CLINICAL PROTOCOL

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Clinical Protocol

A Phase 2 Randomized, Double-Blind, Placebo-Controlled, Single Dose Regimen Study of LMN-101 in Healthy Volunteers Challenged with Campylobacter jejuni

Study Number CAM02

Investigational Product: LMN-101

VHH-derived binding protein designed to bind and inhibit FlaA, flagellin protein of Campylobacter jejuni*, delivered in whole spray-dried, spirulina biomass*

Version 2.0

December 7, 2021

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INVESTIGATOR'S AGREEMENT

A Phase 2 Randomized, Double-Blind, Placebo-Controlled, Single Dose Regimen Study of LMN-101 in Healthy Volunteers Challenged with *Campylobacter jejuni*

I have received and read the Investigators' Brochure for LMN-101. I have read this clinical protocol and agree to conduct the study as outlined in this protocol and according to the International Conference on Harmonisation (ICH) guidelines, The Nuremberg Code, The Belmont Report, The Declaration of Helsinki 2000, US 21 CFR Part 50--Protection of Human Subjects, 32 CFR 219 (The Common Rule), and all regulations pertinent to the Department of Defense, and relevant regional and/or national laws. Confidentiality of all information received or developed in connection with this protocol will be maintained by me, as well as all other personnel involved in the clinical trial who are employed or contracted by me or my organization.

_____ _____

Mohamed Al-Ibrahim, MB, ChB, FACP
Site Principal Investigator

Date

SPONSOR SIGNATURE

_____ _____

Carl Mason, MD
Senior Medical Director

Date

Protocol amendments April 28, 2021, Version 2.0	
General rewrite for clarity and linearity improvements	All
Added Inclusion criteria for screening labs defined as ≤ Grade 1 on CTCAE v. 5.0	Section 7.2
Exclusion for immunomodulating agents, including chemotherapy, extended to 12 months	Section 7.3
Addition of the following conditions to the exclusion criteria: inflammatory bowel disease; autoimmune disease; malignancy; any immunocompromising condition; or history of major gastrointestinal surgery	Section 7.3
Noted that IntegReview is the IRB for review of study documents, amendments, and SAEs. The NMRC IRB has delegated IRB review responsibilities to IntegReview IRB	Sections 7.1, 11.11, and 11.11.2
Duration of collection of types of adverse events clarified	Schedule of Evaluations, Section 11.9
Added management plan for subjects who develop *Campylobacter* bacteremia	Section 8.11
Clarified that all adverse events will be reported regardless of causality	Section 11.2
Definition of causality added	Section 11.4
Included Sponsor requirements for reporting to FDA	Section 11.11.3
Exclusion of grade 3 *Campylobacter* symptoms from the halting criteria is limited to the in clinical research center period	Section 11.14
Statistical Plan updated	Section 12
Duplicate References Removed	Section 14

1. Table of Contents

Abbreviations and Specialist Terms

Abbreviation	Definition
AE	Adverse event
AESI	Adverse events of special interest
CBC	Complete blood count
CFU	Colony-forming unit
CLIA	Clinical Laboratory Improvement Act
CRA	Clinical Research Associate
CRF	Case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CVD	Center for Vaccine Development
DoD	Department of Defense
eCRF	Electronic case report form
ECG	Electrocardiogram
ELISA	Enzyme- linked immunosorbent assay
ETEC	Enterotoxigenic *Escherichia coli*
FQ	Fluoroquinolone
GBS	Guillain-Barré syndrome
GCP	Good Clinical Practice
GE	Glycine extract
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HED	Human-equivalent dose
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
IBD	Inflammatory bowel disease
ICF	Informed consent form
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IP	Investigational product
IND	Investigational new drug
IRB	Institutional Review Board
IVF	Intravenous fluids
MCB	Master cell bank
MIC	Minimum inhibition concentration

Abbreviation	Definition
NF	National Formulary
NMRC	Naval Medical Research Center
NOAEL	No-observed-adverse-event level
ORS	Oral rehydration solution
PAD	Pharmacologically active dose
PBMC	Peripheral blood mononuclear cells
PE	Protective efficacy
PI	Principal Investigator
SAE	Serious adverse event
SD	Standard deviation
SSP	Site-specific procedure
ULN	Upper limit of normal
UMd	University of Maryland
USP	United States Pharmacopoeia
WBC	White blood cell
WRAIR	Walter Reed Army Institute of Research

2. Synopsis

CLINICAL TRIAL SYNOPSIS	
Study Title:	A Phase 2 Randomized, Double-Blind, Placebo-Controlled, Single Dose Regimen Study of LMN-101 in Healthy Volunteers Challenged with *Campylobacter jejuni*
Investigational Product:	**LMN-101** *(VHH-derived binding protein designed to bind and inhibit FlaA, flagellin filament protein of Campylobacter jejuni, delivered in whole, spray-dried spirulina biomass)*
Sponsor:	Lumen Bioscience, Inc.
Research Center:	Pharmaron Clinical Research Center, Baltimore, Maryland
Site PI:	Mohamed Al-Ibrahim, MB, ChB, FACP
Study Objectives:	Primary: To evaluate the frequency of solicited and unsolicited adverse events in subjects that received LMN-101 compared to placebo Secondary: • To evaluate efficacy based on the proportion of subjects with campylobacteriosis in LMN-101 versus placebo recipients after challenge with *Campylobacter jejuni* • To evaluate efficacy based on the proportion of subjects with specific solicited adverse events in LMN-101 versus placebo recipients after challenge with *Campylobacter jejuni* Exploratory: To evaluate in LMN-101 versus placebo recipients after challenge with *Campylobacter jejuni*: • Duration of campylobacteriosis • Duration of specific solicited adverse events • Severity of campylobacteriosis • Severity of specific solicited adverse events • Frequency of recurrence of *Campylobacter jejuni* stool shedding • Frequency of recurrence of campylobacteriosis clinical symptoms • Correlation of the following with severity of illness: serum C-reactive protein, fecal total protein, fecal calprotectin, fecal lipocalin, fecal myeloperoxidase, fecal neopterin, fecal lactoferrin, fecal cytokines, and/or fecal microbiome • Evaluation of systemic absorption of the VHH in LMN-101, and, if VHH is detected in serum, pharmacokinetics of the VHH in LMN-101 and formation of serum anti-drug antibodies to the VHH in LMN-101

Study Design:	Randomized, double-blind, placebo-controlled, single dose regimen study of LMN-101 followed by *Campylobacter jejuni* challenge. Subjects will initially, after documentation of informed consent, begin taking their assigned LMN-101 or placebo regimen three times daily. After two days, subjects will receive the *C. jejuni* challenge inoculum. Subjects will begin an appropriate antibiotic course upon meeting early treatment criteria or 144 hours following *C. jejuni* challenge, whichever is earlier. Subjects will be allowed to leave the clinical research facility 3 days after antibiotics, when all symptoms have resolved or are resolving, and have had ≥ 2 consecutive stool cultures ≥ 12 hours apart negative for *C. jejuni* and are afebrile > 24 hours prior to release and off antipyretics within 24 hours of discharge. Subjects will continue taking their LMN-101 or placebo regimen three times daily for a total of 14 days. Subjects will be provided a diary card/memory aid and thermometer for at-home monitoring of solicited adverse events through Day 24. Subjects will be seen at research facility for protocol-specified evaluations and will also be contacted by telephone 6 months after challenge.
Subject (N):	42 total subjects will be dosed (21 active, 21 placebo). A maximum of 200 subjects will be screened to obtain approximately 60 eligible subjects.
Dose Regimen	LMN-101 - six (6) 500-mg capsules orally three times daily for 14 days, or placebo.
Clinical Monitoring	All participants will be monitored during their in-center portion of the study. Any participant requiring fluid management or other intervention will receive it as clinically appropriate. Participants will be monitored for adverse events and recrudescence after discharge from study center on a daily basis through Day 12 and at subsequent follow-up visits.
Discharge from Clinical Research Unit	Subjects may be released from the clinical research unit approximately 9-10 days following infectious challenge. Release criteria: • Treated with antibiotics for at least 3 days • Clinical symptoms are resolved or resolving • Two stool cultures ≥ 12 hours apart are negative for *C. jejuni* • Afebrile >24 hours prior to release and off antipyretics within 24 hours of discharge.
PK and PD Assessments	Serum samples will be obtained and analyzed to determine if any systemic absorption is observed, as appropriate
Power Calculation	With 21 subjects receiving LMN-101, there is a 66% probability that an adverse event expected to occur in 5% of the population would occur in the study. An event with a 10% rate in the population is 89% likely to occur among the 21 LMN-101 subjects in the study.

	While the sample size was not selected to provide a specific amount of statistical power to detect a statistically significant difference between the two treatment groups, assuming 18 subjects per treatment group complete the study, there is 80% power to detect a large risk reduction in attack rates (e.g., from 80% in the placebo group to 40.6% in the LMN-101 group) at the one-sided significance level of 0.05.
Statistical Methods	Frequencies and percentages of subjects with treatment emergent AEs and SAEs will be summarized by treatment group, MedDRA preferred term and system organ system class, severity, and relationship to study drug. The percentage of subjects with campylobacteriosis or specific solicited adverse events will be calculated for each treatment group using the standard definition of: (# with endpoint / # receiving inoculum) x 100%. The risk ratio (LMN-101 / placebo) will be presented. The risk ratio will be tested using the method of Cochrane-Mantel-Haenszel stratified by enrollment cohort to determine if the risk ratio is statistically significantly different from 1.

CONTACTS IN CASE OF EMERGENCY

Role in Study	Name	Contact Information
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STUDY COLLABORATORS

Role in Study	Name	Contact Information
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CVD Collaborating Investigator	Wilbur Chen, MD, MS, FACP, FIDSA Professor of Medicine Chief, Adult Clinical Studies	Center for Vaccine Development and Global Health (CVD) University of Maryland School of Medicine 685 W. Baltimore St., Suite 480 Baltimore, MD 21201 (410) 706-5328 office (410) 706-1188 direct (410) 706-6156 research concerns (410) 706-6205 fax wchen@som.umaryland.edu

3. Schedule

	Screening		Inpatient															Follow-up						
Day	**-60 to 0**	**-21 to 0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**17**	**24**	**31**	**38**	**59**	**87**	**115**	**190**
Event	**Screen**	**Screen**	**Admit**		**Challenge**																			
Window (days)																	± 2	± 2	± 3	± 3	± 3	± 7	± 7	± 30
Informed consent	X																							
Comprehension test	(X)																							
Inclusion/exclusion		X	X																					
Medical/Surgical history, comorbidities	X		X																					
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Campylobacter prior-exposure test (IgA titer to *C. jejuni* glycine extract)	X																							
Urine tox screen	X		X															X						
Collect blood for IgA and HLA-B27	X																							
Collect blood for HbsAg, HCV, HIV		X																						
Serum/urine pregnancy test[1]		X	X															X						
Collect whole blood for hematology[3]		X	X									X						X						
Collect serum for chemistry [3]		X	X									X						X						
Collect blood for PT, PTT[3]		X																X						
Physical examination (focused PE)		X	X	X	X	X	X	X	X	X	X	X	X	X			(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	
Electrocardiogram[10]		X										X												
Height/weight		X	X																					
TID dosing of study drug[13]			X	X	X	X	X	X	X	X	X	X	X	X	X	X								
Approximate unit discharge day[7]														X										
Review memory/diary card			X	X													X	X						
***Campylobacter* challenge**					X																			
Serum for drug concentrations[2]			XX			X			X			X						X						

	Screening		Inpatient														Follow-up						
Day	**-60 to 0**	**-21 to 0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**17**	**24**	**31**	**38**	**59**	**87**	**115**	**190**
Event	**Screen**	**Screen**	**Admit**		**Challenge**																			
Collect serum for anti-drug antibodies			X															X						
Collect serum for biomarkers[9]			X			X	X	X	X	X	X	X												
Collect stool for biomarkers[9]			X			X	X	X	X	X	X	X												
Blood culture for T ≥ 39°C						X	X	X	X	X	X	X	X	X										
Collect stool for weighing and grading					X	X	X	X	X	X	X	X	X	X										
Collect stool for *Campylobacter* culture[5]					X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	(X)	
Administer antibiotics[6]						(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)								
AEs[8]			X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X			
SAEs			X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X
AESI assessments[8]																		X	X	X	X	X	X	X
Telephone Follow up/ Study completion																								X

[1]Serum beta-hCG or urine pregnancy test must be collected from women of childbearing potential within 14 days before receipt of first dose of investigational product and at Day 1. Serum beta-hCG is also collected on Day 24.

[2]Serum PK is collected before first dose of study drug; 2 ± 1 hours after first dose of study drug. Serum PK is also collected on Day 4; Day 7; Day 10; and Day 24.

[3]Selected clinical laboratory studies to include hemoglobin, platelet count, white blood cell count with differential, sodium, potassium, calcium, magnesium, phosphate, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and total bilirubin. Coagulations studies are prothrombin time (PT) and partial thromboplastin time (PTT).

[5]Stool culture for *Campylobacter jejuni* collected daily while in study center and at follow up clinic visits. If subject is not able to produce a sample on the planned Recrudescence day of the follow-up visit, then the visit should be postponed to a day within the window for that visit (e.g., + 2 days); if patient still cannot produce a sample, then a rectal swab may be taken. If there is no new recrudescence on Day 87 then no stool will be collected for *Campylobacter jejuni* culture on Day 115. If there is a new recrudescence on Day 87 then all subjects who have not recrudesced will have a *Campylobacter jejuni* stool culture completed on Day 115.

[6]Azithromycin 500 mg po daily and ciprofloxacin 500 mg po twice daily for five days will begin when a subject meets early treatment criteria or it has been 144 hours since the *Campylobacter jejuni* challenge, whichever is earlier.

[7]Subjects will be discharged from the clinical research facility when they have had 3 days of antibiotics, all symptoms have resolved or are resolving, and subjects have had ≥ 2 stool cultures ≥ 12 hours apart negative for *C. jejuni* and <u>are afebrile</u> > 24 hours prior to release and off antipyretics within 24 hours of discharge.

[8]Specific solicited adverse events following *Campylobacter jejuni* challenge include diarrhea, fever, nausea, vomiting, abdominal pain, abdominal cramping, tenesmus, mucoid or bloody stools, constipation, headache, lightheadedness, fatigue, lack of appetite, muscle aches, chills, and joint pains will be monitored through Day 24. Subjects will be provided a diary card/memory aid and thermometer for follow-up monitoring of specific solicited adverse events. Adverse events will be collected through Day 59. Adverse events of special interest (AESIs) following *Campylobacter jejuni* challenge include signs or symptoms of Guillain-Barre syndrome, neurodegenerative changes, uveitis, reactive arthritis, and myocarditis/pericarditis and will be collected through Day 190.

