Clinical Study Protocol

A randomized, double-blind, placebo-controlled trial assessing the efficacy of once-daily dosing of Travelan® in a controlled human infection model for ETEC

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Sponsor Name:
Immuron Limited
Unit 10, 25 - 37 Chapman St
Blackburn North VIC 3130, Australia

CLINICAL PROTOCOL SYNOPSIS

Protocol Title	A randomized, double-blind, placebo-controlled trial assessing the efficacy of once-daily dosing of Travelan® in a controlled human infection model for ETEC
IND Number	029087
Investigational Products	1. Travelan® 2. ETEC strain H10407
Sponsor	Immuron Limited
U.S. Sponsor's Representative	Rho, Inc. Rho US Headquarters 2635 E NC Hwy 54 Durham, NC 27713 Phone: 919-408-8000
Manufacturers	**Travelan®** Immuron Limited Address: Unit 10, 25 - 37 Chapman Street, Blackburn North, VIC 3130, Australia **ETEC strain H10407** University of Maryland, Baltimore 685 W. Baltimore St., Suite 480 Baltimore, MD 21201
Principal Investigator	Dr Mohamed Al-Ibrahim, MB, ChB, FACP
Study Site	Pharmaron Clinical Pharmacology Center (CPC) 800 West Baltimore St., 5th and 6th Floor Baltimore, MD 21201, USA
Laboratories	Pharmaron Clinical Pharmacology Center (CPC), Baltimore, MD 21201 Quest Diagnostics, Inc., Baltimore, MD 21227 Center for Vaccine Development & Global Health, Baltimore, MD 21201.Naval Medical Research Command, Silver Spring, MD 20910
Study Objectives	**The primary objective** of the study is to assess the protective efficacy of a single daily dose (1,200 mg) of Travelan® against moderate-to-severe diarrhea following challenge with ETEC strain H10407. **The secondary objectives of the study are:** 1. To assess the safety and tolerability of once-daily dosing with Travelan® (1,200 mg). 2. To assess a variety of clinical endpoints to further evaluate the efficacy of Travelan® to further qualify the degree to which a participant experiences diarrhea **The exploratory objectives of the study are:**

	To measure the mucosal and systemic immune responses to the challenge organism and to obtain and archive samples for future immunologic, proteomic and microbiome analysis.
Study Design	This is a randomized, double-blind, placebo-controlled study to investigate whether once-daily dosing with Travelan® (1,200 mg) protects healthy adult volunteers from moderate-to-severe diarrhea upon challenge with Enterotoxigenic *Escherichia coli* (ETEC) strain H10407. Up to 60 subjects will be randomized to receive either the Travelan® product or a placebo once daily, followed by challenge with approximately $1x10^8$ colony-forming units (CFUs) of ETEC strain H10407. Randomization may be stratified according to blood group based on the correlation between ABO typing and susceptibility to moderate-to-severe diarrhea following challenge with ETEC strain H10407. Subjects will receive Travelan® or placebo caplets once daily in the morning (6 caplets per dose), beginning 2 days prior to experimental challenge with ETEC strain H10407. Subjects will be assigned to groups as per the table below. Travelan®/placebo will be administered for a total of 7 days, or until antibiotic treatment has been initiated. Antibiotic treatment will be initiated after early antibiotic treatment criteria are met or 5 days after challenge. Early antibiotic treatment will commence when any of the following criteria are met and a physician determines it to be warranted: • Severe diarrhea based on volume (800 g in 24 hours) • Diarrhea of any severity AND 2 or more of the following symptoms: severe abdominal pain, severe abdominal cramps, severe nausea, severe headache, severe myalgias, severe arthralgia), any fever (≥ 38.0°C), or any vomiting • Any fever ≥ 39.0°C • Subjects who experience unexpectedly severe events such as symptomatic hypotension (disproportionate to volume loss), renal dysfunction, or altered mental state (e.g. somnolence) at the discretion of the investigators. • A study physician determines that early treatment is warranted for other reasons. The placebo is a commercially-sourced high-protein milk product repackaged and masked to mirror the Travelan® product. Upon admission to the inpatient unit, clinical monitoring will consist of daily medical assessments with adverse event (AE) determination, vital signs at least three times daily, examination and weighing of all stools, stool culture work-up for the challenge strain up to three times daily, and safety laboratory tests. Any subject passing a grade 3-5 stool will be encouraged to start drinking oral fluids at a rate equal to 1.5 times their stool output (or at the same rate as their emesis output as applicable). Intravenous (IV) rehydration will be provided if pre-specified criteria are met.

<table>
<tr><td></td><td>All subjects will be treated with ciprofloxacin (500 mg by mouth twice daily for three days) starting five days after ingesting the H10407 challenge inoculum unless early treatment criteria are met. Subjects will be discharged from the inpatient facility when clinical symptoms are resolved or resolving and two consecutive stool cultures (taken at least 12 hours apart) are negative for the ETEC challenge strain. Subjects may be discharged earlier than Day 8 if they meet criteria.

