FDA ADVISORY COMMITTEE MEETING

APRIL 12, 2007

BACKGROUND INFORMATION PACKAGE

CAPRION PHARMACEUTICALS INC.

MARCH 12, 2007

AVAILABLE FOR PUBLIC DISCLOSURE WITHOUT REDACTION

Caprion Pharmaceuticals Inc.
7150 Alexander-Fleming,
Montreal, Quebec, Canada
H4S 2C8

TABLE OF CONTENTS

TABLES

FIGURES

LIST OF ABBREVIATIONS

A/E	Attaching and Effacing
AUC_{0-t}	Area under the concentration-time curve from time zero to the last non-zero concentration
AUC_{0-inf}	Area under the concentration-time curve from time zero to infinity (extrapolated)
B2F1	STEC strain that produces the Stx2d-activatable variant of Stx2
cαStx1	Chimeric Monoclonal Anti-Shiga Toxin Antibody for Shiga Toxin 1
cαStx2	Chimeric Monoclonal Anti-Shiga Toxin Antibody for Shiga Toxin 2
CD_{50}	50% Cytotoxic Dose
CDC	Centers for Disease Control and Prevention
CHO	Chinese Hamster Ovary
Cmax	Maximum Observed Concentration
CTCAE	Common Terminology Criteria for AE
DMID	Division of Microbiology and Infectious
DNA	Deoxyribonucleic Acid
EHEC	Enterohemorrhagic *E. coli*
ECG	Electrocardiogram
E. coli	*Escherichia coli*
ELISA	Enzyme Linked Immunoabsorbent Assay
Fb	Formulation Buffer
FDA	US Food and Drug Administration
g	Gram
Gb3	Glycolipid Globotriaosylceramide
GI	Gastrointestinal
GRP	Group
h	Hour
HACA	Human Anti-Chimeric Antibody
hMVEC	Human Microvascular Endothelial Cell
HUS	Hemolytic Uremic Syndrome
IDMC	Independent Data Monitoring Committee
IL	Interleukin

IV	Intravenous
kg	Kilogram
L	Liter
LDH	Lactate Dehydrogenase
LEE	Locus of Enterocyte Effacement
LPS	Lipopolysaccharide
mab	Monoclonal Antibody
MHC	Major Histocompatibility Complex
mg	milligram
mL	Millilitre
MMWR	Morbity and Mortality Weekly Report
MTD	Mean Time to Death
n	Number
N	Normal
NA	Not Applicable
ND	Not Done
ng	Nanogram
NIH	National Institute of Health
nl	Nanoliter
O157:H7	Strain of Shiga Toxin-Producing *Escherichia coli* Bacteria
OIA	Optical ImmunoAssay
p	Probability of obtaining a result at least as extreme as that obtained
PCR	Polymerase Chain Reaction
pg	Picogram
PK	Pharmacokinetic
PRBC	Packed Red Blood Cells
RBC	Red Blood Cell
SAE	Serious Adverse Event
SMAC	Sorbitol MacConkey Agar
STEC	Shiga Toxin-Producing Escherichia coli
STME	Shiga Toxin-Mediated Events
STPB	Shiga Toxin-Producing Bacteria

Stx	Shiga Toxin
Stx1 or 2	Shiga Toxin 1 or 2
TNFα	Tumor Necrosis Factor Alpha
T_{max}	Time of Maximum Observed Concentration
$T_{1/2el}$	Half-life Time
VTEC	Vero Toxin-producing Escherichia coli
WBC	White Blood Cells
y	Years
µg	Microgram
µl	Microliter
µmol	Micromole

1. INTRODUCTION

This background information was developed for the Advisory Committee meeting called by the US Food and Drug Administration (FDA) for April 12, 2007. The Committee will discuss clinical trial designs for products that seek indications for the prevention and/or treatment of disease caused by Shiga toxin-producing bacteria.

This background information will cover the following topics:

- Shiga toxins, symptoms and pathogenesis of Shiga toxin mediated disease
- Therapeutic approach
- Proposed indication and clinical endpoints

2. SHIGA TOXINS

Shiga toxins (Stx) represent a group of bacterial toxins that are involved in disease. Shiga toxins are mainly produced by *Escherichia coli* (*E. coli*) and *Shigella dysenteriae* type 1 and sporadically, by *Aeromonas hydrophila*, *Aeromonas caviae*, *Citrobacter freundii*, *Enterobacter cloacae, Shigella flexneri* and *Shigella sonnei*[1, 2, 3].

There are multiple sources of Stx producing pathogens involved in sporadic cases and outbreaks of disease. Shiga toxin producing bacteria (STPB) are associated with a broad spectrum of clinical manifestations in humans ranging from asymptomatic colonization to life threatening hemolytic uremic syndrome (HUS)[4].

