PR, and QT intervals, will be recorded on the eCRF. QTcF (Fridericia's correction) will be calculated and RR will be calculated and recorded on the eCRF. Changes in T-wave

and U-wave morphology and overall ECG interpretation will be documented on the eCRF. T-wave information will be captured as normal or abnormal, U-wave information will be captured in 2 categories: absent/normal or abnormal.

- Biological specimen collection and laboratory evaluations.

  See Section 9.1.9 for all investigations. Any abnormal laboratory results will be flagged and followed up by the Medpace safety group on a real time basis.

9.3. Adverse Events and Serious Adverse Events

9.3.1. Definition of Adverse Events

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related (21 CFR 312.32 [a]).

9.3.1.1. Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording AEs, including SAEs and non-serious AEs; measurement of protocol-specified safety laboratory assessments; measurement of protocol-specified vital signs, ECGs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study. Certain types of events require immediate reporting to the Sponsor, as outlined in Section 9.3.6.

9.3.1.2. Adverse Events

According to the ICH guideline for GCP, an AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, regardless of causal attribution. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition).
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline.
- Any deterioration in a laboratory value or other clinical test (e.g., ECG) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from IMP.
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies).

No dosage modifications or interruptions are planned for patients participating in this study.

9.3.2. Definition of Serious Adverse Events

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Medical Monitor/Sponsor, it results in any of the following outcomes:

- Fatal (i.e., the AE actually causes or leads to death).
- A life-threatening AE (i.e., the AE, in the view of the Investigator, places the patient at immediate risk of death). This does not include any AE that, had it occurred in a more severe form or was allowed to continue, might have caused death.
- In-patient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions).
- Congenital anomaly/birth defect in a neonate/infant born to a mother exposed to IMP.
- A significant medical event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definition above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization.

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE rated as mild, moderate, or severe, see Section 9.3.3.1. The event itself may be of relatively minor medical significance (such as severe headache without any further findings). Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

Serious adverse events are required to be reported by the Investigator to Medpace/Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 9.3.6 for reporting instructions).

9.3.3. Classification of an Adverse Event

9.3.3.1. Severity of an Adverse Event

All AEs will be assessed by the Investigator using the Division of AIDS (DAIDS) Table for Grading Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, (https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf). In general, the following guidelines describe severity:

- **Grade 1 Mild:** Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated
- **Grade 2 Moderate:** Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated
- **Grade 3 Severe:** Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated

- **Grade 4 Potentially Life-Threatening:** Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death
- **Grade 5 Death**

9.3.3.2. Relationship to Investigational Medicinal Product

All AEs must have their relationship to study intervention assessed by the investigator who examines and evaluates the patient based on temporal relationship and his/her clinical judgment.

The following guidance should be taken into consideration:

- Temporal relationship of event onset to the initiation of IMP.
- Course of the event, considering especially the effects of dose reduction, discontinuation of IMP, or reintroduction of IMP.
- Known association of the event with the IMP or with similar treatments.
- Known association of the event with the disease under study.
- Presence of risk factors in the patient or use of concomitant medications known to increase the occurrence of the event.
- Presence of non-treatment-related factors that are known to be associated with the occurrence of the event.

The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the IMP, there is a reasonable possibility that the IMP caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the IMP and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

9.3.3.3. Expectedness

The Investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the IMP. An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure (IB) or is not listed at the specificity or severity that has been observed; or, if it is not consistent with the risk information described in the protocol.

9.3.4. Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a patient presenting for medical care, or upon review by a study monitor. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured

on the appropriate eCRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while in the study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened will be considered as baseline and not reported as an AE. However, if the patient's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

The Investigator or designee will record all AEs (non-serious and serious) with start dates occurring any time after informed consent is obtained until Day 35. At each study visit, the Investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

9.3.5. Adverse Event Reporting

Investigators will seek information on AEs at each patient contact. All AEs, whether reported by the patient or noted by study personnel, will be recorded in the patient's medical record. Adverse events will then be reported on the Adverse Event eCRF as follows:

- Investigators should use correct medical terminology/concepts when recording AEs on the Adverse Event eCRF. Avoid colloquialisms and abbreviations.
- Only 1 AE term should be recorded in the event field on the Adverse Event eCRF.