[9]Biomarkers (e.g., serum C-reactive protein, fecal total protein, fecal calprotectin, fecal lipocalin, fecal myeloperoxidase, fecal neopterin, fecal lactoferrin, fecal cytokines and/or fecal microbiome). Serum for biomarkers will be collected at aliquoted, and frozen on admission and on Days 4, 5, 6, 7, 8, 9, and 10. Stool for biomarkers will be collected on admission prior to challenge, and on Days 4, 5, 6, 7, 8, 9, and 10; the inability of a subject to produce a sample on these days will not be considered a protocol deviation.

[10]Electrocardiogram at screening and Day 10.

<u>Note</u>: Failure to produce a stool on a specific day for any form of analysis will not be considered as a deviation from the protocol. Rectal swabs may be obtained if a subject is unable to produce a stool on the designated day (see footnote 5).

4. Introduction

4.1 Background and study rationale

Diarrhea remains a major cause of mortality and morbidity globally, and this is particularly true in children under five (Troeger *et al.* 2018). Death is due not only to the primary disease itself, but from dehydration and increased susceptibility to other infectious diseases. *Campylobacter jejuni (C. jejuni)* is one of the most common pathogens responsible for these diarrheal diseases.

Campylobacter causes more than 1.5 million illnesses each year in the U.S. (CDC 2017), mostly due to raw or undercooked poultry. Complications can include Guillain-Barré syndrome, irritable bowel syndrome (5-20%), reactive arthritis (1-5%), erythema nodosum, glomerulonephritis, hemolytic anemia, immunoglobulin A nephropathy, and intestinal perforation.

Infectious diseases, caused by enteric pathogens, are one of the most common health threats encountered by armed forces and cause more lost duty time and operational disruption than combat casualties. Military personnel are at higher risk because they often must endure environments that are conducive to the spread of infectious disease. They are called upon to live in close quarters, travel to foreign regions, operate in areas with inadequate sanitation, and live-in situations that force compromises to personal hygiene. Infections caused by *C. jejuni* have become increasingly common in military populations particularly those serving or deployed to Southeast Asia and the Middle East. An additional potential preventive measure for *C. jejuni* infection would be beneficial.

One possible prevention strategy for *Campylobacter* disease involves the use of small therapeutic proteins similar to antibodies made by camels, llamas, and alpacas. Like all antibodies, these proteins can recognize and bind to molecules on pathologic antigens. The antigen-binding domains of these proteins, called variable heavy homodimers (VHHs), can be produced safely in standard biotechnology platforms. Libraries of VHHs can be screened to identify individual clones that recognize specific antigens, including those displayed by enteric pathogens such as *Campylobacter*. Orally delivered VHHs have successfully modified the severity and duration of diarrhea in male infants with rotavirus infections (Sarker *et al.* 2013). They have also been demonstrated to prevent pathogenic infections in animal models, including piglet models of ETEC infection (Harmsen *et al.* 2006, Virdi *et al.* 2013, Virdi *et al.* 2019) and mouse models of rotavirus infection (Garaicoechea *et al.* 2008, Gomez-Sebastian *et al.* 2012, Günaydin *et al.* 2016, Maffey *et al.* 2016, van der Vaart *et al.* 2006).

VHHs are therefore ideal candidates for preventing and treating enteric diseases. However, the current high cost of manufacturing VHHs has prevented their widespread use to date.

Lumen Bioscience has discovered and developed a way to rapidly and stably engineer spirulina (*Arthrospira platensis)*, a type of blue-green algae that is cultivated and consumed worldwide. In ongoing work supported by the Bill & Melinda Gates Foundation, Lumen has demonstrated high-level expression of VHHs in spirulina. Moreover, spirulina containing VHHs with specificity against the FlaA flagellin protein of *C. jejuni* has been shown to fully protect against *Campylobacter jejuni* challenge in two different mouse challenge models developed by researchers at the University of Virginia (Guerrant, *et al.,* 2019, confidential, unpublished data) and the Institute for Research in Biomedicine, Bellinzona, Switzerland (Grassi *et al.,* 2019, confidential, unpublished data).

Lumen developed SP1182, a strain of spirulina that has been stably engineered to express a protein that binds and inhibits the FlaA filament protein on the flagellum of *C. jejuni*. The drug, LMN-101, is orally delivered whole, dried spirulina biomass (i.e., the active biologic is not purified from the spirulina biomass). After spray drying, the spirulina in this biomass is non-viable (likely due to the elevated temperature and the water removal to form the powder), while the binding activity of the campylobacter-binding protein is retained. The drug product consists of the dried powder packaged in vegetable-based capsules for oral delivery.

One benefit of the dried spirulina biomass is protection of the binding protein from gastric acid and proteolytic enzymes during digestion and from degradation during storage. The active biologic agent is a large (55 kDa) macromolecule not expected to be systemically absorbed.

Spirulina was selected as the expression host because it is considered as Generally Recognized as Safe (GRAS) by the FDA, has a well-understood and non-toxic safety profile from its wide use as a food source for humans and animals, has been involved in numerous clinical trials with no reports of adverse events (AEs), and is highly stable as a spray-dried powder.

Taken as a whole, these data provide support for the potential utility for LMN-101 to prevent *Campylobacter jejuni* diarrhea.

4.2 Dosage rationale

A Phase 1 trial in Australia demonstrated LMN-101 was safe and well tolerated at the planned dose for this study, 3000 mg. There were no significant adverse events or significant laboratory abnormalities reported during or following the trial. Rates of adverse events, deemed possibly related, were similar between the two groups. Reported adverse events were mild and consisted of nausea, abdominal pain, diarrhea, gastroesophageal reflux, constipation, pharyngitis, and delayed menstruation. Laboratory evaluation revealed no systemic VHH absorption (Jester et al. 2021).

Several murine *Campylobacter jejuni* challenge studies have demonstrated that the spirulina-VHH no-observed-adverse-effect level (NOAEL) was 13.3 mg biomass, equivalent to 665 mg/kg (Guerrant et al, 2019, confidential unpublished data). The human-equivalent dose (HED) is scaled based on a mg/kg conversion (FDA 2005). The gastrointestinal compartment weight allometrically scales by $W^{0.94}$, approximated as $W^{1.0}$ (FDA 2005), yielding a HED of 665 mg/kg or 46.5 grams/dose. Spirulina has an extensive safety record in the food and nutritional supplement industries. In addition, numerous clinical trials have been conducted with spirulina with no reports of AEs (Karkos *et al.* 2011).

Lumen estimates the minimal pharmacologically active dose (PAD) as determined by the murine preclinical studies using the challenge strain, which was 67 mg/kg (Grassi et al, 2019, confidential unpublished data). Based on the gastrointestinal compartment weight and adjusting for repeated drug administration, the HED is 43 mg/kg, i.e., 3010 mg for a 70-kg person. The planned dose for this study, 3000 mg, is substantially below the calculated NOAEL and incorporates a safety factor of 15.5-fold.

4.3 Controlled human infection *C. jejuni* challenge model

Human challenge studies with *Campylobacter jejuni* were first conducted at the University of Maryland's Center for Vaccine Development using the challenge strains A3249 or 81-176 (Black et al. 1988). Strain 81-176 induced diarrhea at high inoculum doses but was later identified to have several ganglioside

mimics (Guerry 2007). Subsequently, the CG8421 strain was identified and found to lack ganglioside mimicry as determined by structural analysis and genome sequencing (Poly et al. 2008). A new model with this strain was developed as a collaboration among the Naval Medical Research Center (NMRC), University of Vermont, and ACE BioSciences, a Danish biopharmaceutical company working on a candidate *Campylobacter* vaccine. This was to improve understanding of campylobacteriosis and evaluate products designed for primary and secondary *Campylobacter* prevention. For an initial clinical trial, CG8421 was administered to campylobacter-naïve healthy subjects in a dose-ranging in unit trial with a target attack rate of ≥ 75% of subjects meeting a primary endpoint of campylobacteriosis (Tribble et al. 2009).

At least 118 subjects have received an experimental infection with CG8421 (Kirkpatrick et al. 2013, Rimmer et al. 2018, Tribble et al. 2009). This strain has induced moderate to severe diarrhea in the majority of volunteers challenged, without serious AEs, bacteremia, or post-infectious sequelae. One consideration for the *Campylobacter jejuni* challenge strain is the potential for recrudescent infection after apparent clearance from the stool (Baqar et al, 2010, Lindow et al, 2010, Rimmer et al. 2018).

5. Objectives and Endpoints

5.1 Study Objectives

Comparing those subjects receiving LMN-101 versus those receiving a placebo, the objectives are:

Primary

- To evaluate the frequency of solicited and unsolicited adverse events in subjects that received LMN-101 compared to placebo

Secondary

- To evaluate efficacy based on the proportion of subjects with campylobacteriosis in LMN-101 versus placebo recipients after challenge with *Campylobacter jejuni*
- To evaluate efficacy based on the proportion of subjects with specific solicited adverse events in LMN-101 versus placebo recipients after challenge with *Campylobacter jejuni*

Exploratory

To evaluate in LMN-101 versus placebo recipients after challenge with *C. jejuni*:

- Duration of campylobacteriosis symptoms
- Duration of specific solicited adverse events
- Severity of campylobacteriosis symptoms
- Severity of specific solicited adverse events
- Frequency of recurrence of *Campylobacter jejuni* stool shedding
- Frequency of recurrence of campylobacteriosis clinical symptoms
- To correlate severity of illness with:
    - serum C-reactive protein

    - fecal total protein
    - fecal calprotectin
    - fecal lipocalin
    - fecal myeloperoxidase
    - fecal neopterin
    - fecal lactoferrin
    - fecal cytokines
    - fecal microbiome
- To evaluate systemic absorption of the VHH in LMN-101, and, if VHH is detected in serum, pharmacokinetics of the VHH in LMN-101 and formation of serum anti-drug antibodies to the VHH in LMN-101

5.2 Study Endpoints

The endpoints will compare LMN-101 to placebo:

Primary

- Safety: Frequency of solicited or unsolicited adverse events in subjects that received LMN-101 compared to placebo for the protocol-specified duration of collection for each type of adverse event

Secondary

- Efficacy: Proportion of subjects with campylobacteriosis after challenge with *Campylobacter jejuni* strain CG8421
- Efficacy: Proportion of subjects with specific solicited adverse events after challenge with *Campylobacter jejuni* strain CG8421

- "Campylobacteriosis" is defined as:
    - Moderate to severe diarrhea (≥ 4 loose stools or ≥ 401 g of loose stools in 24 hours); or
    - Fever (oral temperature ≥ 38.0°C present on at least two occasions, at least 20 minutes apart) plus at least one of the following symptoms of moderate severity: nausea, vomiting, abdominal cramps, tenesmus; or
    - Fever (oral temperature ≥ 38.0°C present on at least two occasions, at least 20 minutes apart) plus gross blood in ≥ 2 loose stools
- "Specific solicited adverse events" is defined as
    - diarrhea,
    - fever,
    - nausea,
    - vomiting,
    - abdominal pain,
    - abdominal cramping,
    - tenesmus,
    - mucoid or bloody stools,
    - constipation,
    - headache,
    - lightheadedness,

- fatigue,
- lack of appetite,
- muscle aches,
- chills,
- joint pains

Exploratory

- Efficacy:
  - Duration of diarrhea, diarrhea amount (total weight) and stool grade
  - Duration of campylobacteriosis symptoms
  - Duration of specific solicited adverse events
  - Severity of campylobacteriosis symptoms
  - Severity of specific solicited adverse events
  - Frequency of recurrence of *Campylobacter jejuni* stool shedding
  - Frequency of recurrence of campylobacteriosis clinical symptoms
  - Correlation of biomarkers with severity of illness, including serum C-reactive protein, fecal total protein, fecal calprotectin, fecal lipocalin, fecal myeloperoxidase, fecal neopterin, fecal lactoferrin, fecal cytokines, and/or fecal microbiome
- Pharmacology:
  - Detection of measurable VHH in serum
  - If measurable VHH in serum is detected at any timepoint, then assays to measure the following will be performed:

Peak serum VHH concentration following administration of the initial dose and peak serum VHH concentration following a course of treatment
Area under the serum VHH concentration versus time curve (AUC) following administration of the initial dose and following a course of treatment
Induction of serum anti-VHH IgG antibodies

6. Study Design

6.1 General Schema of Study Design

Randomized, double-blind, placebo-controlled, single dose study of LMN-101 followed by *Campylobacter* challenge. The trial will be evaluated based upon a cohort of 21 active and 21 placebo subjects in cohort sizes as determined by the trial site. After the initial cohort has completed the in-center portion of the study, an unblinded statistician will perform an interim analysis.

6.2 Study Duration, Enrollment, and Number of Sites

Estimated duration for the main protocol (e.g., start of screening through final subjects finishing the study) is approximately 9 months. Subjects will be screened for eligibility a maximum of two months prior to the start of the treatment phase. The treatment phase will last 14 days and will be followed by a 6 month follow-up phase. A maximum of 200 subjects will be enrolled and screened to obtain approximately 60 eligible subjects to ensure 42 total subjects are available to be treated (21 active, 21 placebo).

The study center will be Pharmaron Clinical Research Center in Baltimore, Maryland.

6.3 Risks/benefits

Naturally acquired illness caused by *Campylobacter jejuni* ranges from mild to severe watery diarrhea that may contain mucus or blood. Nausea, vomiting, abdominal cramping, headache, abdominal gurgling or gas, anorexia, fever, muscle and/or joint aches, and malaise may occur. Rarely, events of myocarditis, pericarditis, and myopericarditis have been reported following *C. jejuni* infection. For most adults, the illness is not life-threatening but often leads to mild-to-moderate dehydration and significant inconvenience associated with loss of sleep and activity. Recrudescence of *Campylobacter jejuni* infection is also a risk. Study facilities will have personnel and resources capable to manage diarrheal illness and potential complications. Side effects of the antibiotics (azithromycin and ciprofloxacin) used to treat the *Campylobacter jejuni* infection are possible.

Spirulina has a well-described safety profile. However, possible non-serious side effects have been reported with spirulina intended as a nutritional supplement. The FDA MedWatch from January 2001 to July 2009 identified the most common non-serious adverse events from spirulina were nausea, diarrhea, vomiting, fatigue, headache, dizziness, itching, rash, and abdominal cramps. These reports lacked information on the quantity, duration, individual history, or quality of spirulina used and were based on non-GMP spirulina (Marles *et al.* 2011).