2.1 SIGNS AND SYMPTOMS OF SHIGA TOXIN-MEDIATED DISEASE

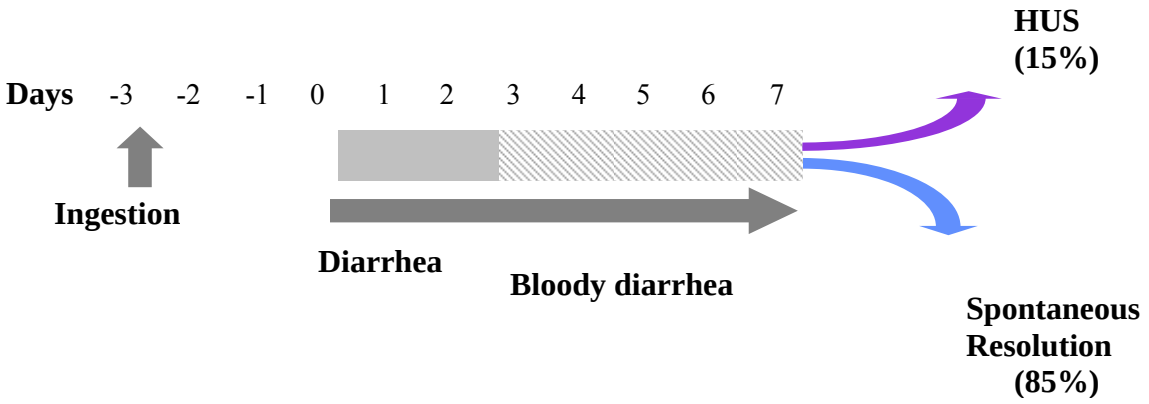
In North America, most cases and outbreaks of infection by STPB have been associated with Shiga toxin – producing *Escherichia coli* (STEC) and more specifically the single *E. coli* serotype O157:H7. These endemic or epidemic cases begin through the ingestion of STEC. Although STEC infection may be asymptomatic, it typically begins with an onset of watery diarrhea between 2 and 12 days after STEC ingestion and is frequently associated with abdominal pain and occasionally with nausea and vomiting[5, 6]. In a large

outbreak of *E. coli* O157:H7 in children <16 years old in Washington state the mean interval between STEC ingestion and onset of diarrhea was 3.7 days and the median was 3 days[7].

Watery diarrhea lasts for 1-3 days after which, in 90% of the cases, it progresses to bloody diarrhea[5]. When bloody diarrhea first develops, a patient has a normal platelet count, creatinine concentration and packed-cell volume with no red-cell fragmentation but approximately 15% of the STEC-infected subjects go on to develop HUS (Figure 1)[5]. Early in the illness, there is evidence that thrombin generation is increased, fibrin deposition is occurring and plasminogen activation is suppressed[5, 8]. Additionally, humans with STEC infection are frequently found to have fecal leukocytes[6, 9], high white blood cell counts early in the illness and elevated c-reactive protein levels[10].

The course of the disease between bloody or non-bloody diarrhea and HUS is not well defined but the HUS is defined as microangiopathic haemolytic anemia (fragmented red blood cells in the peripheral blood smear (schistocytes) and hemoglobin < 105 g/L), thrombocytopenia (platelet count < 150 x $10^3/\mu L$) and nephropathy (serum creatinine > upper normal range adjusted for age and sex and/or hematuria (>1+ by dipstick analysis) and/or proteinuria (≥ 0.3 g/L)). Other extrarenal complications of HUS also subsequently develop such as seizures (in ~14%), intracranial infarction or hemorrhage, retinal hemorrhage and encephalopathy (in ~20%), acute pancreatitis (in ~1%), glucose intolerance (in ~10%), cardiomyopathy (in ~1%) and death (in ~4%)[11].

Figure 1 Progression of *E. coli* O157:H7 (adapted from Tarr *et al.* 2005)[5]



Long-term health effects observed after an outbreak of diarrheal disease associated with *E. coli* O157:H7 and Campylobacter have recently been described[12]. Approximately 3.7 years after the outbreak, in an evaluation of 1958 adults who had no previous history of hypertension or kidney disease, Garg et al. (2005)[12] found hypertension in 27% of individuals who had been asymptomatic during the outbreak and in 32% and 36% of those who had moderate to severe symptoms of acute gastroenteritis, respectively. These data raise serious concerns about the long term risks of *E. coli* O157:H7 infections even in asymptomatic individuals. In children however, gastroenteritis which presented for medical attention during the outbreak was not associated with renal sequelae 4 years later[13]. The difference between adults and children is uncertain however it is possible that longer follow-ups would be required for determining the risk of nephropathy after childhood *E. coli* O157:H7 bacterial gastroenteritis.

Predicting which individual will suffer from Shiga toxin-mediated HUS and related complications based on clinical features of illness is impossible early in infection. One of the biggest clinical issues is that no clinical or biological sign or symptom will allow the treating physician to predict the outcome of the illness in an individual patient. Therefore all infected patients must be considered at risk of HUS[5].

2.2 PATHOGENESIS OF SHIGA TOXIN-MEDIATED DISEASE

The Stx pathogenic cascade begins with bacterial fimbrial attachment to enterocyte receptors. The mechanism by which colonization of the lower gastrointestinal (GI) tract occurs is best described for STEC such as E. coli O157:H7 that contains the locus of enterocyte effacement or "LEE region". The LEE region encodes genes involved in stimulating intestinal epithelial cells to build a pedestal on the cell surface to which the bacteria intimately adhere, called the attaching and effacing lesion, "A/E lesion". STEC that do not contain the LEE region to mediate colonization is not well understood[4, 6]. It is thought that Stx must be absorbed from the intestine to cause disease. How this occurs during STEC infection is unknown[6]. Stx within the intestinal tract crosses the epithelial barrier and enters the bloodstream.