A consistent methodology of non-directive questioning should be adopted for eliciting AE information at all patient evaluation timepoints. Examples of non-directive questions include the following:

"How have you felt since your last clinic visit?"

"Have you had any new or changed health problems since you were last here?"

In addition, solicited AEs will be recorded as per Section 9.2.

The Investigator must record non-serious AEs and report them to the Sponsor according to the timetable for reporting as specified below:

After informed consent has been obtained **but prior to initiation of treatment**, only SAEs caused by a protocol-mandated intervention should be reported (e.g., SAEs related to invasive procedures and urinary catheterization). Any other AE should not be reported.

After initiation of treatment, all AEs and SAEs, regardless of relationship to IMP, will be reported until 28 days after the last dose of IMP (i.e. Day 35).

After a period of 28 days from the last dose of IMP (i.e. Day 35), investigators should report any deaths, SAEs, or other AEs of concern that are believed to be related to prior treatment with IMP.

9.3.6. Serious Adverse Event Reporting

All AEs considered serious by the PI, Sub-Investigator or which meets the definition of an SAE included in Section 9.3.2 must be submitted on an SAE form to the Medpace safety monitor.

The Investigator will immediately report (within 24 hours) to the Medpace/Sponsor any SAE, whether or not considered IMP related, including those listed in the protocol or IB and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event.

All SAEs will be followed until satisfactory resolution or until the site Investigator deems the event to be chronic or the patient is stable. Other supporting documentation of the event may be requested by the Medpace Safety monitoring group/study Sponsor and should be provided as soon as possible. Medpace/Sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the Sponsor's initial receipt of the information. In addition, the Sponsor must notify FDA and all participating investigators in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the Sponsor determines that the information qualifies for reporting.

Study enrollment will be halted pending review of available clinical data for study drug-related SAEs that trigger a DSMC review (see Section 8.2).

9.3.7. Reporting Events to Patients

Study patients will be informed about AEs and SAEs on an individual or aggregate level depending on the potential seriousness of the risk to individuals. Any unexpected AEs and SAEs will be communicated to individuals at study visits or by telephone calls if deemed necessary depending on risk to the individuals. Study patients will be given written top line results when available after the study.

9.3.8. Events of Special Interest

Not applicable.

9.3.9. Reporting of Pregnancy

9.3.9.1. Pregnancies in Female Patients

Female patients of childbearing potential will be instructed to immediately inform the Investigator if they become pregnant during the study (by the EOS visit). A Clinical Trial Pregnancy Reporting Form should be completed by the Investigator and submitted to Medpace/Sponsor within 24 hours after learning of the pregnancy. Pregnancy should not be recorded on the Adverse Event eCRF. The Investigator should discontinue IMP, if applicable, and counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy.

9.3.9.2. Pregnancies in Female Partners of Male Patients

Male patients will be instructed through the Informed Consent Form to immediately inform the Investigator if their partner becomes pregnant during the study (by the EOS visit). A Clinical Trial Pregnancy Reporting Form should be completed by the Investigator and submitted to Medpace/Sponsor within 24 hours after learning of the pregnancy. Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male patient exposed to IMP. The pregnant partner will need to sign an Authorization for Use and Disclosure of Pregnancy Health Information to allow for follow-up on her pregnancy. Once the authorization has been signed, the Investigator will update the Clinical Trial Pregnancy Reporting Form with additional information on the course and outcome of the pregnancy. An Investigator who is contacted by the male patient or his pregnant partner may provide information on the risks of the pregnancy and the possible effects on the fetus, to support an informed decision in cooperation with the treating physician and/or obstetrician.