Therapeutic antibiotics for use in this study are licensed approved medications that have been used extensively and shown to be very safe with only rare side effects. The most commonly reported side effects for ciprofloxacin are gastrointestinal symptoms (nausea, vomiting, and diarrhea) in as many as 5 individuals in 100. Other reported symptoms in less than 1 individual in 100 include rash, dizziness, and headache. Rarely, allergic reactions to these medications have been observed. Ciprofloxacin is not recommended for use in pregnancy due to concerns of joint damage to the unborn child (based on studies in young animals). Pregnancy is exclusionary for study participation and is documented through testing prior to study interventions and provided discussion on methods to prevent pregnancy during study. Fluoroquinolones, including ciprofloxacin, are associated with an increased risk of tendinitis and tendon rupture in all ages. The risk of developing fluoroquinolone-associated tendinitis and tendon rupture is further increased in individuals over 60 years of age, in individuals taking corticosteroid drugs, and in individuals with kidney, heart, or lung transplants, all of whom are either not included or specifically excluded from this study. Ciprofloxacin can rarely prolong the QT interval, so an ECG is done at screening to ensure that there is no pre-existing QT prolongation. Clostridium difficile-associated diarrhea (CDAD/ pseudomembranous colitis) has been reported with use of nearly all antibacterial agents.

The most common adverse events in response to azithromycin are vomiting, diarrhea, nausea, or abdominal pain. Azithromycin can rarely prolong the QT interval, so an ECG is done at screening to ensure that there is no pre-existing QT prolongation.

If the subject does not tolerate azithromycin or ciprofloxacin, the subject will be given an alternative antibiotic (either amoxicillin-clavulanate or cefpodoxime). The most common amoxicillin-clavulanate side effects may include nausea, vomiting, or diarrhea; rash or itching; vaginal itching or discharge; or

diaper rash. The most commonly reported side effects for cefpodoxime include oral candidiasis, abdominal cramps, diarrhea, nausea, and vomiting.

Good nursing practice will be utilized during blood draws, which minimizes the risk to the subject. Handwashing and sanitary disposal of feces (including pretreatment with bleach) are the main elements of personal hygiene and will minimize the spread by person-to-person infection; handwashing will be emphasized to the subjects, and subjects will be instructed not to share food or beverages. Subjects and staff will be trained in proper techniques of handwashing. Subjects will be instructed as to the importance of completing the 5-day course of antibiotics, and this instruction will be documented. Risk of secondary transmission is highly unlikely due to antibiotic treatment and because subjects are required to submit two confirmed negative stool samples prior to discharge.

A less common, but potentially life-threatening, complication of *C. jejuni* infection is Guillain-Barré syndrome (GBS), a post-infectious polyneuropathy that is a leading cause of paralysis, and the related syndrome Miller-Fisher syndrome (Heikema *et al.* 2013, Keithlin *et al.* 2014). Although many infectious agents are associated with GBS, *C. jejuni* is the most frequent pathogen associated with the syndrome (Baker *et al.* 2012). The association between GBS and *Campylobacter jejuni* infection is supported by serologic, culture, and experimental data. Published data from a large case-control study of campylobacter-associated GBS showed evidence of *C. jejuni* infection in 26% of the 103 GBS and Miller-Fisher syndrome patients compared to 2% of household controls, and 1% of hospital controls (Rees *et al.* 1995). The pathogenesis of GBS following *Campylobacter jejuni* infection is hypothesized to involve "molecular mimicry," where peripheral nerves share epitopes with some *C. jejuni* antigens (Godschalk *et al*. 2004). Thus, an immune response, which is initially mounted against the infection, may be misdirected to peripheral nerves in some convalescing individuals. Based on this knowledge, a detailed characterization of strain CG8421 documented the lack of ganglioside mimicry and therefore posed no risk of GBS or Miller-Fisher syndrome (Poly *et al.* 2008).

Like Salmonella, Shigella, and Yersinia, *Campylobacter jejuni* enteritis has been associated with development of a reactive arthritis/arthropathy (RA) (Ajene et al, 2013). This risk is increased in individuals who have the HLA-B27 antigen. All subjects will be screened for HLA-B27, and a positive result will be an exclusion criterion for the study.

There is a risk of functional bowel disorders, including irritable bowel syndrome, following *Campylobacter jejuni* enteritis (Riddle et al, 2012).

There is a minimal risk of pain, hematoma, or infection at the site of venipuncture. The maximum amount of blood drawn from a subject in total, and daily, will fall within applicable regulations.

There may be physical, psychological, and social risks if subjects test positive for hepatitis B, hepatitis C, and/or HIV. Subjects testing positive will be counseled and referred for treatment.

Medical records associated with this protocol are subject to provisions of the Privacy Act of 1974, 5 U.S.C., Section 552A, and AR 340-21. All data and medical information obtained about subjects will be considered privileged and held in confidence. Subjects will not be identified by name in any published report/presentation of the results. Complete confidentiality cannot be promised to individual subjects who are military personnel because appropriate medical command authorities may require reporting information bearing on the health of their personnel. Representatives of the Sponsor, investigational

site IRB, or FDA may inspect the records of this research as part of their responsibility to oversee research and ensure protection of subjects. Study results and data may be published in scientific/medical journals; the identity of individual subjects will not be disclosed.

7. Study Population

7.1 Recruitment, screening, and enrollment of study volunteers

Volunteers will be recruited using standard procedures for Pharmaron clinical trials. All study-related advertisements will be reviewed and approved by the IntegReview IRB. Subjects who express interest in participating in the study will be asked to complete an IRB-approved pre-screen questionnaire by telephone to assess general health status and basic eligibility. A maximum of 200 potential volunteers determined to be generally healthy and meeting basic eligibility requirements will be scheduled for an in-person screening. Volunteers for challenge studies are carefully screened on the details of the protocol, their time commitment, and the nature of the confinement on a quarantine ward. During the in-person screening, volunteers will take a test to make sure that they understand the study and its requirements.

Informed consent is a continuous process that includes the written subject informed consent document. Subjects will receive an oral presentation of the study in language they can understand (i.e., using lay terms as appropriate). Each prospective subject will be given the written, IRB-approved informed consent, allowed ample time to read the consent, allowed to ask questions about the study, have his/her questions answered, and given time to decide if he/she would like to participate in the study. To document subject's understanding of informed consent, immediately before the consent is signed, the person obtaining consent will administer a brief quiz or comprehension test. Incorrect answers will be discussed with subjects to reinforce the consent and subjects will be given one additional opportunity to take the test. A final acceptable test score is 70% or more answered correctly. Subjects failing after two attempts are not eligible for study enrollment. No coercion or influence is allowed in obtaining subject's consent.

Before subjects participate in any study-related activity, including procedures to determine eligibility, consent forms will be signed and dated by subjects as well as by the PI or designee. Subjects will receive copies of the signed consent prior to participation. As part of the consent process, subjects will also be asked to read and sign a Medical Records/Lab Results Release, and other IRB-approved study documents, and will be provided with an opportunity to ask questions.

7.2 Inclusion Criteria

1. Male or non-pregnant female between 18 and 50 years of age, inclusive, at time of informed consent
2. Willingness to participate after written informed consent obtained
3. Available for all planned clinical visits (for physical examinations, blood draws, and stool collections) and follow-up monitoring (9 or 10 clinic visits and 1 phone interview 6 months post-challenge)
4. Agreement to follow the restrictions of the study. Willing and able to follow the study directions and procedures, including the rules and procedures of the clinical research unit.

5. Demonstrated comprehension of the protocol procedures including knowledge of *Campylobacter* illness by passing a written examination (passing grade ≥ 70%).
6. General good health, without significant medical illness or abnormal physical examination findings as determined by the PI.
7. Laboratory values are Grade 1 or lower using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 as defined below:
    a. Absolute neutrophil count ≥ 1500/µL
    b. Lymphocyte count ≥ 800/µL
    c. Platelet count ≥ 125,000/µL
    d. Hemoglobin ≥ 13.0 g/dL in males (≥ 11.0 g/dL in females)
    e. Serum creatinine ≤1.5x ULN
    f. ALT and/or AST ≤ 1.5x ULN
    g. Total bilirubin ≤ 1.5x ULN
8. Females of childbearing potential must commit to use one of the following highly effective methods of birth control consistently for at least 1 month prior to screening through study completion:
    a. Stable hormonal contraception with inhibition of ovulation; or
    b. Intrauterine device (IUD); or
    c. Bilateral tubal occlusion; or
    d. Surgical sterilization (vasectomy) of male partner at least 6 months prior to study; or
    e. Sexual abstinence (inactivity).
9. To be considered of non-childbearing potential, females should be surgically sterilized (bilateral tubal ligation, hysterectomy, or bilateral oophorectomy at least 2 months prior to study) or be post-menopausal and at least 1 year since menses with follicle-stimulating hormone ≥ 40 units.
10. Males should use condoms for contraception and refrain from donating sperm through Day 64.
11. BMI between 18.5 and 33.5 inclusive
12. Complies with current Pharmaron Covid-19 policies and procedures

7.3 Exclusion Criteria

1. Significant medical condition or laboratory abnormalities that in the opinion of the Principal Investigator preclude participation in the study.
2. History of Covid symptoms or positive Covid test within 2 weeks prior to admission date.
3. Alcohol or illicit drug abuse/dependency
4. Positive serology results for HIV, HBsAg, or HCV with confirmatory assays.
5. Pregnancy or breastfeeding
6. Personal or documented family history of Guillain-Barré syndrome or neuromuscular disease; or an inflammatory arthritis such as reactive arthritis, ankylosing spondylitis, or rheumatoid arthritis; inflammatory bowel disease; autoimmune disease; malignancy (not including basal cell carcinoma); any immunocompromising condition; or history of major gastrointestinal surgery.
7. Evidence of neurological abnormalities.
8. History of reactive arthritis or evidence of inflammatory arthritis on exam.
9. Fever within the 2 weeks prior to time of enrollment.
10. Evidence of IgA deficiency (serum IgA < 7 mg/mL or below the limit of detection of assay).
11. HLA-B27 positive

12. Allergy or prior intolerance to two or more of the following antibiotics: azithromycin, ciprofloxacin, levofloxacin, erythromycin, ampicillin, or amoxicillin/clavulanate.
13. Allergy or prior intolerance to spirulina or spirulina products.
14. Fewer than 3 stools per week or more than 3 stools per day as the usual frequency.
15. History of moderate to serious diarrhea while traveling in a developing country within the last 3 years.
16. History of myocarditis or pericarditis.
17. History of major abdominal surgery or unexplained abdominal scar. Ok if appendectomy or cholecystectomy (one year post)
18. Regular use of antidiarrheal, antacids, loperamide, bismuth subsalicylate diphenoxylate, or similar medication affecting bowel motility (regular defined as at least weekly).
19. Use of proton pump inhibitors, H2 blockers, or other antacids within 48 hours preceding initiation of LMN-101 or placebo.
20. Use of antibiotics during the 7 days preceding initiation of LMN-101 or placebo.
21. Use of spirulina, or spirulina containing products, other than the study drug in the 30 days preceding initiation of LMN-101 or placebo.
22. Use of any investigational product within 30 days preceding initiation of LMN-101 or placebo or planned use during the active study period.
23. Use of any medication known to affect the immune system (e.g., systemic corticosteroids, chemotherapy, monoclonal antibody biologic response modifiers) within 12 months preceding initiation of LMN-101 or placebo or planned use during the active study period (excluding inhaled steroids with spacer).
24. History of prior exposure to *Campylobacter* including by vaccination or infection in previous trials, or serum immunoglobulin A (IgA) titer to C. *jejuni* glycine extract >1:4000.
25. Other dietary or environmental exposures that may place the subject at high risk for prior *Campylobacter* exposure (to be determined on a case-by-case basis by the PI).
26. Employment as a food handler; childcare worker; or caregiver for elderly, immunocompromised individuals, or other at-risk population.
27. History of major mental illness such as schizophrenia, major depression or suicidal ideation
28. Any other criteria which, in the Principal Investigator's opinion, would compromise the ability of the subject to participate in the study, the safety of the study or the results of the study.
29. Potential participant's 12 – lead electrocardiogram demonstrating pathologic abnormalities including non-sinus rhythm, pathologic Q waves, significant ST-T wave changes, corrected QT interval (QTc) using Fridericia correction (QTcF) at screening and Day -1 (admission) >450 msec.
30. At screening, systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg.

8. Study Schedule

8.1 Screening (Two months prior to study start)

Subjects will be screened for enrollment within two months before Day 1. Subjects will be screened according to predefined inclusion criteria as described in section 7.2. See section 7.3 for a list of exclusion criteria. The following procedures will be performed at screening:

- Informed Consent

- Comprehension test
- Medical history, including existing medical conditions
- Record concomitant medications
- Vital signs including heart rate, respiratory rate, blood pressure, and temperature
- *Campylobacter* prior exposure test
- Urine toxicology screen
- Collect blood samples for immunoglobulin and HLA-B27 tests

8.2 Study-Specific Screening (Three weeks prior to start)

Note: Study team will have flexibility to perform screening and study-specific screening procedures at any point prior to admission to research clinic

Visit will include the following procedures:

- Assessment of inclusion/exclusion criteria
- Record concomitant medications
- Collect blood samples for Hepatitis B, Hepatitis C, and HIV tests
- Serum or urine pregnancy test for female subjects of childbearing age
- Selected clinical laboratory studies to include hemoglobin, platelet count, white blood cell count with differential, sodium, potassium, calcium, magnesium, phosphate, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and total bilirubin. Coagulations studies are prothrombin time (PT) and partial thromboplastin time (PTT).
- Physical examination
- Vital signs
- Electrocardiogram
- Height/weight

8.3 Treatment Phase

Day 1: Admission to research clinic

Assessment of inclusion/exclusion criteria
Medical history, including existing medical conditions
Record concomitant medications
Urine toxicology screen
Serum or urine pregnancy test for female subjects of childbearing age
Selected clinical laboratory studies to include hemoglobin, platelet count, white blood cell count with differential, sodium, potassium, calcium, magnesium, phosphate, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and total bilirubin.
Physical examination
Height/weight
Collect serum for anti-drug antibodies
Collect serum for drug concentrations
Collect stool sample
Subjects will receive three doses of their assigned IP throughout the day
While in unit, doses will be co-administered with 50 mL of bicarbonate buffer
Subjects will be monitored for at least 60 minutes for any AEs, SAEs, or allergic reactions, then have a set of vital signs taken.
Subjects will be given a diary and thermometer and instructed on use

Day 2

Record concomitant medications
Physical examination
Subjects will receive three doses of their assigned IP throughout the day
While in unit, doses will be co-administered with 50 mL of bicarbonate buffer
Subjects will be monitored for at least 60 minutes for any AEs, SAEs, or allergic reactions, then have a set of vital signs taken.
Staff will collect and review the diary with the subjects

Day 3: Challenge day

Subjects will consume a light breakfast and fast for 90 minutes before the challenge procedure. Sixty minutes into the fast, they will take a dose of IP with 50 mL USP-grade bicarbonate buffer solution then continue to fast. At 90 minutes, subjects will drink 120 mL of USP-grade bicarbonate buffer solution, followed approximately 1 minute later with the *C. jejuni* CG 8421 strain challenge, 5×10^5 CFU. Subjects will be observed, and vital signs measured approximately 30 minutes after dosing. Subjects will fast for an additional 90 minutes after receiving the challenge. Subjects will receive afternoon and evening doses of IP. The following procedures will also be performed:

Subjects will be monitored for AEs, specific solicited AEs, and SAEs
Record concomitant medications
Physical examination
Vital signs including additional vital signs 30 minutes after challenge
Collect, weigh, and grade stool sample
Collect stool sample and culture for presence of *C. jejuni*

Day 4 - Day 9

Clinical research staff will monitor the subject's general and gastrointestinal health, assess systemic signs and symptoms, and determine any adverse events by medical interview and focused physical examinations. Additionally, subjects will be examined for symptoms and signs of dehydration.