<table><tr><td>Product</td><td>Number of subjects</td><td>Dose (daily)</td></tr><tr><td>Travelan®</td><td>30</td><td>1,200 mg</td></tr><tr><td>Placebo</td><td>30</td><td>1,200 mg</td></tr></table></td></tr>
<tr><td>Endpoints</td><td>The primary efficacy endpoint of this study is prevention and/or reduction of moderate-severe diarrhea, defined as ≥4 Grade 3-5 stools in any 24-hour period post-challenge or ≥401 grams of Grade 3-5 stools in any 24-hour period post-challenge.

Secondary endpoints are chosen to assess the safety and tolerability of the Travelan® product, and support the primary endpoint in determining the protective efficacy of the Travelan® product by further quantifying and qualifying the degree to which a participant experiences diarrhea. Additional comparisons between the placebo and test article groups are outlined below:

• Presence of Travelan®-associated AEs during the study period
• Maximum 24-hour loose stool (Grades 3-5) output
• Total loose stool (Grades 3-5) output
• Percent of subjects with severe diarrhea
• Percent of subjects with diarrhea of any severity
• Percent of subjects with fever, nausea, vomiting, anorexia, or abdominal pain/cramps rated as moderate-to-severe
• Percent of subjects who indicate they would have reduced their daily activity if they had been vacationing or traveling for business because of their ETEC illness
• Time to diarrhea onset and diarrhea resolution
• Number of CFUs of the challenge strain per gram of stool at 48 hours post-challenge
• Percent of subjects requiring early antibiotic treatment
• Percent of subjects requiring IV fluids
• ETEC disease severity score to grade the severity of all adverse events</td></tr>
<tr><td>Study Duration</td><td>Volunteers will complete 1-3 screening visits that may occur up to 60 days prior to enrollment according to protocol. Consenting and eligible volunteers will spend approximately 12 days on the inpatient isolation unit. They will be asked to return for 2 outpatient follow-up visits at 15 and 29 days post-challenge, and complete a telephone assessment 6 months post-challenge. The total participation for an individual volunteer is up to 9 months.</td></tr>
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	Duration for screening, study intervention, CHIM, follow-up, immunology studies, analysis, and reporting after all volunteer contact is complete is 1 to 1 ½ years.
Eligibility Criteria	Inclusion Criteria: 1. Male or female between 18 and 50 years of age, inclusive at time of screening visit. 2. General good health, without significant medical illness, abnormal vital signs or physical examination findings, or clinical laboratory abnormalities, as determined by the principal investigator (PI) in consultation with theMedical Monitor and Sponsor. 3. Demonstrate comprehension of the protocol procedures, requirements, and CHIM this will be evaluated by completing a multiple choice comprehension assessment (passing grade ≥ 70%) during screening and in the consenting process. 4. Willing to participate, as evidenced by signing the informed consent document. 5. Available for all planned follow-up visits. 6. Negative serum pregnancy test at screening and negative serum and/or urine pregnancy test on the day of admittance to the inpatient unit for all female participants. All females must agree to use an efficacious hormonal or barrier method of birth control during the study. Efficacious methods of birth control include hormonal birth control methods (oral contraceptive pills, patches, vaginal rings, long-acting reversible contraception, surgical sterilization, condoms with spermicide, or abstinence from intercourse with a male partner. Female participants unable to bear children must have this documented (e.g., tubal ligation or hysterectomy). 7. A negative Covid-19 PCR test is required on the day of admission to the unit to comply with Pharmaron's Covid-19 policy (subjects reporting to admission for Cohort 1 who test positive for COVID-19 may rescreen for Cohort 2) 8. Acceptable hematology and blood chemistry levels as assessed by the PI. i.e., Serum creatinine <1.3 mg/dL. AST, GGT, amylase, lipase, alkaline phosphatase not to exceed 1.5x upper limit of normal (ULN) 9. Vital signs will be assessed in the supine position and must be within the following ranges: • Oral body temperature between 35-37ºC inclusive • Systolic blood pressure between 90-140 mmHg inclusive • Diastolic blood pressure between 55-90 mmHg inclusive • Pulse rate between 45-90 bpm inclusive Exclusion Criteria: *General health issues* 1. Presence of a significant medical condition (e.g., psychiatric conditions such as significant anxiety, depression, or somatization disorder; gastrointestinal disease, such as peptic ulcer, symptoms or evidence of active