Stxs are 70-kilodalton holotoxins that comprise a single enzymatically active A-subunit and five receptor binding B-subunits. The B-subunits recognize and bind to a family of glycolipids called Glycolipid Globotriaosylceramide (Gb3). Gb3 resides in the plasma membrane of certain eukaryotic cells, such as the kidney, with the carbohydrate directed towards the outside of the cell where it interacts with Stx. The Stx-Gb3 complex is internalized and enters the cytoplasm where the A-subunit inhibits protein synthesis and induces apoptosis. Proximal tubule and mesangial cells, as well as macrophase/monocytes respond to Stxs with an increase in active cytokines and chemokines.

Cytokines and chemokines generated locally in the kidneys are likely to have two separate actions. These are to activate glomerular endothelial cells to express Gb3, so that they can bind and respond to Stxs, and to make the endothelial cell surface more prothromic and adherent to neutrophils (Figure 2)[14]. The process of neutrophil migration from the systemic circulation across the intestinal epithelium to the gut lumen can cause transient epithelial barrier damage, allowing systemic uptake of gut luminal contents[6]. Recently, STEC-induced neutrophil migration has been shown to enhance Stx uptake across intestinal epithelium in vitro[6, 15], suggesting that inflammation occurring within

the host GI tract during STEC infection may promote systemic Stx uptake. Lipopolysaccharide is also absorbed from the gut during STEC infection, and may play a role in pathogenesis of HUS[6, 14, 16]. Additionally, it has been shown that Stxs may indirectly activate platelets[14]. It has been hypothesized that intestinal absorption of Stx does not occur as a one time event but is continuous even after the onset of disease[17].

Figure 2 Pathogenesis of STEC (adapted from Andreoli *et al.* 2002)[14]

1. Ingestion of STPB

Colonization of the gut (Stx independent)
Watery diarrhea

2. Bloody diarrhea

Tissue damage (Stx-dependent)

3. Hemorrhagic colitis

Systemic toxaemia

4. Host cytokines and chemokines generated

Stx and cytokine action on endothelium

5. Microvascular thrombosis in target organs (primarily kidney) resulting in HUS

One study suggests that the sequence of events leading to initiation of the thrombotic cascade precedes renal tubular injury and therefore, may underlie the initial pathophysiologic events leading to renal injury[8]. This is likely due to the fact that the processes of inflammation and coagulation are intimately linked through tissue factor. This is supported by the finding of elevated tissue factor circulating levels in children with STEC-induced HUS[18] and by studies demonstrating upregulation of tissue factor activity in proximal tubular epithelia and glomerular endothelial cell cultures in response to Stx[19, 20]. These studies also indicate that kidney damage may be due to both the direct and indirect action of Stx on renal cells.

There is limited evidence for complement-mediated injury to the host in STEC-associated HUS. This may be attributed to the rapid internalization of toxin, as demonstrated with Vero cells[21], and that large amounts of Stx-antibody complexes may not be formed.

Although HUS can occur in persons of any age, young children are most often affected. HUS is the most important cause of acute renal failure in children[17] and may lead to end-stage renal disease requiring dialysis or kidney transplantation. The reason for increased sensitivity of children to HUS is not known. Explanations such as stage of development of the intestinal epithelium[22] or compromised immune system as is seen in Factor H-related HUS[23] have been postulated however the role of any or all of these factors is yet to be clearly elucidated.

Infecting strains of bacteria can produce either Stx type 1 (Stx1), Stx type 2 (Stx2) or both. Stxs do not appear to carry equivalent risks of causing disease. Strains that produce only Stx1 have the lowest risk, while strains that make only Stx2 have the highest risk and strains that produce both Stx1 and Stx2 carry an intermediate risk. However, the association is far from absolute[5,10]. In a prospective point of care study in Seattle, Washington of children with STEC, stx_1 was detected in 26% of the patients, stx_2 in 23% of the patients and stx_1 and stx_2 in 51% of the patients[24]. HUS has been reported with each toxin alone or in combination[25].

2.2.1 Evidence from Animal Models

The mechanism of action of the toxins has been evaluated in animal models, but none of these models reproduce the pathophysiology of the human disease. The relevant animal models for this section are summarized below:

Enteropathic and encephalopathic manifestations:

- In rabbits, intravenous Stx administration results in lesions in the gut, spinal cord and central nervous system, corresponding to the location of Gb_3 in rabbits. In one study, 24 hours after injecting Stx2 intravenously, all rabbits developed hemorrhagic diarrhea and limb paralysis. Severe hypotension developed within 35 hours and circulatory failure subsequently provoked the death of the rabbits[26].

Enteropathic and nephropathic manifestations

- Dutch Belted rabbits can be infected with STEC (Enterohemorrhagic *E. coli* (EHEC) O153 or O157:H7) to induce an illness with both enteric and systemic/renal pathology. The dose of organisms required to cause illness is massive compared to the human infection, diarrhea is seen in all animals though bloody diarrhea is infrequent, clinical pathological assessment and composite scoring of renal histopathology are variable, and the pace of illness is very different with some animals ill enough to be euthanized by day 3 post inoculation[27].