The following procedures will be performed:

Collect serum for drug concentrations on Day 4 and Day 7
Start antibiotic treatment at 144 hours after challenge or when they meet early treatment criteria, whichever is earlier. Azithromycin 500 mg po daily and ciprofloxacin 500 mg po twice daily for five days.

The following procedures will be performed daily (hereafter "daily procedures"):

Subjects will be monitored for AEs, specific solicited AEs, and SAEs
Record concomitant medications
Physical examination
Vital signs
Subjects will receive three doses of their assigned IP co-administered with 50 mL of bicarbonate buffer throughout the day
Collect serum for biomarkers

Collect stool for biomarkers
Collect, weigh, and grade stool sample
Collect stool sample and culture for presence of *C. jejuni*
Blood culture for temperatures ≥ 39°C

Day 10

Perform daily procedures
Selected clinical laboratory studies to include hemoglobin, platelet count, white blood cell count with differential, sodium, potassium, calcium, magnesium, phosphate, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and total bilirubin.
Collect serum for drug concentrations
Electrocardiogram
Review discharge criteria

Day 11 - Day 12

Subjects will be monitored for AEs, specific solicited AEs, and SAEs
Record concomitant medications
Physical examination
Vital signs
Subjects will receive three doses of their assigned IP co-administered with 50 mL of bicarbonate buffer throughout the day
Collect, weigh, and grade stool sample
Collect stool sample and culture for presence of *C. jejuni*
Blood culture for temperatures ≥ 39°C
Review discharge criteria

Discharge Day

Subjects will be discharged from the research clinic when the following criteria are met:
at least three days after commencing antibiotics;
when all symptoms have resolved or are resolving;
subjects have had ≥ 2 consecutive stool cultures ≥ 12 hours apart negative for *C. jejuni*.
Subjects will receive remaining antibiotics and remaining IP doses to take at home
Subjects will take home a diary card/memory aid and thermometer to monitor for solicited adverse events, including diarrhea, fever, nausea, vomiting, abdominal pain, abdominal cramping, mucoid or bloody stools, headache, fatigue, lack of appetite, muscle aches, chills, and joint pains.

Day 17 ± 2 days

Assess for AEs and SAEs
Record concomitant medications
Physical examination
Vital signs
Review subject's diary
Collect stool sample and culture for presence of *C.* jejuni

8.4 Follow-up Phase

The following procedures will be performed during follow-up visits (hereafter "follow-up visit procedures"):

Assess for SAEs and AESI
Record concomitant medications
Collect stool sample and culture for presence of *C. jejuni*
Vital signs

Day 24 ± 2 days

Follow-up visit procedures
Assess for AEs
Urine toxicology screen
Serum or urine pregnancy test for female subjects of childbearing age
Selected clinical laboratory studies to include hemoglobin, platelet count, white blood cell count with differential, sodium, potassium, calcium, magnesium, phosphate, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and total bilirubin.
Coagulations studies are prothrombin time (PT) and partial thromboplastin time (PTT).
Review subject's diary
Collect serum for drug concentrations
Collect serum for antidrug antibodies

Day 31 (± 3 days)

Follow-up visit procedures
Assess for AEs

Day 38 (± 3 days)

Follow-up visit procedures
Assess for AEs

Day 59 (± 3 days)

Follow-up visit procedures
Assess for AEs

Day 87 (± 7 days)

Follow-up visit procedures

Day 115 (± 7 days)

Follow-up visit procedures

Day 190 (± 30 days)

Research clinic staff will call subjects approximately six months following challenge. SAEs and AESIs will be assessed and recorded.

8.5 Recrudescent infection and recrudescent follow-up plan

Monitoring for *C. jejuni* recrudescence utilizes clinical and microbiological active surveillance. Post-discharge clinic visits (with stool culture collection) will occur approximately on Days 17, 24, 31, 38, 59, and 87 (14-, 21-, 28-, 35-, 56-, and 84-days post challenge). Stools will be cultured to determine the presence of *Campylobacter jejuni* organisms. As with the initial clinic visits, if a subject fails to produce a stool on the planned day of a follow-up visit then the sample collection should be postponed to a day within the window for that visit (e.g., ± 2 days). If they still cannot produce a sample, then a rectal swab may be taken.

In the event of a recrudescence in any subject by Day 87, all subjects still on study who have not recrudesced will be contacted to attend a further visit with stool collection on Day 115 (± 4 days). If there is no recrudescence in any subject by day 87 then the day 115 visit will not be required.

Recrudescence of infection: Defined as positive stool culture for the *C. jejuni* strain used for inoculation occurring after a complete course of antibiotics, clinical improvement, and clearance of stool cultures (a minimum of 2 *C. jejuni* culture negative stool specimens at least 12 hours apart).

Prior studies, using the same *C. jejuni* strain, have demonstrated that between 4-18% of subjects receiving the investigational inoculum experienced a recrudescence of *Campylobacter jejuni* infection. The recrudescence events were detected and confirmed as strain CG8421 through tests on stool specimens obtained at clinic visits, despite $\geq$ 2 negative stool cultures for *C. jejuni* prior to discharge from the clinical research unit. In all cases but one, additional antibiotics (azithromycin and ciprofloxacin for 10 days) were provided, and the infection was cleared. One subject cleared, however, experienced a second recrudescence. This volunteer was lost to follow-up prior to completing enhanced follow-up schedule.

Recrudescent follow-up plan

If a recrudescent event occurs, the subject will be assessed as soon as possible with a medical interview and physical examination. The subject will be followed for a total of 6 months from the date of documented infection recurrence. The date of production of the stool demonstrating recrudescence will be re-designated as Recrudescence Day 0 for follow-up purposes.

Recrudescent follow-up will include:

Medical interview and physical examination as soon as possible.
Repeat stool collection will occur with testing for stool microbiology and expanded to include routine *C. difficile*, stool bacteriology and parasitology if the subject has symptoms. The subject will be tested for evidence of immunocompromise; hypogammaglobulinemia testing and a repeat HIV test will be performed.
The strain will be confirmed by clinical microbiology and PCR testing to confirm it is the study strain.
The *C. jejuni* isolate will be tested to ensure continued antibiotic susceptibility.
Combination antibiotic treatment with ciprofloxacin 500 mg po BID and azithromycin 500 mg po QD will be given for a total of 10 days.
If a subject experiences side effects from the azithromycin and ciprofloxacin therapy requiring an alternative agent, another effective antibiotic, based on documented challenge strain susceptibility, will be used. Alternative antibiotic doses are as follows: amoxicillin/clavulanate (Augmentin) 875 mg

PO BID for five days or cefpodoxime 400 mg PO BID for 5 days. The cGMP challenge strain CG8421 has documented susceptibility to ampicillin by prior testing. Decisions regarding antibiotic use will be determined by the study clinician on a case-by-case basis, performed in discussion with the Medical Monitor.

On Recrudescence Days 14, 21, 28, 35, 56, and 84, the subject will undergo a medical interview and physical assessment along with stool collection for microbiology. On Recrudescence Days 56 and 84, stool will also be collected for exploratory endpoints.

Follow-up will be completed with a telephone interview on Recrudescence Day 190 (± 30 days) to inquire about new-onset serious health events.

If any previously recrudesced subject experiences a second recrudescence prior to Recrudescence Day 84, that subject will restart the follow up sequence as above.

8.6 Assessment of subject compliance with investigational products

A member of the study team will observe the ingestion of the first dose of investigational product (IP). The subject will be given a diary to take home after their discharge to document subsequent doses of the IP. While in unit, the subject will be administered the IP, *C. jejuni* challenge, and antibiotics under direct observation. If subject is to complete the antibiotic course at home, then the subject will be questioned regarding compliance at the next study visit and diary review.

8.7 Concomitant medications

Only concomitant medications approved by the study physician will be used during the study. Subjects taking regular medication (e.g., birth control pills) prior to enrollment will be allowed to continue unless it is specifically excluded as part of the inclusion/exclusion criteria. Subjects requiring non-approved or excluded medication will not be eligible for enrollment. Any medication ordered during the trial (e.g., ondansetron, acetaminophen, azithromycin, ciprofloxacin, or alternative antibiotics) will be documented in source documents and on the appropriate page of the case report forms. Approved medications that were being taken prior to and during the trial will also be documented.

8.8 Early termination

Subjects have the right to withdraw from the study at any time and for any reason without affecting the right to treatment by the Principal Investigator for study-related conditions. The Principal Investigator also has the right to withdraw subjects in the event of intercurrent illness, AEs, or for administrative/social reasons.

Should withdrawals occur, efforts will be made to ensure subject safety. In case of subject withdrawal, for whatever reason, a final trial evaluation must be completed (if possible) stating the reasons. Withdrawals due to non-attendance must be followed-up by the Principal Investigator to the extent possible to obtain the reason for non-attendance. Subjects withdrawing from the study prior to receiving the challenge dose (Day 3) will stop taking the investigational product and be asked to complete a final visit for safety about 28 days (± 5 days) after the last dose. Subjects withdrawing after receiving the *C. jejuni* challenge dose will receive antibiotics (two days dosing under direct observation prior to discharge) for their own at-home treatment and will be educated on the importance of complying with treatment. Attempts will be made to follow all subjects for safety through Day 190.

8.9 Randomization

Subjects will be randomized in a 1:1 ratio to one of two treatment groups (LMN-101 or placebo) per cohort as detailed in the Pharmacy Manual. Subjects who fulfill all inclusion/exclusion criteria and are eligible to participate will be assigned the next sequential treatment number by the Principal Investigator. The treatment number corresponds to a given treatment assignment known to the study pharmacist but blinded to the subject, the PI, and the remaining members of the clinical research site and collaborators.

Sealed, code-break envelopes will be provided to the research pharmacist if a given subject has a serious adverse event that requires unblinding of treatment assignment.

If a given subject drops out of the study or becomes ineligible before the *Campylobacter jejuni* challenge, they will be replaced on the study by a subject given the same treatment assignment.

8.10 Blinding

Individual subject assignments to a given treatment group will remain blinded to investigators, subjects, and personnel involved in collecting, cleaning, and analyzing the data until completion of the trial phase and validation of the clinical and outcome data.

8.11 Fluid and bacteremia management

8.11.1 Oral fluid replacement

Any subject passing grade 3-5 stools will be encouraged to drink fluids at a rate equal to or greater than their stool output. If willing, the volunteers will be provided with Oral Rehydration Solution, as an optimal replacement. Other oral fluids may be substituted.

8.11.2 Criteria for IV fluid replacement

During the trial, a subject will be considered for intravenous (IV) fluid replacement if determined necessary by study physician (e.g., diarrhea with nausea/vomiting and unable to replace by oral route).

8.11.3 Criteria for serum electrolyte monitoring

Study physicians will assess the need for testing based on clinical status and previous laboratory results. Serum electrolyte levels will be followed in any subject who meets criteria for intravenous fluid replacement.

8.11.4 Criteria for blood culture surveillance

Blood cultures will be collected if a subject has a confirmed temperature of $\geq$ 39.0°C. The blood culture will be processed using standard practices through the Quest microbiology laboratory. If a blood culture is positive for Campylobacter, it will be repeated until there are 2 negative sets of blood cultures.

8.11.5 Management plan for Campylobacter jejuni bacteremia.

Subjects who develop *Campylobacter jejuni* bacteremia will be treated with empiric intravenous ciprofloxacin in conjunction with oral azithromycin. Intravenous amoxicillin-clavulanate is an alternative antibiotic. Therapy will be modified as indicated based on antibiotic susceptibility testing of the

Campylobacter jejuni isolates. Decisions regarding changes in antibiotic use will be determined by the study clinician on a case-by-case basis, performed in discussion with the Medical Monitor.

8.12 Planned antibiotic treatment phase

Antibiotics will be supplied through the study site investigational pharmacy. Azithromycin (500 mg by mouth once daily for 5 days) concurrently with ciprofloxacin (500 mg by mouth BID for 5 days) will be used as the treatment regimen for all subjects, unless the study physician or designee determines that intravenous antibiotics are required for a specific participant. The challenge strain is susceptible to both antibiotics. If a recrudescent event occurs, both ciprofloxacin and azithromycin will be given for a total of 10 days. If a subject experiences side effects from the azithromycin and/or ciprofloxacin therapy requiring an alternative agent, another effective antibiotic, based on documented challenge strain susceptibility, will be used. Alternative antibiotic doses are as follows: amoxicillin/clavulanate (Augmentin) 875 mg PO BID for five days or cefpodoxime 400 mg PO BID for 5 days. The cGMP challenge strain *C. jejuni* CG8421 has documented susceptibility to ampicillin by prior testing. Decisions regarding changes in antibiotic use will be determined by the study clinician on a case-by-case basis, performed in discussion with the Medical Monitor.

Antibiotic treatment is scheduled to start approximately 144 hours ± 1-hour post-challenge. Early treatment will commence if the following criteria are met:

8.12.1 Criteria for early treatment with antibiotics

Subjects meeting any of the following criteria will be treated with antibiotics prior to the planned treatment day (Day 9) by the study PI. The study PI may also determine the best route for antibiotic administration.

Severe diarrhea (> 6 grade 3-5 stools in 24 hours or > 800 g of grade 3-5 stools in 24 hours).
Fever (oral temperature ≥ 38.0°C present on at least two occasions, at least 20 minutes apart) with at least one associated symptom (nausea, vomiting, abdominal cramps, tenesmus, or gross blood in ≥ 2 loose stools).
A study physician determines early treatment is warranted for other reasons.

Any subjects meeting discharge criteria will be released with the remaining antibiotic treatment to be taken at home.

9. Study Evaluations

9.1 Specimen preparation, handling, and shipping

Research microbiology, including the preparation of live inoculum and culturing of stool specimens, will be carried out at Center for Vaccine Development (CVD) Microbiology Laboratory.

No greater than 500 mL of blood will be collected per subject during any 8-week period of the study in keeping with FDA and Red Cross guidelines.

Stool samples for fecal biomarkers (for example, fecal total protein, fecal calprotectin, fecal lipocalin, fecal myeloperoxidase, fecal neopterin, fecal lactoferrin, fecal cytokines, and/or fecal microbiome) will

be placed into a sealed container with an ice pack in a cool bag. Samples will be transported to the CVD laboratory for processing, storage, and testing.