	gastritis/dyspepsia, gastroesophageal reflux disease, inflammatory bowel disease, or irritable bowel syndrome (as suggested by medical history or medical diagnosis); history of major gastrointestinal surgery; or laboratory abnormalities that in the opinion of the investigator preclude participation in the study. Significant medical conditions include HIV, active Hepatitis B or C infection, ongoing immunosuppression for any reason, autoimmune disease, any underlying cardiac, pulmonary, endocrine, or renal conditions, any gastrointestinal illness (chronic reflux, inflammatory bowel disease, ulcer), any diabetes mellitus, and other such illnesses that can put a volunteer at increased risk. Exclusionary laboratory abnormalities include any abnormality that is grade 2 or above, or any two grade 1 laboratory abnormalities. 2. Immunosuppressive illness or evidence of IgA deficiency (serum IgA levels outside the normal range). This includes any disease that requires immunosuppressive medication such corticosteroids, monoclonal antibodies that target key aspects of the immune system (e.g. rituximab or TNF-blockers, or any autoimmune disease). 3. Positive serology results for HIV, HBsAg, or HCV antibodies, and confirmatory tests if appropriate. 4. Positive urine drug screen (positive for the presence of amphetamines, barbiturates, opiates, phencyclidine, cocaine, benzodiazepines, methadone, and propoxyphene at screening and at the discretion of the study physician, with the exception of stable persons with a diagnosis of ADHD that is well-controlled with a prescribed amphetamine. 5. History of alcohol abuse in the past 3 months or drug abuse in the past year 6. Significant abnormalities in screening laboratory hematology, serum chemistry or electrocardiogram, as determined by the PI or PI in consultation with the Medical Monitor and Sponsor. Significant ECG abnormalities include the following: a. PR > 220 msec b. QRS complex > 120 msec c. QTcF > 450 msec (male) or >460 msec (female) 7. Serum bilirubin exceeds upper limit of normal 8. Use of any medication known to affect immune function (e.g., corticosteroids and others) within 30 days preceding receipt of the investigational products or planned to be used during the active study period. Any regular systemic corticosteroid will be exclusionary, while topical, intranasal, and inhaled steroids will be permitted. 9. Nursing or lactating on the day of admittance to the inpatient unit. 10. Inability to tolerate 150 ml of sodium bicarbonate buffer. 11. Recent vaccination (including licensed vaccines) or receipt of an investigational product (within 30 days before challenge through 30 days following the challenge dose). 12. History of diarrhea (> 3 unformed or liquid stools over a 24-hour period) in the 2 weeks prior to the planned inpatient phase.

	13. Fewer than 3 stools per week or more than 3 stools per day as the usual frequency, or loose or liquid stools other than on an occasional basis. 14. Regular use of laxatives or any agent that increases gastric pH (regular defined as at least weekly). 15. Use of proton pump inhibitors, H2 blockers, or antacids within 48 hours of dosing. 16. A fever (≥38.0°C) in the 2 weeks prior to time of challenge. 17. Use of antibiotics during the 30 days before bacterial dosing or receipt of more than 3 courses of antibiotics over the two months prior to dosing. 18. Blood or plasma donation of one pint or more within 30 days preceding the receipt of the investigational products. 19. Lactose intolerance or allergy to milk or milk products. 20. Employment as a health care worker, food handler, childcare worker, or caregivers for elderly or immunocompromised individuals. *Challenge-specific exclusionary conditions based on potential increased risk or complicating outcome ascertainment.* 21. Allergy to fluoroquinolones, trimethoprim-sulfamethoxazole, doxycycline , or ampicillin/penicillin (excluded if allergic to two of four). 22. History of microbiologically confirmed ETEC infection in the last 3 years. 23. Occupation involving handling of ETEC currently, or in the past 3 years. 24. Symptoms consistent with travelers' diarrhea defined as ≥3 unformed or liquid stools over a 24 hour period concurrent with travel to countries where ETEC infection is endemic (most of the developing world) within 3 years prior to dosing, OR planned travel to endemic countries during the length of the study. ETEC endemic countries include all countries in Asia (except for Japan and South Korea) the Middle East, Africa, Mexico, Central and South America. 25. Vaccination for or ingestion of ETEC, cholera, Shigella, or *E. coli* heat-labile toxin within 5 years prior to dosing. 26. Any prior experimental infection with ETEC strain H10407, or prior experimental infection with other ETEC strains or other bacterial enteric pathogens (Salmonella, *Shigella*, and *Campylobacter*) within the past 5 years.
Sample Size Estimate/Analysis	The hypothesis is that Travelan® will confer ≥70% protective efficacy against moderate-to-severe diarrhea upon challenge with ETEC strain H10407 (in comparison to the placebo group). Using a Fisher's exact conditional test for two proportions with a one-sided *alpha*=0.05 and a moderate-to-severe diarrhea rate of approximately 70% in placebo recipients, a sample size of 30 subjects per study arm (Travelan® or placebo) yields an approximate 80% power to detect a significant difference in the moderate-to-severe diarrhea rate in placebo and Travelan® subjects when the efficacy of the Travelan® product is >70%. Of note, a one-sided *alpha* is utilized based on the extensive existing data supporting a 1-way effect of bovine colostrum products on disease rates.