9.3.9.3. Abortions

Any spontaneous abortion should be classified as an SAE (as the Sponsor considers spontaneous abortions to be medically significant events), recorded on the Adverse Event eCRF, and reported to the Medpace/Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 9.3.6). Any induced abortion due to maternal toxicity and/or embryo-fetal toxicity should also be classified as and SAE , recorded on the Adverse Event eCRF, and reported to Medpace/Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 9.3.6).

Elective abortion not associated with toxicity (e.g., induced abortion for personal reasons) does not require expedited reporting but should be reported as outcome of pregnancy on the Clinical Trial Pregnancy Reporting Form.

9.3.9.4. Congenital Anomalies/Birth Defects

Any congenital anomaly/birth defect in a child born to a female patient or female partner of a male patient exposed to IMP should be classified as an SAE, recorded on the Adverse Event eCRF, and reported to Medpace/Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 9.3.6).

9.4. Unanticipated Problems

9.4.1. Definition of Unanticipated Problems

The Office for Human Research Protections (OHRP) considers unanticipated problems (UPs) involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the patient population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places study patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

9.4.2. Unanticipated Problem Reporting

The Investigator will report UPs to the reviewing IRB and to Medpace/Sponsor lead PI. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PIs name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB and to Medpace/Sponsor within 24 hours of the Investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to Medpace/Sponsor as soon as possible, but in no event later than 10 working days after the Investigator first becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the OHRP within timeline in accordance with policy of the IRBs receipt of the report of the problem from the Investigator.

A Sponsor/representative who conducts an evaluation of an UP shall report the results of such evaluation to the FDA and to all reviewing IRBs and participating investigators within 10 working days after the Sponsor first receives notice of the UP. Thereafter the Sponsor shall submit such additional reports concerning the effect as FDA requests (21 CFR 812.150[b][1]).

9.4.3. Reporting Unanticipated Problems to Patients

Study patients will be informed about UPs on an individual or aggregate level depending on the potential seriousness of the risk to individuals.

10. STATISTICAL CONSIDERATIONS

10.1. Statistical Hypotheses

This is a Phase 1b study and primary outcomes are evaluation of safety, tolerability and PK. No formal hypothesis will be tested.

10.2. Sample Size Determination

A total of approximately 30 patients will be randomized in a 2:1 ratio to LBP-EC01 or placebo. This is a FIH study and the sample size should be sufficient to establish early safety and PK at a single dose level.

10.3. Populations for Analyses

The following data sets will be evaluated:

- Safety Population: Patients who took at least 1 dose of IMP.
- PK Population: Patients who took at least 1 dose of IMP with at least 1 analyzable PK sample.
- PD Population: Patients who met enrollment criteria, who have a baseline PK assessment, and who took at least 1 dose of IMP.

10.4. Statistical Analyses

10.4.1. General Approach

A separate Statistical Analysis Plan (SAP) will be developed prior to database lock.

Pharmacokinetics

- Individual and mean plasma concentrations and urine concentrations at each sampling time point for LBP-EC01 will be presented by listings and descriptive summary statistics.

Pharmacodynamics

- Change from baseline (Day 1) in *E. coli* quantitative culture concentration at Day 2, Day 3, Day 5, Day 7 (EOT), Day 14 and Day 28 in the urine will be summarized by treatment groups.
- All other PD endpoints (time to 1 log reduction in urinary E. coli count, recurrence of *E. coli* colonization or incidence of infection) will be tabulated.

10.4.2. Safety Analyses

Safety and tolerability will be presented as:

- Listings, summary tables, and graphs (individual plots and/or mean plots) by treatment group will be provided for safety and tolerability assessments.
- Laboratory results will be presented as change results from baselines as shift tables.

Adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA); each AE will be counted once only for a given patient unless severity increases. The severity, frequency, and relationship of AEs to IMP will be presented by System Organ Class (SOC) and preferred term groupings and will include start date, stop date, severity, relationship, expectedness, outcome, and duration. Adverse events leading to premature discontinuation from the IMP and SAEs will be presented in a listing.

10.4.3. Baseline Descriptive Statistics

Baseline characteristics, including demographics and laboratory measurements will be summarized using descriptive statistics.

10.4.4. Planned Interim Analyses

No interim analysis is planned.

10.4.5. Exploratory Analyses

Differences between indwelling and intermittent urinary catheterization populations will be compared.

Any persisting microbiological isolates will be fully explored with bacteriophage host range, antibiotic resistance patterns and genetic sequencing of isolates.

11. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

11.1. Regulatory, Ethical, and Study Oversight Considerations

11.1.1. Informed Consent Process

11.1.1.1. Consent/assent and Other Informational Documents Provided to Patients

Consent forms describing in detail the IMP, study procedures, and risks will be given to the patient and written documentation of informed consent is required prior to starting screening interventions. The following consent materials are submitted with this protocol.

11.1.1.2. Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the patient's agreeing to participate in the study and continues throughout the patient's study participation. Consent forms will be IRB-approved and the patient will be asked to read and review the document. The Investigator will explain the research study to the patient and answer any questions that may arise. A verbal explanation will be provided in terms suited to the patient's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research patients. Study patients will have the opportunity to carefully review the written consent form and ask questions prior to signing. The study patient s should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The patient will sign the informed consent form prior to any procedures being done specifically for the study. Patients must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent form will be given to the patients for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the patient undergoes any study-specific procedures. The rights and welfare of the patients will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

11.1.2. Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause as described in Section 8. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to patients, Investigator, the IND, Sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform patients, the IRB, and Sponsor and will provide the reason(s) for the termination or suspension. Patients will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements

- Data that are not sufficiently complete and/or evaluable
- Poor protocol compliance

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the Sponsor, IRB and/or FDA.

11.1.3. Confidentiality and Privacy

Patient confidentiality and privacy are strictly held in trust by the participating investigators, their staff, and the Sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to patients. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor. All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the Sponsor, representatives of the IRB, regulatory agencies may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the patients in this study. The clinical study site will permit access to such records.

The patient's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or Sponsor requirements.

Patient's research data, which are for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Medpace Data Coordinating Center. This will not include the patient's contact or identifying information. Rather, individual patients and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by Medpace Data Coordinating Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Sponsor or Medpace Data Coordinating Center.

11.1.4. Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the Medpace Data Coordinating Center. After the study is completed, the de-identified, archived data will ultimately be transmitted to and stored by the Sponsor.

With the patient's approval and as approved by local IRBs, de-identified microbiological and biological samples will be stored at the Medpace and IHMA bio-sample repository. These samples could be used to further examine the causes of urinary tract and other infections to improve anti-microbial and bacteriophage therapy. The PK analysis data may be shared with future PK studies for population kinetics analyses.

During the conduct of the study, an individual patient can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to bio-sample storage may not be possible after the study is completed.

11.1.5. Safety Oversight

Safety oversight will be under the direction of the Medpace Safety Monitoring safety group, Medpace Medical Monitor and the Sponsor Medical Monitor. A DSMC will review the available clinical data if the conditions outlined for suspending enrollment and treatment in Section 8 are met. The DSMC will include 3 suitably qualified individuals. The composition of the DSMC will be two medically trained representatives from the CRO (Medpace) and a delegate from the Sponsor.

Periodic Review for Trends:

Locus will be accountable for overall safety surveillance for the compound, including adverse event trend analysis and signal detection. Medpace will assist with the safety analyses and safety surveillance for this protocol by gathering and compiling all safety data for this study and facilitating regular safety reviews based on enrollment and occurrence of any AEs.