Post challenge, at each non in unit visit through Day 87 (Day 115 if there is any recrudescence) stool samples for *Campylobacter jejuni* culture and/or fecal biomarkers will be placed into a sealed container with an ice pack in a cool bag and brought to the clinic within 8 hours of production (per Laboratory Manual).

9.2 Clinical laboratory evaluations

Standard clinical laboratory tests for the purpose of inclusion and exclusion of potential subjects and as clinically indicated for safety monitoring will be carried out at Pharmaron Laboratories, a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. Quest will perform *Campylobacter jejuni* blood cultures.

The stool bacteriology performed post-inoculation will be carried out in the CVD Microbiology Laboratory, which is CLIA-certified. Until the start of antibiotics, up to two stool cultures per subject will be performed daily. Beginning 24 hours after the start of antibiotics, up to three specimens per day will be cultured per subject. If the subject cannot provide a stool sample within the time window for each visit day, then a rectal swab may be taken.

9.3 Clinical evaluations

The severity grading for the adverse events listed below is to ensure consistency with other bacterial enteric disease experimental infection studies.

Clinical definitions

Campylobacteriosis: Clinical illness meeting at least one of the following patterns with documented *C. jejuni* infection occurring within 144 hours of challenge:

Moderate to severe diarrhea (≥ 4 loose stools or ≥ 401 g of loose stools in 24 hours); or
Fever (oral temperature ≥ 38.0°C, present on at least two occasions, at least 20 minutes apart) with at least one of the following symptoms of moderate severity: nausea, vomiting, abdominal cramps, tenesmus; or
Fever (oral temperature ≥ 38.0°C present on at least two occasions, at least 20 minutes apart) with gross blood in ≥ 2 loose stools

Infection: Positive *C. jejuni* stool culture in two stool cultures > 24 hours post-inoculation or a positive *C. jejuni* stool culture > 24 hours post-inoculation with associated clinical symptoms meeting the campylob*a*cteriosis endpoint definition.

Grading system for stools:

Grade 1: firm, formed (normal)
Grade 2: soft, formed (normal)
Grade 3: viscous opaque liquid or semi-liquid which assumes the shape of the container
Grade 4: watery, non-viscous, opaque liquid which assumes the shape of the container
Grade 5: clear or translucent, watery, or mucoid liquid which assumes the shape of the container

Diarrhea:

One loose/liquid stool (grade 3-5) of ≥ 300 g within 144 h of *Campylobacter jejuni* challenge*;*

OR

At least two loose/liquid stools totaling ≥ 200 g during any 48-hour period within 144 hours of challenge with *Campylobacter jejuni*

OR

≥ 3 loose or liquid stools in a 24-hour period regardless of volume.

Diarrhea severity (determined within 144 hours of challenge): diarrhea severity is determined by frequency, within a 24-hour period, and by total diarrhea (grade 3-5 stools) volume during episode. The grade selected is the higher severity by either frequency or volume. Table 2 displays the grading scheme:

Table 2: Severity grading scheme.

Severity Grade	Parameter
Mild	2-3 or more grade 3-5 stools in a 48-hour period totalling 200-400 g or more or a single grade 3-5 stool of 300 g in 24 hours
Moderate	4-5 grade 3-5 stools in 24 hours or 401-800 g of grade 3-5 stools for total episode
Severe	> 6 grade 3-5 stools in 24 hours or > 800 g of grade 3-5 stools for total episode

Dysentery: Gross blood observed in ≥ 2 stools Grade 3-5

Recrudescence of infection: Positive stool culture for the *C. jejuni* strain used for inoculation occurring after a complete course of antibiotics, clinical improvement, and clearance of stool cultures (2 specimens, a minimum of 12 hours apart), with or without symptoms of infection.

Hypovolemia: Confirmed (after 5 minutes) supine systolic blood pressure (BP) < 90 mmHg with associated symptoms or significant lightheadedness on standing with a confirmed postural change in BP or pulse. Postural vital signs will be measured lying and 2 minutes after standing. A significant change will be any of the following: decrease in systolic BP or diastolic BP of > 20 mmHg; increase in pulse of > 30 beats/min; or upon Principal Investigator discretion.

Fever: An oral temperature ≥ 38.0°C (100.4°F) present on at least two occasions 20 minutes or more apart.

Arthralgia: Persistent pain or stiffness in a joint without any evidence of swelling or inflammation. If a subject does have arthralgia, then a targeted history will be taken to determine whether there is an attributable cause for the pain, such as exercise.

Arthritis: Persistent pain or stiffness in a joint with evidence of inflammation by physical exam.

Reactive arthritis: An arthritis that develops soon after an infection elsewhere in the body without a microorganism being isolated from a joint. Symptoms typically begin within 1 to 4 weeks after an infectious event. Reactive arthritis is generally monoarticular or oligoarticular. It is generally an asymmetric, additive arthritis, with new joints becoming involved over a period of days to 1 to 2 weeks.

The joints of the lower extremities are most commonly affected, including knees, ankle, subtalar, metatarsophalangeal, and interphalangeal joints. The wrists and fingers may also be involved. Tendonitis and fasciitis, particularly involving the Achilles tendon and plantar fascia, are characteristic. Joints are painful and can be swollen. Morning stiffness is a prominent feature. Symptoms should last greater than one month. Involvement of the skin, eyes, and cardiovascular system are seen, but have not been reported with campylobacter-associated disease. If suspected, subjects will be evaluated by a Board-certified rheumatologist. Procedures will be established prior to the study to ensure timely evaluation and management of any subject with possible post-infectious reactive arthritis. There is no specific diagnostic test. HLA-B27 is present in in 30-50% of those with reactive arthritis and is an exclusionary criterion for this study.

Guillain-Barré syndrome (GBS): The diagnosis of GBS requires progressive weakness of the arms and legs and areflexia. The diagnosis of GBS is supported by symmetric and progressive symptoms, recovery beginning 2 to 4 weeks after progression ceases, mild or no sensory symptoms, cranial nerve involvement, autonomic dysfunction, absence of fever at the onset, elevated cerebrospinal fluid (CSF) protein with < 10 white cells, and typical electrodiagnostic features. If suspected, subjects will be evaluated by a Board-certified neurologist. Procedures will be established prior to the study to ensure timely evaluation and management of any subject with possible post-infectious GBS. The development of GBS will be reported as a SAE.

9.4 Pharmacokinetics

The serum levels of VHH will be evaluated on the following time schedule: before the first dose; 2 ± 1 hours after the first dose; Day 4, Day 7, Day 10, and Day 24.

Anti-drug antibodies: Serum will be collected at baseline and Day 24 for potential determination of the presence of anti-VHH antibodies. Anti-VHH antibody determinations will be performed if measurable systemic absorption of VHH is demonstrated. If anti-drug antibodies are confirmed, additional testing on the samples may be performed (e.g., titer and neutralizing antibodies) to further understand the process.
Exploratory biomarkers: Biomarkers in the stool may be explored (e.g., fecal total protein, fecal calprotectin, fecal lipocalin, fecal myeloperoxidase, fecal neopterin, fecal lactoferrin, fecal cytokines and/or fecal microbiome) as well systemic inflammatory responses (e.g., serum C-reactive protein). Serum, plasma, whole blood, and/or stool may be banked for future research evaluation.

10. Investigational product

10.1 Investigational product

The investigational product, LMN-101 and placebo, will be supplied by Lumen Bioscience, Inc. (Seattle, Washington, USA). LMN-101 is VHH-derived binding protein designed to bind and inhibit FlaA (a flagellin filament protein of *C. jejuni*), delivered in whole, spray-dried, spirulina biomass.

The LMN-101 drug substance is manufactured using a stable spirulina cell line that was engineered to constitutively express the VHH protein aa682 under cGMP using recombinant DNA processes and controls. The cells are cultured using chemically defined, animal product-free basal medium under photoautotrophic growth conditions, utilizing bicarbonate and CO_2 as the carbon source. After

harvesting and rinsing with a dilute solution of trehalose, cells are spray dried to produce drug substance. The drug substance is fully tested against established and validated specifications prior to release for further processing and use in clinical studies. Spirulina drug substance is produced in the identical manner except for lacking the VHH protein.

LMN-101 drug product is manufactured at Lumen's cGMP manufacturing facility in Seattle, Washington USA. The drug substance is provided in pre-formulated capsules. The LMN-101 drug product is tested for potency and purity defined by established and validated specifications. Lumen's cell banking protocols are designed to comply with ICH guideline Q5D for microbial cells. Spirulina cells expressing aa682 (or wild-type spirulina without aa682) are cultured indoors in photobioreactors enclosing disposable polyethylene bags. Spirulina's preferred growth regimen (high pH, high salinity, high temperature, and absence of any exogenous energy source other than light) suppresses the growth of contaminants and thus safely allows high production rates under sanitary conditions. The collected biomass is rinsed by a dilute solution of trehalose and collected by filtration. The resulting paste is spray dried to produce a fine powder. The material is dried to less than 6% moisture and is stored at room temperature in a sealed container. For this study, doses of LMN-101 will be delivered as a spray-dried, trehalose-stabilized spirulina powder with or without VHH in size 00 white, opaque, capsules without additional excipients. Doses of placebo will be delivered as identical-appearing cornstarch NF with coloring in size 00, white, opaque, capsules. No capsules contain any materials of human or animal origin.

10.2 Dosing regimen of investigational product

Subjects will receive LMN-101, 3000 mg (taken as six LMN-101 500-mg capsules each containing 500 mg of spirulina-VHH, strain SP1182) orally three times daily for 14 days (n=21 subjects), or identical-appearing placebo (n=21 subjects).

10.3 Dispensing of investigational product

The investigational pharmacy will dispense LMN-101 and/or placebo according to the Pharmacy Manual. All will be identical in appearance and dispensed according to the subject's treatment number. Dispensing packets, with appropriate labels will be generated for each individual subject. The process for preparing LMN-101 and placebo, as well as blinding and packaging, is detailed in Pharmacy Manual.

10.4 Dosage form of investigational product

LMN-101 ingredients are spray-dried spirulina biomass (with the aa682 protein) and trehalose. Placebo is identical-appearing, starch-based powder. LMN-101 and placebo doses are packaged in size 00, white, opaque capsules (Capsugel, Morristown, NJ, US). No capsules contain any materials of human or animal origin. While in unit, the IP doses will be co-administered with 50 mL of sodium bicarbonate solution.

10.5 Route of administration of investigational product

LMN-101 and placebo must be administered orally and may be taken with fluids or food. While in unit, doses will be co-administered with 50 mL of bicarbonate buffer. Bicarbonate buffer consists of 2 g USP-grade sodium bicarbonate in a total of 150 mL water. Subjects will drink 50 mL of buffer, followed approximately 1 minute later by IP. The IP may be taken with additional water. Bicarbonate buffer will be provided for subjects for doses post discharge. Subjects will be encouraged to drink 50 mL of buffer, followed approximately 1 minute later by IP.

10.6 Timing of dosing of investigational product

Capsules are to be self-administered by the subject three times daily. The subject will be provided a diary by the research clinic to record study drug administration, date, and time.

10.7 Packaging and labeling of investigational product

Investigational product will be provided in individual dose packets, labeled appropriately. While not in unit, subjects will be provided the requisite number of doses of study drug supply until their next scheduled study visit as detailed in the Pharmacy Manual. Product labeling states:

> **CAM02 Investigational Drug LMN-101 500-mg capsules – ACTIVE or Placebo, 6 capsules per container**
> For oral use only, Take as directed
> Lot No: LMN-101-500mg XXXXXXX (blinding code)
> Expiration Date: MONTH YEAR Store at 15-25° C (59-77° F)
> Manufactured by Lumen Bioscience, Inc.
> 1441 North 34th Street, Suite 300 Seattle, WA 98103 USA
> Tel: +1 (206) 899-1904
> Caution—New Drug--Limited by United States law to investigational use only
> Keep out of reach of children

10.8 Storage and handling of investigational product

All Investigational Products will be kept in a locked area with limited access. Capsules containing investigational product should be stored at room temperature, 59-77°F (15-25°C) and protected from moisture, light, and extreme heat during storage. Capsules contain no preservatives. Any damaged or partially used capsules should be returned to the investigational pharmacy and discarded using appropriate drug disposal procedures and documented.

Subjects should be instructed to maintain investigational product away from excessive heat (104°F [40°C]) and moisture.

Only subjects enrolled in the study may receive the investigational product, and the challenge strain, in accordance with all applicable regulatory requirements. Upon the completion of the study, this material will be subjected to final inspection and reconciliation. At that time, all unused, partially used, and fully used (empty) container(s) along with a packing slip must be destroyed as per Pharmacy Manual. Documentation of destruction will be retained by the investigational pharmacy with the study files.

10.9 *C. jejuni* challenge strain CG8421

A human challenge model for studies investigating *C. jejuni* has already been established. It uses the strain *C. jejuni* CG8421. In addition to mitigating the risk of GBS by lacking ganglioside mimicry, the CG8421 strain model has an acceptable safety profile, with a consistently high attack rate and robust immune responses when using a low concentration of inoculum (5×10^5 CFUs). When tested previously, clinical disease after CG8421 infection consisted of moderate-to-large volume diarrhea with symptoms of headache, myalgia, and abdominal cramping. Fever occurred in 39-53% of subjects depending on study and cohort. There were no significant clinical differences between individuals who received 10^6

and 10^5 CFUs of *C. jejuni*, although subjects who received the lower dose had a slightly longer incubation period and a lower total volume of loose stools (Tribble *et al.* 2009). There were no serious adverse events related to CG8421 and no hypotension/shock or post-infectious sequelae. After initiation of antibiotics in subjects, symptoms resolved, and stool cultures cleared rapidly. However, after receiving ciprofloxacin monotherapy, 4% of subjects developed recrudescent infection, despite prior resolution of symptoms and clearance of CG8421 from stool cultures. Due to this, the antibiotic regimen was increased to combination azithromycin and ciprofloxacin for 5 days based on recommendations of an independent Expert Advisory Committee. Despite this, in the most recent study the recrudescence rate was 18% (Rimmer *et al.* 2018). Shedding was eliminated with further dual antibiotic therapy (ciprofloxacin and azithromycin for 10 days).

The challenge model that will be used for this study will use 5 x 10^5 CFU of *C. jejuni* CG8421.

10.10 Preparation of challenge strain

The *C. jejuni* challenge strain underwent cGMP production at Charles River Laboratories (358 Technology Drive, Malvern, PA 19355, 610-640-4550) in October 2006. A Master Cell Bank (MCB) was prepared from the research seed upon release of the strain. The cGMP cell bank was released based on the criteria outlined in Table 4.