Nephropathic and encephalopathic manifestations:

- Mice fed STEC, or given Stxs intravenously develop acute tubular necrosis[28, 29]. For example, mice infected with a non pathogenic mouse *E. coli* strain transformed with a plasmid carrying the *stx*$_1$ gene exhibited neurological signs as well as histopathological changes of the intestine and the kidneys within 3 days after infection as well as elevated serum cytokines including interleukin-1beta (IL-1β) and Tumor Necrosis Factor Alpha (TNFα); the control group fed the control strain did not exhibit any pathological sign or symptom[30]. Although colonic mucosal damage can be detected in mice fed the strain transformed with a plasmid carrying *stx*$_1$, mice do not develop hemorrhagic diarrhea.

Nephropathic and/or thrombotic manifestations:

- In the greyhound toxaemia dog model, thrombocytopenia and acute renal failure are the direct consequence of STEC administered orally through contaminated food[14].
- In the baboon model, injection of Stx1 results in thrombocytopenia and microangiopathic haemolytic and acute nephropathy that is similar to that observed in humans with Shiga toxin-mediated HUS[14, 31]. Although GI mucosal damage can be detected, baboons do not develop hemorrhagic diarrhea.
- A murine toxaemia model of HUS uses intraperitoneal co-injection of purified Stx2 plus lipopolysaccharide (LPS)[32]. The time course of disease is rapid with severe

alteration of white blood cells (WBCs) and renal platelet aggregation occurring in the first 12 hours, haemolytic anemia and severe thrombocytopenia within 12 to 36 hours, and renal function and fibrin deposition increasing over 12 to 72 hours. Biological processes (apoptosis, immune and inflammatory response, etc.) and molecular functions (complement, chemokine/cytokine, receptor binding activity, etc.) as determined by microarray expression analysis are immediately induced (2 to 4 hours).

- Piglets infected in the first few hours of life with STEC (mostly Stx2 producers) by oral gavage present with renal lesions similar to the histopathological lesions observed in human HUS in more than 2/3 of the cases. Control animals fed Stx negative *E. coli* never presented such lesions. The mapping of the renal lesions in STEC infected animals coincided with the distribution of Gb_3 receptors in piglets.
- In another study, left kidneys of rats were perfused with Stx1 from the renal artery through the renal vein and then revascularized; right kidneys would serve as controls. On day 1, apoptosis and induction of TNFα gene expression were noticed to occur in the medulla of the Stx1-perfused kidneys. On day 3, extensive tubular injuries were observed by light microscopy. In addition, aggregated platelets and monocytic infiltrates in both glomeruli and the medullary interstitium were detected by immunostaining. These results indicate that Stx1 caused glomerular platelet aggregation, tubular damage, and acute deterioration of renal function by acting directly on renal cells[33].

None of the animal models precisely mimic human infection in timing, dose of organism, pace of disease, and development of enteropathic, nephropathic and thrombotic manifestations. The lack of a suitable animal model exhibiting all the cellular events associated with Shiga toxins poses challenges to investigate the early cellular events following enteric colonisation leading to HUS.

2.3 SHIGA TOXIN DETECTION

Currently there is no analytical method to detect circulating levels of either toxin in the blood. Diagnostic procedures are based on the detection of Stx in fecal extracts or

cultures. Procedures for Stx detection differ in complexity, speed, sensitivity, specificity and cost[4]. Strain heterogeneity makes diagnostic testing problematic, because a common, inexpensive, but relatively insensitive way of screening stool samples for STEC O157:H7 takes advantage of its inability to ferment sorbitol (using Sorbitol MacConkey agar (SMAC)) but detects only 50% to 60% of the cases caused by STEC O157:H7 and misses the remaining 50% to 40% of the cases along with all of the non-O157:H7 STEC strains[34].

Other non-serotype restricted diagnostic methods have been developed such as screening of stool specimens for the presence of Stxs by either plate Enzyme Linked Immunoabsorbant Assay (ELISA) or latex agglutination, enhancing sensitivity to the 80-90% range although at significantly increased expense. Polymerase Chain Reaction (PCR) based methods for detecting Stx genes in stool samples are in the research phase[6]. These procedures generally require at least 24 to 72 hours to obtain results, and difficulties in isolating the colony at the clinical site, a pre-requisite for PCR, have been encountered rendering the feasibility of such a test questionable on a large scale.

A rapid point of care stool assay has been developed by Inverness Medical – Biostar Inc. The Biostar OIA® SHIGATOX assay takes approximately 15 minutes to conduct and involves the qualitative detection of Stx1 and/or Stx2 in fecal samples by optical immunoassay technology but does not differentiate between the toxins (http://www.fda.gov/cdrh/pdf6/K061889.pdf.). This test however has proven to be very sensitive and specific when compared to a commercial ELISA test (Meridian Premier™ EHEC) and a cytotoxic assay[35].

An ELISA that could differentiate between the two toxins has been recently approved by the FDA (ImmunoCard STAT® EHEC) but unlike the Biostar OIA® SHIGATOX Assay, results are available within 20 minutes after overnight broth enrichment http://www.mdeur.com/products/751630.htm.

In conclusion, compared to other commercially available assays, the Biostar OIA® SHIGATOX is the only test that can cost-effectively and rapidly detect Shiga toxins in human diarrheal fecal specimens without the need of a laboratory facility.