This periodic review will occur monthly once 20% of subjects are randomized and may include the following data:

- Laboratory and Clinical data
- Serious Adverse Events and Adverse Events from the clinical database

Data Management and Biostatistics from the CRO, if applicable, will compile the data to be reviewed. If a potentially unreported SAE is identified, the Medpace Medical Monitor (MM) or Lead Safety Physician will alert the Clinical Safety Manager to distribute the related safety information to the DSMC. Medpace Clinical Safety will follow up with the site and Clinical Research Associate (CRA) adhering to the reporting guidelines outlined in Section 9.3. Monthly reviews of the compiled safety data will take place by the DSMC.

Communication of Significant Findings:

If deemed medically significant, the Medpace MM or Lead Safety Physician will alert Locus of overall study trends during review. Documentation of the reviews will be maintained in the Trial Master File.

If a concerning trend in safety data or significant safety signal is identified by the DSMC, it will be submitted to participating regulatory agencies, Investigators, and IRBs as applicable. This information will be submitted no later than calendar day (CD) 7 (death/life-threatening cases) or CD 15 (for all other cases), after the determination by Locus that the information qualifies for reporting.

11.1.6. Clinical Monitoring

Clinical site monitoring will be conducted to ensure that the rights and well-being of study patients are protected, that the reported study data are accurate, complete, and verifiable, and that the conduct of the study is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by Medpace clinical monitors.
- On-site and centralized monitoring will be performed. Frequency (e.g., early, for initial assessment and training, versus throughout the study. Monitoring will be

targeted verification of regulatory documentation, eligibility criteria endpoint, safety and other key data variables including PK assessments and microbiology.

- The Sponsor will be provided electronic copies of monitoring reports within 15 days of visit.
- Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
- Independent audits may be conducted by the Sponsor to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

11.1.7. Quality Assurance and Quality Control

The contract research organization (CRO; Medpace) will be responsible for data management of this study, including quality checking of the data. Sites will be responsible for data entry into the Electronic Data Capture (EDC) system.

A comprehensive validation check program will verify the data. Discrepancies will be generated automatically in the system at the point of entry or added manually for resolution by the Investigator.

Medpace will produce a Data Handling Manual that describes the quality checking to be performed on the data. Laboratory electronic data will be sent directly to Medpace, using Medpace's standard procedures to handle and process the electronic transfer of these data.

System backups for data stored by Medpace and records retention for the study data will be consistent with Medpace's standard procedures.

In the event of discrepant data, Medpace will request data clarification from the sites, which the sites will resolve electronically in the EDC system.

Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., GLP, Good Manufacturing Practices).

The investigational site will provide direct access to all study-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by local and regulatory authorities.

11.1.8. Data Handling and Record Keeping

11.1.8.1. Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data for this study will be captured via an online EDC system. The data collected in the source documents is entered onto the study eCRF. An audit trail will maintain a record of initial entries and changes made; reasons for change; time and date of entry; and user name of person authorizing entry or change. For each patient enrolled, an eCRF must be completed and electronically signed by the PI or authorized delegate from the study staff. If a patient withdraws from the study, the reason must be noted on the eCRF. If a patient is withdrawn from the study because of a treatment-limiting AE, thorough efforts should be made to clearly document the outcome.

The Investigator should ensure the accuracy, completeness and timeliness of the data reported to the Sponsor/CRO in the eCRFs and in all required reports.

The eCRFs will be submitted electronically to the Sponsor/CRO and should be handled in accordance with instructions from the Sponsor/CRO.

At the end of the study, the Sponsor will receive patient data for the study by site and will share this data with the study investigators for his or her site in a readable electronic format which must be kept with the study records. Acknowledgement of receipt of the data is required.

Study monitors will perform ongoing source data verification to confirm that critical protocol data (i.e., source data) entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents.

Source documents (paper or electronic) are those in which patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, patient-reported outcomes, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, patient files, and records kept at pharmacies, laboratories, and medicotechnical departments involved in a clinical trial.

Before study initiation, data to be entered directly into the eCRFs (i.e., no prior written or electronic record of the data) and considered source data must be defined in the Clinical Monitoring Plan.