Table 4: Release Criteria for C. jejuni cGMP MCB

Parameter	Methodology	Specification
Viability	Plating on Mueller-Hinton agar, incubation in a jar with campylobacter-specific gas mix at 37°C for 48 h.	≥ 10^6 CFU/vial
Purity	Plating on the appropriate selective agar to include sheep blood and Sabouraud-Dextrose agar. Incubation of plates under aerobic conditions, 37°C 18-24 h.	No contaminating organisms on agar plates
Identity	16S RNA analysis and *C. jejuni*-specific PCR	*C. jejuni*

10.11 Packaging and labeling of challenge strain

The CG8421 challenge strain is stored as 1-mL aliquots in 2-mL cryostorage tubes (1 mL per tube) held at -80°C ± 10°C in the WRAIR Pilot Bioproduction Facility (PBF). The cryotubes are labeled as shown in Figure 2.

Campylobacter jejuni strain CG-99-8421 CRL # 498006-1

Mfg. Date 07 Oct 06 Vial # xxx of 210 Store at -70 + 10°C

Prepared by Charles River Laboratories Malvern PA

Figure 2: Cryotube labels

10.12 Storage and transportation of challenge strain

The vials will be transferred on dry ice from the WRAIR Pilot Bioproduction Facility to CVD Microbiology Laboratory, logged in, and stored at -80°C ± 10°C in a locked and temperature-monitored ultra-low temperature freezer. Any use of these vials will be done under the supervision of qualified and trained CVD Microbiology Laboratory personnel and tracked in a strain accountability log. Any vials remaining at the end of the study will be returned to NMRC or disposed by autoclaving.

10.13 Preparation, administration, and inoculum size of challenge strain

The preparation of the challenge strain is outlined in "ED_CampyRif01_SSP402" version 1.0 November 3, 2014, "Site-Specific Procedure (SSP)". An appropriate number of master seed vials that have been stored at -80°C ± 10°C in Mueller-Hinton broth with 15% glycerol will be thawed at room temperature. One hundred microliters of the master seed lot will be spread onto an appropriate number of Mueller-Hinton agar plates for confluent growth and incubated overnight (21 ± 1 hour) at 40 ± 2°C under microaerobic conditions achieved using a BBL CampyPak Plus (BD). After overnight growth, *C. jejuni* identity will be confirmed by purity testing, morphology, gram stain, oxidase testing, catalase testing, and confirmation of darting motility. Bacterial biomass will be harvested by suspension in sterile PBS (0.01M sodium phosphate, 0.138M sodium chloride, 0.0027M potassium chloride, pH 7.4) and adjusted to the appropriate OD600 to achieve the target infectious dose. Challenge inoculum will be verified by enumeration of viable counts on Mueller-Hinton agar and Campy CVA in triplicate on the sample both the pre- and post-dosing (the mean of the pre- and post-dose CFU will be used for the estimated inoculum size). Purity will be determined by plating the challenge inoculum on sheep blood agar and incubating 2 plates at 37°C and 2 plates at 42°C for 24-48 hours under aerobic conditions.

C. jejuni CG8421 will be administered with bicarbonate buffer (2 g in a total of 150 mL). Study participants are fasted for at least 90 minutes before and 90 minutes after challenge. At challenge, study participants will drink 120 mL of buffer, followed approximately 1 minute later by 30 mL of buffer containing CG8421. The CG8421 will be added to the bicarbonate immediately before dosing.

10.14 Antibiotics

Ciprofloxacin, 500-mg tabs, and azithromycin, 500-mg tabs, will be supplied through the investigational pharmacy for oral administration for 5 days (daily for azithromycin, twice daily for ciprofloxacin). If the study physician determines that alternative or IV antibiotics are required, these will be supplied through the pharmacy. This five-day course of antibiotic therapy was recommended by an Expert Advisory Committee.

10.15 Assessment of subject compliance with investigational products

A member of the study team will witness the ingestion of the first dose of investigational product (IP). The subject will be given a diary to take home to document subsequent doses of the IP. While in unit, the subject will be administered the IP, challenge, and antibiotics under direct observation. If subject is to complete the antibiotic course at home, then the subject will be questioned regarding compliance at the next study visit and diary reviewed.

10.16 Missed doses of LMN-101 or placebo

Any missed doses of IP during the non in unit portion will be documented and the subject will be asked to bring in the medicine container and untaken pills. The returned doses will be documented in the subject chart, the bottles will be tagged to be returned to the investigational pharmacy, and documentation maintained in the study binder for drug accountability.

Any subject missing the two doses of IP immediately prior to challenge will not be eligible for challenge. Missed dose(s) will be documented along with the reason for the missed dose.

If a dose is refused due to illness during the study, the missed dose will be clearly documented, and the extra dose(s) will be returned to the pharmacy with tracking at the end of the in-unit phase of the study. If a subject vomits within one hour of the dose, attempts will be made to re-dose the subject. This will be clearly documented in the subject's records.

If a subject refuses a dose not due to illness, staff will notify the PI or designee, and a reason will be documented.

11. Safety Management

11.1 Adverse events

The Principal Investigator is responsible for documentation of AEs according to the detailed guidelines below. Subjects will be instructed to contact the Principal Investigator or designee immediately should they manifest any signs or symptoms they perceive as serious during the study period as well as prompt notification for any new onset of gastrointestinal symptoms irrespective of severity (through Day 59). Approximately six months after receiving the *C. jejuni* challenge dose, the subjects will be contacted by phone to document any intervening medically significant new chronic illnesses or serious adverse events. These data will be documented in the subject's source documents and CRFs, then summarized in an appendix to the final clinical study report.

11.2 Definitions

An AE is any untoward medical occurrence or change in a clinical trial subject having received a biologic or medicinal product (trial related) and does not need to have a causal relationship with this treatment. This includes any noxious, pathological, or unintended change in anatomical, physiologic, or metabolic functions as indicated by physical signs, symptoms, and/or clinically significant laboratory abnormalities. This may occur in any phase of the clinical study whether associated with the study product and whether considered product related. This includes an exacerbation or worsening of pre-existing conditions or events; intercurrent illnesses, injuries, or vaccine or drug interaction; or worsening of abnormal clinical laboratory values. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation need not be considered AEs. Discrete episodes or worsening of chronic conditions occurring during a study period should be reported as AEs to assess changes in frequency or severity. Stable, pre-existing conditions and/or elective procedures are not AEs. AEs will be documented in terms of signs and symptoms observed by the Principal Investigator or reported by the subjects at each study encounter, with a medical diagnosis stated. Pre-existing conditions or signs and/or symptoms (including any which are not recognized at study entry but are recognized during the study period) present in a subject prior to the start of the study should be recorded in the Medical History form within the subject's CRF. AEs that occur after informed consent is obtained, but prior to test article receipt, will be documented in the Medical History form within the subject's CRF as instructed by the clinical research monitor.

A serious adverse event (SAE) is any untoward medical occurrence or effect at any dose that results in:

- Death
- Is immediately life threatening

- Results in persistent or significant disability/incapacity
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in congenital anomaly/birth defect in the offspring of a study subject
- Is an important medical event that may jeopardize the individual or may require intervention to prevent one of the other outcomes listed above.

Although not considered SAEs, cancers should be reported in the same way as SAEs.

Pertinent definitions include:

Life threatening:

An AE is life threatening if the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

Disabling/incapacitating:

An AE is incapacitating or disabling if it results in a substantial disruption of the subject's ability to carry out normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhea, influenza, injection site reactions and accidental trauma (e.g., sprained ankle).

Although not an SAE, the FDA has requested that all instances of recrudescence be reported analogous to SAE reporting.

Hospitalization:

In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency department for treatment that would not have been appropriate in the physician's office or outpatient setting. Hospitalization for either cosmetic surgery or elective surgery related to a pre-existing condition which did not increase in severity or frequency following initiation of the study, or for routine clinical procedures that are not the result of an adverse event, need not be considered as AEs and are therefore not SAEs. Since this is an in-unit study, the planned in unit period is not to be recorded as a SAE. An extension of the planned hospitalization due to a potentially life-threatening health condition will be reported as a SAE. An extension of the hospitalization a day or two to fulfill discharge criteria is not considered as a SAE.

Routine Clinical Procedure:

A procedure which takes place during the study and does not interfere with the test article administration or any of the ongoing protocol specific procedures.

If any adverse event is reported during an elective procedure, that occurrence must be reported as an adverse event, either 'serious' or 'non-serious' according to the usual criteria. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE should be considered serious.

11.3 Assessment of severity

Severity of the following adverse events (grades 0-4) should be assessed as defined in Table 5 below to ensure consistency with previous experimental infection studies with other enteric pathogens. Adverse

Events not represented in the below criteria will be coded according to Common Terminology Criteria for Adverse Events (CTCEA), Version 5.0.

Table 5: Adverse Event definitions and parameters for in unit setting

Adverse Event		**Parameter**
Diarrhea	1	2-3 or more grade 3-5 stools in a 48-hour period totalling 200-400 g or more or a single grade 3-5 stool of 300 g in 24 hours
	2	4-5 grade 3-5 stools in 24 hours or 401-800 g of grade 3-5 stools for total episode
	3	> 6 grade 3-5 stools in 24 hours or > 800 g of grade 3-5 stools for total episode
	4	Life-threatening
Body Temperature	1	100.4-101.1°F (38.0-38.4°C)
	2	101.2-102.0°F (38.5-38.9°C)
	3	102.1-104.9°F (39.0-40.5°C)
	4	Life-threatening hyperthermia
Vomiting	1	One episode within any 24-hour period
	2	Two episodes within any 24-hour period
	3	More than two episodes within any 24-hour period
	4	Life-threatening consequence of emesis
1 = mild; 2 = moderate; 3 = severe; 4 = life-threatening		

11.4 Assessment of causality

Every effort should be made to explain AEs and assess causal relationships, if any, to administration of the IP, *Campylobacter jejuni* challenge, treatment antibiotics, or other study procedures. AEs occurring on study days prior to receipt of the challenge inoculum will be assessed as having no relationship with the challenge strain. AEs occurring after receipt of the challenge will be assessed as to their relationship with the IP, *Campylobacter jejuni* challenge strain, treatment antibiotics or other study procedures, if applicable. The degree of certainty with which an AE can be attributed to these products (or alternative causes, e.g., natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the event can be understood in terms of one or more of the following:

- Reaction of similar nature having previously been observed with *Campylobacter jejuni* challenge strains or antibiotic administration
- Published literature accounts supporting causality
- Temporal relationship with administration

The Principal Investigator will separately assess causality of all AEs as either ‘probably related’, ‘possibly related’, or ‘unrelated’ to the IP, *Campylobacter jejuni* challenge strain, treatment antibiotics, and other

study procedures. Non-serious and serious adverse events will be evaluated as two distinct types of events given their different medical nature. If an event meets the criteria for a serious adverse event it will be examined by the Principal Investigator to the extent possible to determine ALL contributing factors applicable to the event.

Other possible contributors include:

- Underlying disease
- Other medication
- Protocol-required procedure
- Other cause (specify)

"Probably related" to IP is defined as evidence to suggest a causal relationship with IP, and the influence of other factors is unlikely. The event occurs within a reasonable time after administration of the study drug, is unlikely to be attributed to other drugs or participant's underlying clinical condition and follows a clinically reasonable response on withdrawal of the study drug. Withdrawal and re-challenge data are not required to fulfill this definition.

"Possibly related" to IP is defined as some evidence to suggest a causal relationship with IP, for example, the event occurred within a reasonable time after administration of the study drug. Other factors may have contributed to the event, for example, other drugs or the participant's underlying clinical condition.

"Unrelated" to IP is defined as an event which has a temporal relationship to study drug that makes a causal relationship improbable, for example, the event did not occur within a reasonable time after administration of the study drug and in which other drugs or participant's underlying clinical condition provides plausible explanation.

11.5 Anticipated adverse events

This study is evaluating a challenge with live *Campylobacter jejuni* bacteria, and therefore all the symptoms of *Campylobacter jejuni* infection are expected with severity grades ranging from 1-3. Serious adverse events and Grade 4 adverse events related to campylobacteriosis will be considered unexpected for the purposes of IND safety reporting. The most common effects of *Campylobacter jejuni* infection are moderate to severe diarrhea, which may lead to dehydration, electrolyte abnormalities and the need for oral or intravenous rehydration, abdominal cramping, and fever. Nausea with or without vomiting, chills, gross blood in stools, loss of appetite, headache, muscle aches, and bloating may also occur. Recrudescent events have been documented in up to 4 -18% of previous subjects receiving this dose.

Adverse events of special interest are not expected due to their low likelihood of occurrence, but they will be monitored. These include Guillain-Barre syndrome, neurodegenerative changes, arthritis, symptomatic uveitis, and myocarditis/pericarditis. GBS is thought to not be possible following challenge with the challenge strain due to the absence of the portion of the *Campylobacter jejuni* that cross reacts with neurologic tissue.

Fluoroquinolones, including ciprofloxacin, are associated with an increased risk of tendinitis and tendon rupture in all ages. The risk of developing fluoroquinolone-associated tendinitis and tendon rupture is

further increased in older individuals usually over 60 years of age, in individuals taking corticosteroid drugs, and in individuals with kidney, heart, or lung transplants, all of whom are excluded from this study.

11.6 Out of unit adverse events

Potential symptoms associated with the IP and study participation will be solicited from subjects using a Diary Card.

Additionally, subjects will be asked daily about number of stools and if abnormal. If stool is reported as abnormal the clinic staff will then ask the subject questions to determine the grade of the stool and assess for diarrhea.

- Hematology will be assessed about 7 days after starting the IP.
- Chemistry will be assessed about 7 days after starting the IP.

11.7 In unit participant surveillance

Subjects will continue to receive the IP during the in-unit phase of the study, however, once the challenge is given, the solicited adverse events experienced will be attributed to the challenge.

Expected symptoms of campylobacteriosis will be specifically solicited daily during the in-unit period.

"Specific solicited adverse events" are defined as

- diarrhea,
- fever,
- nausea,
- vomiting,
- abdominal pain,
- abdominal cramping,
- tenesmus,
- mucoid or bloody stools,
- constipation,
- headache,
- lightheadedness,
- fatigue,
- lack of appetite,
- muscle aches,
- chills,
- joint pains

Symptoms that will be assessed objectively include:

- Diarrhea
- Fever
- Dysentery (confirmed by hemoccult testing)

Open-ended questions will also be used to capture any other symptoms during the study period. All AEs should be recorded on the appropriate AE form of the subject's CRF and recorded irrespective of severity or whether or not they are considered related to the IP or challenge inoculum. AEs occurring

after receipt of the challenge (Day 3) will be assessed as to their relationship with the IP, the challenge strain, and the treatment antibiotics (if treatment has started).

11.8 Out of unit surveillance

11.8.1 Discharge to Day 59

Subjects will continue taking their assigned IP through the third dose on Day 14. Assessment of specific solicited AEs will continue through Day 24. Assessment of AEs will continue through Day 59. Subjects will have memory aids or diaries to help them capture adverse events through Day 24.