2.4 COST AND EPIDEMIOLOGY OF SHIGA TOXIN DISEASE

Even though approximately half of STEC infections do not require medical care a small percentage of cases go on to develop HUS with sequelae such as chronic kidney failure requiring lifelong dialysis or a kidney transplant. The average cost per case varies greatly by severity of illness, ranging from $26 for an individual who does not obtain medical care, $1,142 for an individual that seeks medical care but does not develop HUS and to $211,084 for an individual who develops HUS (± End Stage Renal Disease). The cost for a patient who dies from HUS is approximately $6,6 million. For STEC O157, the average cost per case in 2005 was estimated to be $5,872.[36, 37]. The high cost of illness due to STEC infections suggests that additional efforts to control this pathogen are warranted. However, despite much effort, outbreaks continue to occur. One such outbreak reported in 2006 in the US resulted in 102 hospitalization (51%), 31 cases of HUS (16%) and 3 deaths (1.5%).

The natural history of STPB disease is brief with only approximately one week between ingestion of the bacteria and the occurrence of HUS (with some cases lasting up to two weeks)[5]. Therefore, the prevalence, the number of cases of disease present in a population at any given time, of both Shiga toxin producing bacterial infections and HUS is low in the US because of the low rate of occurrence of infection and the acute nature of the condition.

One key publication by the Centers for Disease Control and Prevention (CDC) provides epidemiological figures of the number of incident STEC infections[38]. Mead et al.[38] evaluated the total number of STEC infections annually to be 110,220. The estimated number of STEC infections is based on a correction factor of 20 fold to take into account under reporting. The incidence of *E. coli* O157:H7 infection is decreasing according to

CDC FoodNet (Center for Disease Control (A) 2006). Comparing 1996-1998 with 2005, the estimated incidence of STEC O157:H7 decreased by 29%.

Since 1995 more than two dozen reports of outbreaks of HUS due to STEC infections in the US have been reported in the literature but nationwide estimates can not be derived from these reports. Estimates of HUS incidence are derived from notification and epidemiological surveillance systems. According to the CDC April 22nd, 2005 issue of Morbity and Mortality Weekly Report (MMWR) (Center for Disease Control (B) 2005), a total of 178 cases of post-diarrheal HUS were reported from 32 states during 2003; of these, 118 (66%) occurred among children aged <10 years.

Rangel et al.[39] reviewed all *E. coli* O157:H7 cases reported to CDC from 1982 to 2002. In that period, 49 states reported 350 outbreaks, representing 8,598 cases, 1,493 (17%) hospitalizations, 354 (4%) hemolytic uremic syndrome cases, and 40 (0.5%) deaths. The transmission route for 183 (52%) outbreaks was foodborne, 74 (21%) unknown, 50 (14%) person-to-person, 31 (9%) waterborne, 11 (3%) animal contact, and 1 (0.3%) laboratory-related. The paper also acknowledges the high under reporting rate.

Due to the low prevalence of STEC infection, the hospitalization, HUS and death rates are mostly based on estimates. For example, the HUS rate was estimated to be 1.5% for all STEC infected cases in the US[40] and 15% in STEC infected children in an outbreak[5]. The under reporting rates and the definition of HUS used in registries can influence these estimates. Nevertheless, it is recognized that children are more susceptible to developing the disease.

3. THERAPEUTIC APPROACHS

For a therapeutic intervention, the natural approach would be to treat or prevent HUS, especially in children. However, there are important challenges to the development of a therapeutic intervention:

1) The number of HUS cases is extremely low. Choosing prevention of HUS as the primary efficacy endpoint requires a very large and costly trial of approximately 870 patients, excluding drop-outs (435 treated and 435 placebo patients for a z-test with one-sided significance level of 0.025 to have 80% power, assuming a HUS incidence rate of 10% in placebo patients and 50% reduction in HUS incidence in treated patients). These considerations render the feasibility of such a study questionable, particularly since detection of STEC infections requires screening of 50-100 diarrhea episodes for every case meeting entry criteria.

2) Treating patients with manifest HUS may be an option however, at this stage the disease is already well established and the chances of reversing it and thereby showing treatment benefits are very low.

From these issues, it is apparent that studying HUS, the end spectrum of the disease, is not the most appropriate avenue. We therefore propose to aim at the earlier stages of STEC disease. The advantages of targeting STEC disease rather than ***established*** HUS are:

1) Subjects with STEC diarrhea prior to the development of extraintestinal manifestations and HUS are more likely to benefit from with Stx neutralizing antibodies.

2) There is good experimental and pathological evidence to suggest that STEC-associated hemorrhagic colitis is mediated by Stx. Amelioration or prevention of Stx-induced colitis, if accompanied by a similar trend towards reduction of rates of HUS (mild, "incomplete" and full-blown), can be used as an indication of efficacy of the product. This is substantiated by the fact that severity of enteritis was associated with the prognosis of HUS. In a retrospective review of children (n = 509) hospitalized with HUS, the analysis of Kaplan-Meier survival curves demonstrated a better prognosis for return of normal renal function in the children with watery diarrhea but without prolapse ($p = 0.009$) than in children with bloody diarrhea or prolapse[41].

3) The incidence of STEC diarrhea is greater than that of HUS. Therefore, a study evaluating the effect of antibody therapy on the course of STEC disease, instead of the prevention of HUS, is more feasible.