Source documents that are required to verify the validity and completeness of data entered into the eCRFs must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section 11.1.8.3.

To facilitate source data verification, the investigators and institutions must provide the Sponsor direct access to applicable source documents and reports for trial-related monitoring, Sponsor audits, and IRB/EC review. The investigational site must also allow inspection by applicable Health Authorities.

11.1.8.2. Use of a Computerized System

When clinical observations are entered directly into an investigational site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with Health Authority requirements pertaining to computerized systems used in clinical research. An acceptable

computerized data collection system allows preservation of the original entry of data. If original data are modified, the system should maintain a viewable audit trail that shows the original data as well as the reason for the change, name of the person making the change, and date of the change.

11.1.8.3. Study Records Retention

Study 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 until at least 2 years have elapsed since the formal discontinuation of clinical development of the IMP. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the Sponsor. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

11.1.9. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or MOP requirements. The noncompliance may be either on the part of the patient, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, Section 5.1.1
- 5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site Investigator to use continuous vigilance to identify and report deviations within 2 working days of identification of the protocol deviation, or within 2 working days of the scheduled protocol-required activity. All deviations must be verified in study source documents, and the CRAs will report them via the monitoring visit report (MVR) which will be entered into a ClinTrak SM listing. The sponsor will have access to this listing and the MVR. Protocol deviations must be sent to the reviewing IRB per their policies. The site Investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the Monitoring Plan and Protocol Deviation Plan.

11.1.10. Publication and Data Sharing Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor prior to submission. This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the Investigator.

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating Investigator will be designated by mutual agreement.

Any formal publication of the study in which contribution of Sponsor personnel exceeded that of conventional monitoring will be considered as a joint publication by the Investigator and the appropriate Sponsor personnel.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Any inventions and resulting patents, improvements, and/or know-how originating from the use of data from this study will become and remain the exclusive and unburdened property of the Sponsor, except where agreed otherwise.

11.1.11. Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11.2. Additional Considerations

Not applicable

11.3. Abbreviations

AE	Adverse Event
AMR	Antimicrobial Resistance
BP	Blood Pressure
BID	Twice Daily
CD	Calendar Day
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CMP	Clinical Monitoring Plan
CR	Carbapenem-Resistant
CRISPR	Clustered Regularly Interspersed Short Palindromic Repeats
CRA	Clinical Research Associate
CRO	Contract Research Organization
CrPhage	CRISPR-Enhanced Bacteriophage
DNA	Deoxyribonucleic Acid
DSMC	Drug Safety Management Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Forms
EDC	Electronic Data Capture
EOS	End of Study
EOT	End of Treatment
ESBL	Extended Spectrum Beta-Lactamase

FDA	Food and Drug Administration
FIH	First In Human
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
Ig	Immunoglobulin
IL	Interleukin
IND	Investigational New Drug
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IV	Intravenous
IVRS	Interactive Voice/Web-Based Response System
IU	Intraurethrally
MBL	Medpace Bioanalytical Laboratory
MedDRA	Medical Dictionary for Regulatory Activities
MDR	Multi-Drug Resistant
MM	Medical Monitor
MOP	Manual of Procedures
NCT	National Clinical Trial
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
OTC	Over-The-Counter

PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
(q)PCR	(Quantitative) Polymerase Chain Reaction
QTcF	QT Interval With Fridericia's Correction
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCI	Spinal Cord Injury
SoA	Schedule of Assessments
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States
UTI	Urinary Tract Infection
XDR	Extensive Drug Resistant

11.4. Protocol Amendment History

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located on the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
1.3	04-Oct-2019	Updated to preclinical information, more detailed description of drug product, exclusion criteria and safety management sections	Updates based on FDA feedback.
1.4	01-Oct-2020	Updated to clarify that PD analysis will be conducted with urine samples across all timepoints collected	Administrative clarification

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