11.8.2 Day 59 to Day 190

Additionally, subjects will be educated to telephone the investigative site if they experience any symptoms of diarrhea (3 loose stools/24 hours) or fever ≥ 38°C (100.4°F) with any associated severe symptoms (nausea, abdominal cramps, vomiting, myalgia, headache, arthralgia, or gross blood in their stool). Clinical staff will solicit at each visit any clinically apparent signs or symptoms of SAEs or AESIs (Guillain-Barre syndrome (GBS), neurodegenerative changes, arthritis, uveitis, and myocarditis/pericarditis).

11.9 Recording adverse events

A study investigator will evaluate all AEs either observed by the Principal Investigator or one of his/her clinical collaborators or reported by subjects spontaneously or in response to a direct question. Specific solicited adverse events (defined as diarrhea, fever, nausea, vomiting, abdominal pain, abdominal cramping, tenesmus, mucoid or bloody stools, constipation, headache, lightheadedness, fatigue, lack of appetite, muscle aches, chills, and joint pains) will be recorded through Day 24. The AE recording period is from Day 1 through Day 59. SAEs and AESIs will be collected through Day 190. The nature of each event, date of onset, outcome, severity, and relationship to the challenge strain and/or antibiotic should be established. Details of any symptomatic/corrective treatment should be recorded on the appropriate page of the eCRF. Subjects should be asked non-leading questions initially when soliciting AEs, followed by more direct questions as necessary. AEs already documented in the eCRF, i.e., at a previous assessment, should be reviewed at subsequent follow-up assessments and updated as necessary. If an AE changes significantly in frequency or intensity during a study period, a new record of the event will be started. Each solicited symptom/sign will be recorded separately as an AE. In some instances, the PI may classify some clinical syndromes as AE, rather than the individual signs and/or symptoms.

11.10 Follow-up of adverse events and assessment of outcome

Outcome should be assessed as:

- Resolved
- Resolved with sequelae
- Severity change
- Ongoing at study conclusion
- Died
- Lost to follow up

Investigators should follow-up all AEs until Day 59 this may include repeat safety laboratory analysis. At the clinical visits on Day 87, and Day 115, and the telephone call on Day 190, the Principal Investigator will follow the subject as outlined assess for the onset of SAEs and AESIs.

11.11 Reporting serious adverse events

All SAEs must be reported immediately to the sponsor by the Principal Investigator, whether or not regarded as possibly attributable to the test articles, antibiotics, *Campylobacter jejuni* challenge strain, or other study procedures. Serious adverse events and Grade 4 adverse events related to campylobacteriosis will be considered unexpected for the purposes of IND safety reporting. Any unexpected fatal or life-threatening suspected adverse reaction(s) will be reported to the FDA by the Sponsor. SAE reports will be provided to the Sponsor, Medical Monitor, and the IRB (IntegReview IRB); contact information is provided on cover pages. The Principal Investigator must report SAEs within 24 hours of becoming aware of the event by telephone or e-mail (if appropriate) to the study contacts as described in the protocol. This initial notification should include minimal, but sufficient information to permit identification of the reporter, the subject, the test articles, SAEs, and date of onset. The Principal Investigator should not wait for additional information to fully document the event before notifying. The report is then to be followed by submission of a completed SAE Report Form provided by the Sponsor as soon as possible but not more than 3 working days past the initial report, detailing relevant aspects of the SAE in question. All Principal Investigator actions and event outcomes must also be reported immediately.

Related and unanticipated SAEs or related severe (grade 3 or greater) unanticipated AEs will be sent to the IRBs and Sponsor within 24 hours. SAE Report Forms are to be used for documentation of these various aspects regarding the event. Hospital records and autopsy reports should be obtained if applicable.

11.11.1 Sponsor notification by Principal Investigator

The Principal Investigator must report all SAEs immediately (within 24 hours of identification), whether or not the event is considered related to the study product, and provide to the Sponsor the following information via email (preferred) or telephone:

- Protocol IND number, investigational product, Principal Investigator name, and contact number
- Subject identification number and product blinding code from IP label
- Serious adverse event, onset date, date of investigational product administration, severity, relationship to the test articles, antibiotics, *Campylobacter jejuni* challenge strain, or other study procedures, and subject's current status

AND

Email the following documents to Medical Monitor, Lumen Bioscience:

- Cover sheet
- Adverse Event Case Report Form
- Supplemental Serious Adverse Event Report Form
- Concomitant Medication Case Report Form or a list of concomitant medications

- Medical record progress notes including pertinent laboratory/diagnostic test results

The Principal Investigator will assess all SAEs as being either related or unrelated to the administered product. The Sponsor contact is noted on the cover page of this protocol.

The Principal Investigator must report these additional immediately reportable events within 24 hours of identification to the Sponsor:

- Any withdrawal of consent during the study
- Pregnancy or intent to become pregnant
- A protocol deviation that jeopardizes the safety of a subject or scientific integrity of the study

11.11.2 Institutional Review Boards

Unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and all subject deaths should be promptly reported by phone or email to the IntegReview IRB. A written report will follow the initial notification.

Investigators are required to forward safety information provided by the Sponsor's representative to the IRB. All SAEs will be reported to the IRBs within 24 hours of the Principal Investigator becoming aware of the event.

Table 6: Contact Information

IRB	Telephone/Fax	Email	Address
IntegReview IRB	(512) 326-3001 (512) 697-0085	vgovea@integreview.com	3815 S. Capital of Texas Hwy Suite 320 Austin, TX 78704

11.11.3 FDA notification by Sponsor

The Sponsor will be responsible for notifying the FDA of any unexpected fatal or life-threatening suspected adverse reactions 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 the 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.

In each IND safety report, the Sponsor must identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information. The Sponsor must report any suspected adverse reaction that is both serious and unexpected. The Sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between a drug (including antibiotics) and the adverse event, such as: (A) A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome); (B) One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture); (C) An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events

that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in a drug treatment group than in a concurrent or historical control group.

11.12 Follow-up of serious adverse events

All SAEs must be documented and followed until the event either resolves, subsides, stabilizes, disappears, or is otherwise explained, or the subject is lost to follow-up, but not longer than 6 months after the last receipt of test article. All follow-up activities have to be reported, if necessary, on one or more consecutive SAE report forms, in a timely manner. All fields with additional or changed information must be completed and the report form should be forwarded to the study contact for reporting SAEs as soon as possible, but not more than 7 calendar days after receipt of the new information. Clinically significant laboratory abnormalities will be followed up until they have returned to normal or until stable. Reports relative to the subsequent course of an AE noted for any subject must be submitted to the Sponsor. The outcome of SAEs should be assessed in the same manner as all AEs.

11.13 Treatment of adverse events

Treatment of an AE is determined by the Principal Investigator and according to the best treatment currently available. The applied measures should be recorded in the CRF for the subject.

11.14 Discontinuation/withdrawal from study

Based on prior experience with *C. jejuni* challenge studies, it is expected that some subjects will have severe (grade 3) AEs (such as severe diarrhea) but not grade 4.

AEs which will prompt stopping the investigational product administration for all subjects and review include:

- Any individual experiencing any SAE considered at least possibly related to the investigational products; or
- If two or more subjects experience the same or similar unanticipated adverse event that is Grade 3 or higher; or
- If any subject experiences an AESI (GBS, uveitis, arthritis, or myocarditis/pericarditis); or
- If the accumulation of SAEs and/or severe AEs collectively raises a safety concern in the opinion of the PI, Medical Monitor, or Sponsor.

This does not include two or more subjects experiencing anticipated severe (grade 3) AEs (such as severe diarrhea, abdominal cramps, fever, or other symptoms included in the clinical definition of campylobacteriosis) specifically during the in-unit period (but specifically DOES include all grade 3 AEs that occur post clinical research center discharge).

The decision to restart will be made by consensus of a review committee comprised of the Independent Medical Monitor, the sponsor's medical expert, and the Principal Investigator. The decision to stop the study will be communicated to the IRB and, similarly, the decision to re-start a study will also be communicated to the IRB.

AEs which will prompt an Independent Medical Monitor review include:

- If one or more subjects experiences a moderate-to-severe symptomatic recrudescence.

Administration of the investigational product will be discontinued for any subject that develops:

- A serious adverse event; or
- An adverse event that is Grade 3 or higher and considered at least possibly related to the investigational product (excluding in-unit events related to campylobacteriosis as described in the protocol); or
- The Principal Investigator deems that stopping the investigational product administration is in the best interest of the subject.

Additional reasons for individual subject withdrawal include:

- The subject does not wish to continue with the study; or
- The subject is lost to follow-up.

Withdrawal/stopping of the trial due to these last two points, if not AE related, does not need to be reported other than as part of routine annual reporting to the FDA and IRBs.

11.15 Right to terminate the study

Both Lumen Bioscience and the Principal Investigator have the right to terminate this study at any time, and to arrange an appropriately agreed upon schedule for termination, if necessary. This information will be provided to each subject or legally authorized representative during the informed consent process.

12. Statistical considerations

12.1 Statistical hypothesis

The primary endpoint of the study is to compare the frequency of solicited and unsolicited adverse events in subjects that received LMN-101 compared to placebo. Event rates will be reported for the two treatment arms, but no statistical hypothesis tests will be performed for the primary endpoint due to the uncertain frequency of AEs in the placebo arm and the anticipated low rate of AEs in the treatment arm.

The secondary endpoint is to compare LMN-101 to placebo in the proportion of subjects with campylobacteriosis after challenge with *Campylobacter jejuni* strain CG8421. The null hypothesis, that the risk ratio for campylobacteriosis (LMN-101 / placebo) is one, will be tested against the 2-sided alternative at the 0.10 level of significance.

12.2 Sample size considerations

The sample size for this study was selected to be large enough so that an adverse event that occurs with 5-10% frequency with LMN-101 administration in the context of *Campylobacter jejuni* exposure is likely to be observed in the study. However, practical and ethical considerations for this human challenge study were also important in limiting the size of the study. With 21 subjects receiving LMN-101, there is a 66% probability that an adverse event expected to occur in 5% of the population would occur in the

study. An event with a 10% rate in the population is 89% likely to occur among the 21 LMN-101 subjects in the study.

While the sample size was not selected to provide a specific amount of statistical power to detect a statistically significant difference between the two treatment groups, assuming 18 subjects per treatment group complete the study, there is 80% power to detect a large risk reduction in attack rates (e.g., from 80% in the placebo group to 40.6% in the LMN-101 group) at the one-sided significance level of 0.05.

12.3 Population

The Safety Analysis Set will be based on all subjects who received study treatment (LMN-101 or Placebo). Subjects will be analyzed according to the treatment they received, even if this differs from the treatment they were randomized to (an as-treated approach).

The Efficacy Analysis Set will be based on all subjects who received study treatment and complete the *Campylobacter jejuni* challenge without significant protocol deviations (a per protocol approach). Subjects will be analyzed according to the treatment they received, even if this differs from the treatment they were randomized to.

12.4 Statistical Analyses

12.4.1 General Considerations

Continuous variables will be summarized by treatment group with mean, SD, median, minimum, and maximum (or geometric mean and geometric CV% for log-transformed variables). Frequency distributions of categorical variables will be presented by treatment group.

Inferential statistics will not be used in the analysis of safety variables but will be applied to the secondary and exploratory efficacy endpoints using 1-sided tests of significance at the 0.05 level of significance and/or two-sided 90% confidence intervals. It is expected that the study will be enrolled as two separate cohorts, and cohort will be used as a stratification factor in statistical tests when applicable.

The study analysis will be conducted using SAS, v9.4 or higher.

12.4.2 Safety Analysis

The analysis of safety and tolerability will be completed on the safety analysis set. Safety will be evaluated from reported AEs, changes in clinical laboratory values, changes in vital signs and changes in ECG parameters.

Safety data, including AEs, vital signs, ECG, and laboratory tests, will be listed by study subject.

The incidence of all reported AEs and treatment-related AEs will be tabulated by treatment group. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and severity graded according to Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. An AE will be considered treatment emergent if it occurs or worsens after the first dose of randomized study treatment.

Frequencies and percentages of subjects with treatment emergent AEs and SAEs will be summarized by MedDRA preferred term, system organ system class, severity, and relationship to study drug. In the

event of multiple occurrences of the same AE with the same preferred term in the same subject, the AE will be reported as the number of AEs with the AE counted only once. Treatment emergent AEs leading to death and causing premature discontinuation of study drug will also be summarized.

Campylobacter jejuni challenge-related solicited adverse events, including diarrhea, fever, nausea, vomiting, abdominal pain, abdominal cramping, tenesmus, mucoid or bloody stools, constipation, headache, lightheadedness, fatigue, lack of appetite, muscle aches, chills, and joint pains, will be monitored from the time of challenge through Day 24. These will also be summarized by treatment group.

Monitoring for Adverse Events of Special Interest (AESI) after *Campylobacter jejuni* challenge will include clinically apparent signs or symptoms of Guillain-Barre syndrome (GBS), neurodegenerative changes, arthritis, uveitis, and myocarditis/pericarditis at each of the non in unit visits, and during the final phone call.

Clinical laboratory results and changes from baseline in clinical laboratory results will be summarized by treatment group. Shift tables will be constructed to tabulate categorical shifts in laboratory values from baseline to the minimum and maximum post-baseline values by treatment group. Categories will be based on the laboratory normal ranges and CTCAE Grade 3 toxicity thresholds.

Vital signs and ECG parameters, and changes from baseline in vital signs and ECG parameters, will be summarized by treatment group. Additionally, QTc measures (Fridericia's formula) will be summarized in the following categories:

1. QTc >450 to ≤480 msec
2. QTc >480 to ≤500 msec
3. QTc >500 msec
4. QTc increase of >30 and ≤ 60 msec from baseline
5. QTc increase of >60 msec from baseline

Serum will be collected at baseline and Day 24 for presence of anti-VHH antibodies. Anti-VHH antibody determinations will be performed and summarized if systemic absorption of VHH is demonstrated.

Tolerability will be assessed by the proportion of participants completing study drug and remaining on study (other than subjects dropping off for reasons other than study drug) and free from possibly drug-related and dose-limiting SAEs to the end of Day 59.

12.4.3 Analysis of Efficacy

The planned statistical evaluation of efficacy will compare the prospectively defined clinical and microbiological endpoints of campylobacteriosis rate and severity, diarrhea output (weight), severity, dysentery (presence/absence), and fever between treatment groups.

Campylobacteriosis will be summarized using frequencies and percentages by treatment group as well as 90% confidence intervals around the percentages. The percentage of subjects with campylobacteriosis will be calculated for each treatment group using the standard definition of: (# with endpoint / # receiving inoculum) x 100%. The campylobacteriosis risk ratio (LMN-101 / placebo) will be presented. The risk ratio will be tested using the method of Cochrane-Mantel-Haenszel stratified by

enrollment cohort to determine if the risk ratio is statistically significantly different from 1; similar methodology will be used to construct a 90% asymptotic confidence interval around the risk ratio.

Summary tables will also be created to detail quantitative and temporal features of the illness such as diarrhea stool frequency and volume, maximum temperature observed, and time to illness.