In conclusion, early interruption of the Stx-mediated cascade is expected to alleviate the severity of the illness, the rate of complications and the incidence/ duration of hospitalizations even if sample size considerations make it difficult to prove statistically whether HUS can be prevented. Therefore, we propose to target the early phases of the disease, i.e. children who seek medical care for STEC infection prior to HUS.

3.1 SHIGA TOXIN MEDIATED EVENTS

As described in sections 2.1 and 2.2, STEC infection is strongly associated with colitis (enteropathy) and thrombotic microangiopathy and other extra-intestinal manifestations typical of STEC disease. We call these manifestations "Shiga toxin mediated events" (STME). The methodology for the definition and grading of the STMEs was presented in three different international meetings last year[42, 43, 44]. In the absence of practical methods to detect circulating or tissue-bound Stx in affected patients, STMEs are proposed as markers of Shiga toxemia, i.e. evidence of local (colon) and systemic effects of biologically active toxin. The list of STMEs and the STME grading system are presented in two categories in Figure 3. The proposed scale of STMEs is an adaptation of the "Common Terminology Criteria for Adverse Events" ((CTCAE version 3.0) universally used in oncology trials for the grading of drug toxicity). We named this scale the **STPB Disease Severity and Progression Scale**:

Figure 3 STPB Disease Severity and Progression Scale

Enteropathy (hemorrhagic colitis)					
	0	**1**	**2**	**3**	**4**
Diarrhea (daily stool frequency)	Baseline (no diarrhea)	< 5	5 - <10	10 - <15[1]	≥15[2]
Abdominal pain/cramps[3]	None	Mild	Moderate	Severe or requiring pain medication	Unbearable
Bloody diarrhea	No visible blood	Occasional/ small amounts of blood	Blood mixed with stool, streaks of fresh blood	Frank blood (hemorrhage)	Hemorrhage requiring colonoscopy or surgery

[1] Or ≥1 stool every hour over a ≥6 hour period
[2] Or ≥1 stool every ½ hour over a ≥6 hour period
[3] Wong-Baker Faces (visual analogue) pain scale[45]

Thrombotic Microangiopathy and Nephropathy (TMAN)					
	0	**1**	**2**	**3**	**4**
Hemoglobin[4], [5] [g/l]	≥115	<115 - 105	<105 - 90	<90 - 65	<65 or PRBC[6]
Platelets [N/nl]	≥150	<150 - 125	<125 - 75	<75 - 25	<25 or Platelet transfusion/ bleeding
Hematuria (Dip stick analysis)[7]	None or trace	small	moderate	large	-
Serum creatinine (age-adjusted) [µmol/l]	Normal (for age)	>1 - 2x upper normal	>2 - 4x upper normal	>4x upper normal	Dialysis

[4] With elevated Lactate Dehydrogenase (LDH) (above upper normal) and/or reduced haptoglobin (below normal range) [5] For all patients with anemia, exclude other causes of anemia, particularly due to iron deficiency (microcytic anemia, low mean corpuscular volume) and hemoglobinopathies, [6] Transfusion of packed red blood cells (PRBC) [7] Or: Red blood cells (RBC) per µl urine (0 = ≤ 5, 1 = >5-10, 2 = >10-30, 4 = >30)

To evaluate the utility of the proposed STMEs, Caprion conducted a prospective observational study in subjects with STEC infection last year, under conditions similar to the proposed pivotal trial (Section 6). At two sites in South America (Santiago, Chile and Cordoba, Argentina children from 1-18 years of age (mean 4 +/- 3.3 years) with bloody or non-bloody diarrhea for no more than 3 days were eligible to participate in this study. The evaluation included laboratory tests (biochemistry, haematology and urinalysis at Days 0 (recruitment), 1, 2, 3, 7 and 56) and the description of the signs and symptoms for a period of up to 56 days.

Data from 33 subjects with STEC infection were analyzed. The stools of these subjects were Shiga toxin positive as demonstrated by two independent tests (Biostar OIA® SHIGATOX Assay and Meridian Premier™ EHEC) which use different antibodies to detect the Shiga toxins. All these cases were SMAC negative (STEC non-O157:H7). There were more male subjects (55%) compared to female subjects (45%), and all were Hispanic in origin. Twenty-six subjects (79%) had non-bloody diarrhea, seven (21%) subjects had bloody diarrhea and none had HUS at the start of the study.

The number of subjects at each evaluable timepoint who experienced a STME with a score greater than zero, based on the STPB Disease Severity and Progression Scale (Figure 3), is presented in Figures 4 through 9. Bars represent the number of patients with the indicated sign or symptom. Day 0 equals the day of enrolment (within three days after the onset of diarrhea). It is noteworthy that none of the patients became thrombocytopenic.

Figure 4 Number of Subjects with Diarrhea

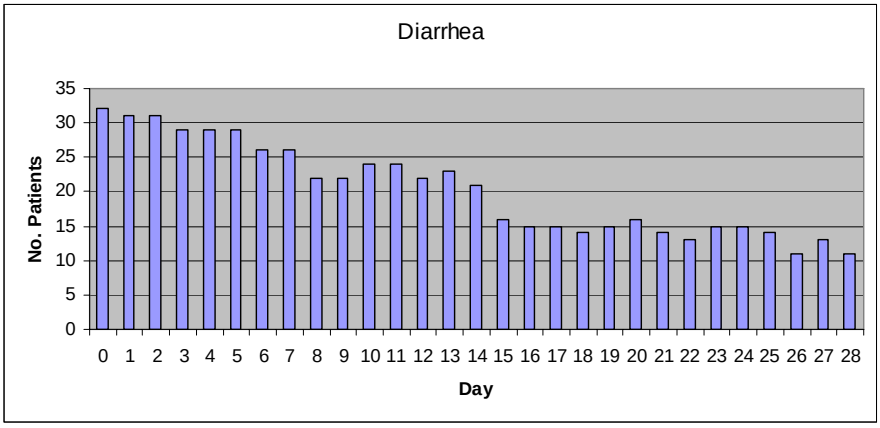


Figure 5 Number of Subjects with Abdominal Cramps

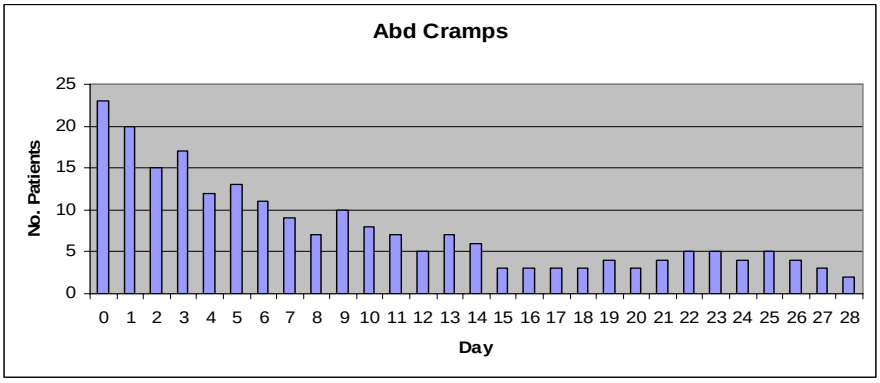


Figure 6 Number of Subjects with Bloody Diarrhea

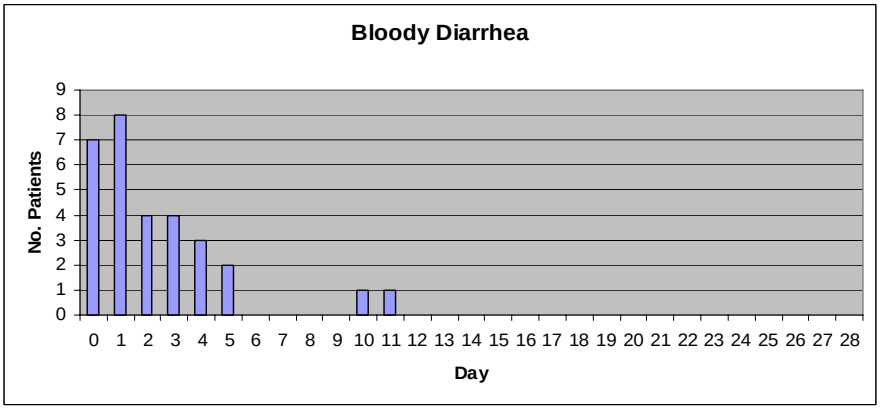
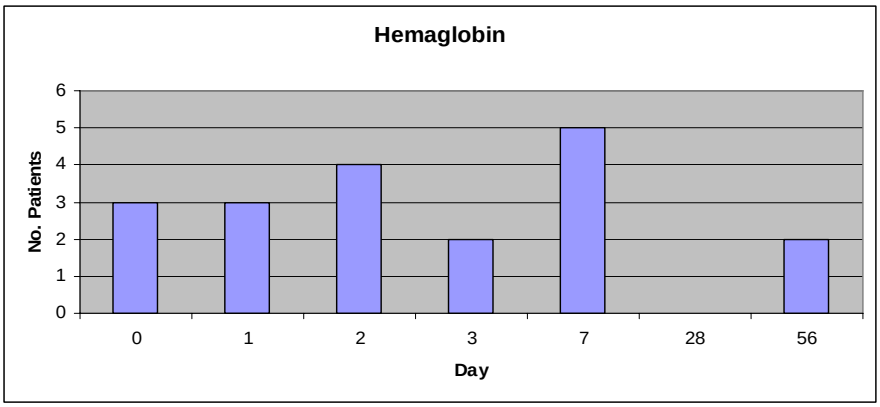


Figure 7 Number of Subjects with Abnormal* Hemoglobin



* Abnormal is defined as ≥grade 1 on the STPB Disease Severity and Progression Scale