The median duration of any campylobacteriosis clinical symptoms, diarrhea, and dysentery will be estimated with Kaplan-Meier survival analysis methodology and compared between treatment groups using the log-rank test.

The geometric mean total weight of loose stools will be calculated for each treatment group and compared using an analysis of variance model with terms for treatment group and enrollment cohort. Subjects with zero loose stool output will be imputed as the logarithm of half the smallest quantifiable output.

The median, 25th percentile and 75th percentile of the maximum stool grade for each subject and the *Campylobacter jejuni* severity scale will each be calculated and compared between treatment groups using the Cochran-Mantel-Haenszel row mean score test stratified by enrollment cohort.

Rates of recurrence of *Campylobacter jejuni* stool shedding and campylobacteriosis clinical symptoms will be calculated and compared between treatment groups using the Cochrane-Mantel-Haenszel test stratified by enrollment cohort.

Stool and blood biomarkers may also be summarized in a tabular format and graphed to demonstrate kinetics of response. Qualitative (responder rates) and quantitative assessments (log-transformed values) will be analyzed. All statistical tests will be interpreted in a two-tailed fashion using $p < 0.10$ to represent a significant difference.

12.4.4 Planned Interim Analysis

The initial cohort will be comprised of 21 subjects. After the initial cohort has completed the in-center portion of the study, an unblinded statistician will perform an interim analysis to allow a data safety monitoring board to determine if the trial should continue based on safety.

13. Data handling and recordkeeping

The primary source document for this study will be the subject's phase one unit's chart. The Principal Investigator will maintain complete and accurate documentation for the study, including any medical records provided by potential participants, records detailing the progress of the study for each subject, laboratory reports, eCRFs, signed informed consent forms for each study subject, drug disposition records, correspondence with the phase one unit's IRB, the study monitor and the Sponsor, adverse event reports, and information regarding subject discontinuation and completion of the study. All required study data will be clearly and accurately recorded by authorized study personnel in the eCRFs. Only designated study site personnel shall record or change data in an eCRF. The Principal Investigator will be responsible for the procurement of data and for quality of data recorded in the eCRFs. Original observations entered directly into the eCRFs are considered source data. Study-specific procedures detail how each form will be completed. The study coordinator will ensure accuracy of the case report forms. All source documents will be retained at the site.

For this study, the phase one unit will utilize the Medidata Rave data system for the collection of the study data in an electronic format. The data system, as implemented by the phase one unit, will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CRF Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the electronic data system. The applicable source data will be electronically transcribed by the site designee onto the eCRF (data entry screens) in the database system. The Principal Investigator is ultimately responsible for the accuracy of the data transcribed on the eCRF. Data monitoring and management will be performed in the electronic data system by the clinical research associate and the designated data management group.

A detailed data management plan will be written and approved by the study team and the PI. The plan will be drafted prior to study initiation but will be finalized before study close-out and database lock.

14. Clinical trial material accountability

The Principal Investigator is responsible for maintaining accurate records of the processing and use of all clinical trial materials, including the receipt, disposition, and return and/or disposal of these supplies. All used, unused, or undispensed study medication must be accounted for by the Principal Investigator.

Accurate records of challenge strain *C. jejuni* preparation will be kept. When the challenge strain is prepared for the study, the batch/lot number, date used, strain characteristics, and viability profile will be recorded. Colony forming units per mL of suspension will be determined for the challenge strain before and after administration to eligible study subjects.

Drug accountability will be conducted during monitoring visits to ensure appropriate receipt, storage, dispensing, and documentation of returned Clinical Trial Materials.

15. Quality control (QC)/quality assurance (QA)

15.1 QC/QA monitoring

During the study and at the close-out of the study, a monitor from Lumen Bioscience or its representative will have regular contacts with the investigational site, for the following:

- Provide information and support to the Investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, data is being accurately recorded in the CRFs, and investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the CRFs with the subject's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each subject (e.g., clinic charts)
- Record and report any protocol deviations not previously sent to Lumen Bioscience
- Confirm AEs and SAEs have been properly documented on CRFs and confirm any SAEs have been forwarded to Lumen Bioscience or its representative and those SAEs that met criteria for reporting have been forwarded to the IRBs and/or FDA

The monitor will be available between visits if the Investigator(s) or other staff needs information or advice. The PI assumes ultimate responsibility for the conduct of the study and remains readily accessible throughout the duration of the study.

15.2 Audits and inspections

Authorized representatives of Lumen Bioscience, a regulatory authority, or FDA may visit the site to perform audits or inspections, including source data verification. The purpose of a Lumen Bioscience audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization, and any applicable regulatory requirements. The Investigator should contact Lumen Bioscience immediately if contacted by a regulatory agency about an inspection.

The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, subject charts and study source documents, and other records relative to study conduct.

To ensure compliance with GCP and all applicable regulatory requirements, Lumen Bioscience may conduct one or more quality assurance audits.

15.3 Protocol deviation management

Other than minimal-risk changes, all unanticipated major problems involving human subjects or others will be reported promptly to the IRBs, and no such changes will be made to the research without IRB approval unless necessary to eliminate apparent immediate hazards to human subjects. Minor minimal-risk deviations necessitated during the trial will be made on site as needed and documented for subsequent review within a reasonable time period.

Deviations from the protocol that potentially impact on subject safety will be promptly reported to the Medical Monitor, IRBs, and the Sponsor. Other deviations will be reported at the time of continuing review.

15.4 Clinical research monitoring

Lumen Bioscience monitoring responsibilities will be provided by an independent Clinical Research Associate (CRA). Monitoring will be conducted according to an approved monitoring plan, and according to applicable SOPs. The CRA or other authorized representatives of the Sponsor may inspect all documents and records maintained by the Principal Investigator, including, but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subject in this study. The clinical study site will permit access to such records. The Principal Investigator will obtain, as part of informed consent, permission for authorized representatives of the Sponsor, or regulatory authorities, to review, in confidence, any records identifying individuals in this clinical study.

The Principal Investigator will notify the Sponsor within 24 hours following contact by a regulatory agency. The Principal Investigator and study coordinator will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The Principal Investigator will provide the Sponsor with copies of all correspondence that may affect the review of the

current study or his/her qualification as a Principal Investigator in clinical studies conducted by the Sponsor. The Sponsor will provide any needed assistance in responding to regulatory audits or correspondence. The Principal Investigator will permit independent auditors (employees of the Sponsor or an external company designated by the Sponsor) to verify source data validation of the regularly monitored clinical trial. The auditors will compare the entries in the CRFs with the source data and evaluate the study site for its adherence to the clinical study protocol and GCP guidelines and applicable regulatory requirements. The Sponsor will arrange local monitoring prior to beginning, at initiation, during the study, and at closeout by the study monitor or designee.

15.5 Medical Monitor (Sponsor)/Independent Medical Monitor

The Medical Monitor shall be available for consultation with the Principal Investigator and serves as liaison between the clinical study site and the Sponsor. The study investigators will consult with the Medical Monitor on issues related to subject enrollment and continued participation as needed. The Medical Monitor is required to review all unanticipated problems involving risk to subjects, SAEs, and all subject deaths associated with the protocol, and provide an unbiased written report of the event. At a minimum, the Medical Monitor should comment on the event outcomes, and in the case of a SAE or death, comment on the relationship to participation in the study. The Medical Monitor should indicate concurrence or non-concurrence with the details of the report provided by the Principal Investigator. Reports for events determined by either the Principal Investigator or Medical Monitor to be related or unrelated to participation and reports of events resulting in death should be promptly forwarded to the IRBs.

In addition to the Sponsor's Medical Monitor, an Independent Medical Monitor will function as an independent safety advocate for subjects per Army Regulation 70-25 and DoD Instruction 3216.02. An Independent Medical Monitor is required to review all unanticipated problems involving risk to subjects or others, SAEs, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the Independent Medical Monitor should comment on the outcomes of the event or problem and, in the case of an SAE or death, comment on the relationship to participation in the study. The Independent Medical Monitor should also indicate whether he or she concurs with the details of the report provided by the Principal Investigator. Reports for significant events determined by either the Principal Investigator or Independent Medical Monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the IRB.

16. Regulatory, ethical and study oversight considerations

16.1 Institutional Review Board

The PI must verify that the IRB has approved the clinical protocol, Informed Consent Form, and recruitment materials for the investigation prior to conducting study evaluations. Initial IRB approval, and all materials approved by the IRB for this study including the Informed Consent Form and recruitment materials, must be maintained by the Investigator and made available for inspection.

All amendments to the protocol, consent form, and/or questionnaires, including a change of PI, will be submitted to the IntegReview IRB for review and approval prior to implementation. The PI is responsible for informing the IRB of any amendment to the protocol.

The PI is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. Lumen Bioscience will provide this information to the PI.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB according to FDA regulations and guidelines.

16.2 Written informed consent

The PI or research staff designee will ensure that the subject or legally authorized representative is given full and adequate oral and written information about the nature, purpose, possible risks, and potential benefits of the study. The subject or legally authorized representative must also be notified that the subject is free to discontinue from the study at any time. The subject or legally authorized representative should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent, or the signed and dated informed consent of a legally authorized representative, must be obtained before conducting any study procedures. The PI must maintain the original, signed Informed Consent Form. A copy of the signed Informed Consent Form must be given to the subject or legally authorized representative.

16.3 Subject risk-mitigation strategies

Subjects will be questioned and examined daily for evidence of infection and diarrhea complications. Vital signs will be recorded at least three times per day. Based on prior studies, infected subjects tend to develop illness with incubation periods of approximately 1-3 days. Therapeutic benefit seems to be optimal if treatment is given within the first three days of symptom onset.

The risk of diarrhea complications will be minimized by a conservative approach to timing of antibiotic administration — well within an interval that has been shown to be efficacious — and daily clinical monitoring. Stool output will be closely monitored. All subjects will be treated no later than Day 6 post-dosing. The subjects will be monitored for evidence of relapsed *Campylobacter jejuni* infection by clinical symptoms and repeat stool cultures on Days 14, 21, 28, 35, 56, and 84.

Aggressive fluid management will be undertaken to ensure the most common complication, dehydration, does not occur. The procedures to institute early oral and/or intravenous rehydration therapy are detailed in section 5.11. In addition to rehydration therapy, prospectively defined criteria and procedures to institute early antibiotic therapy are also fully described above. To ensure clinical resolution and limit the potential for secondary spread upon discharge, predefined discharge criteria have been established. Subjects will be discharged from the clinical research center phase of the study when they have had two days of antibiotics, clinical symptoms are resolved or resolving, and two consecutive stool cultures ≥ 12 hours apart are negative for *C. jejuni*.

Systemic or severe gastrointestinal complications rarely occur with *Campylobacter jejuni* infection. The following clinical findings necessitate immediate consideration and management of complicated enteritis:

- Physical examination compatible with an acute abdomen
- Severe GI bleeding (any evidence of GI blood loss other than hemoccult positivity only, with evidence of hemodynamic instability, decrease in hemoglobin, hypovolemia)
- Sepsis (high fever: temperature >102°F (39°C), rigors, hemodynamic instability)

Any of these findings require prompt clinical management and discussion with the Sponsor's Medical Monitor and the Independent Medical Monitor. The Pharmaron inpatient clinical facility is located 3 blocks from the University of Maryland Medical Center. Arrangements are in place for transportation and admission of sick subjects from the inpatient clinical facility to the University of Maryland Medical Center.

The *C. jejuni* strain has the potential for risk to both the environment and to the research personnel. *Campylobacter* spp. are common commensals of many birds and mammals with the usual route of transmission to humans through consumption of contaminated meat, poultry, milk, and occasionally large waterborne outbreaks. Therefore, the risk to the environment regarding potential transmission outside of the clinical research facility is low. There is a minimal risk of acquiring *C. jejuni* infection associated with subject inoculum administration, care activities on the ward, or processing *C. jejuni*-infected stool. The risk to the environment will be reduced by ensuring that all human waste products from in center participants are disinfected with bleach prior to disposal, ensuring all subjects comply with discharge criteria (two consecutive negative stool cultures ≥ 12 hours apart for *C. jejuni*), emphasizing importance of hand washing for subjects and staff, ensuring proper disposal/cleaning of linen, and cohorting subjects in the clinical research facility while shedding *C. jejuni.* Additionally, subjects will not be discharged until they are no longer shedding the challenge strain as per procedures outlined in the protocol.

If a recrudescent infection is detected, the follow-up clock will be reset for the individual who recrudesced. The risk of recrudescent infection will be minimized by the participation of only immunocompetent individuals, the use of an extended duration of concurrent antibiotics (five days), monitoring of clinical symptoms, and frequent evaluation of stool cultures. Follow-up stool cultures after discharge will be performed to demonstrate continued eradication of infection.

If a subject is found to have recrudescence of infection, the following steps will be taken:

- Testing will be performed to rule out *C. difficile*-associated diarrhea. The strain will be confirmed by clinical microbiology and PCR testing to confirm that it is the study strain. Antibiotic sensitivity will be confirmed by the Clinical Microbiology Laboratory.
- The subject will be provided a second course of azithromycin 500 mg PO daily for 10 days and ciprofloxacin 500 mg PO BID for 10 days (assuming the strain remains susceptible) and treated symptom.

If a subject cannot tolerate the planned antibiotic treatment , or if resistance emerges to both antibiotics, an alternative antibiotic treatment will be Augmentin (875 mg PO BID for 10 days) or the oral third-generation cephalosporin, cefpodoxime (400 mg PO BID for 10 days). Decisions regarding

antibiotic use and combination of these second-line agents will be made by the Principal Investigator on a case-by-case basis in consultation with the Medical Monitor.

The subject will have stool cultures performed after the completion of antibiotic therapy, and then at recrudescence Days 14, 21, 28, 35, 56, and 84 (Day 0 = day of production of the stool demonstrating the recrudescence) to confirm clearance.

The subject will have a telephone call approximately 180 days following the start of the recrudescent episode to assess for any new chronic or serious adverse health events.

16.4 Benefits

There is no benefit that can be guaranteed to subjects for participating in this research study. However, there is potential societal benefit of the development of a *Campylobacter* preventative treatment.

16.5 Subject compensation

Compensation for participation will be provided only for completed study procedures designated for compensatory payment. If a subject is eligible to participate in the investigational protocol after screening, the subject will be compensated for participation time and travel. Subjects will not be paid for missed follow-up visits and may forfeit some or all of their bonus as a result of missed visits or non-compliance.

16.6 Provisions to protect privacy of subjects and confidentiality of data

The Principal Investigator will exercise all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of subjects' identities. On exported electronic source data or any other documents submitted to the Sponsor, subjects will only be identified by subject number. Documents not for submission to the Sponsor, e.g., subject identification log and original ICF, will be maintained by the Principal Investigator in strict confidence.

16.7 Safeguards for vulnerable subjects

This study will not include individuals less than 18, incarcerated, or unable to meet the requirements to sign the informed consent form.

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