Figure 8 Number of Subjects with Hematuria

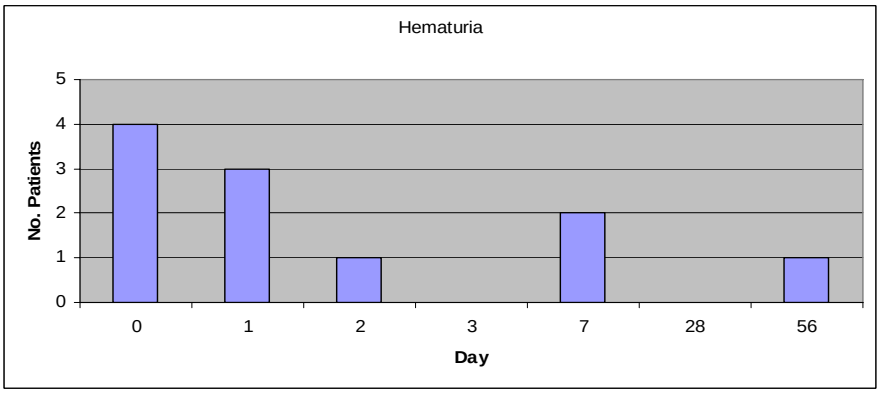
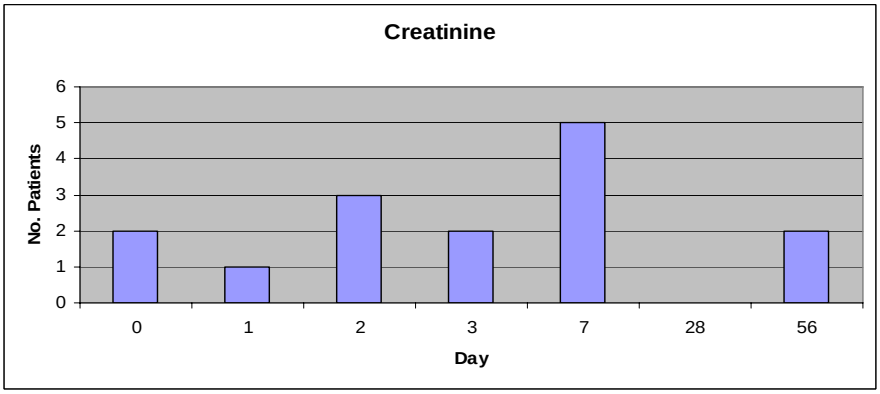


Figure 9 Number of Subjects with Abnormal* Creatinine



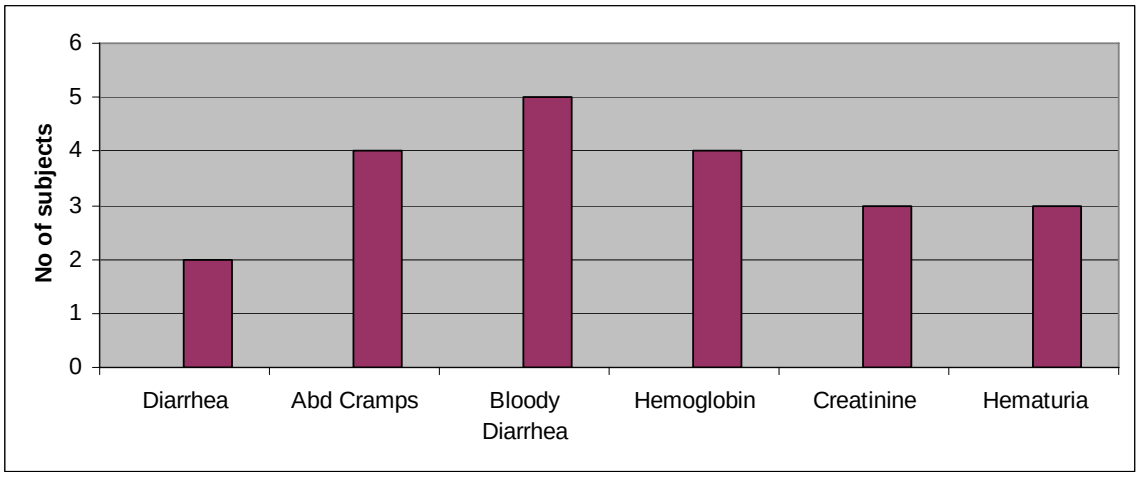
* Abnormal is defined as ≥grade 1 on the STPB Disease Severity and Progression Scale

In addition to evaluating the number of patients with a STME at any given time, we also recorded the worsening of signs and symptom (STME progression). STME progression was defined as:

- STMEs that occurred for the first time at a grade ≥1 following Day 0, that is, the STME was not observed on Day 0 (STME of grade 0), but after Day 0;
- STMEs that were present on Day 0 (STME grade ≥1) but increased in severity following Day 0. An increase in severity was defined as a ≥2 point increase on the STPB Disease Severity and Progression Scale.

Based on this definition, Figure 10 below illustrates the number of patients with documented STME progression during the first two weeks of the observational study. The time of evaluation was limited to the first 14 days of the study because of the acute nature of the disease.

Figure 10 Progression of STMEs in the STEC Observational (Epidemiology) Study (n=33)



For the evaluation of a therapeutic intervention in this indication, we propose to examine the number of subjects who experience progression of one or more STMEs. A subject experiencing multiple progressive STMEs will be counted only once. In the South

American epidemiology study, a total of 21/33 subjects (64%) experienced at least one STME that progressed during the first 14 days of the study, based on the above definition.

In this study, STMEs were relatively mild, typically qualifying as grade 1 or 2 on the STPB Disease Severity and Progression Scale. None of the bloody diarrhea episodes were severe, and no case of HUS was observed. Although the number of subjects in this study was too low to represent the complete spectrum of STEC disease manifestations, the findings are informative with respect to the following points:

1) The STPB Disease Severity and Progression Scale has been applied to describe the natural STEC disease in the setting of a prospective study;
2) The low morbidity of the identified STEC cases from Chile and Argentina, as reflected in the low STPB scores, contrasts with the degree of severity expected from reports in peer-reviewed literature, particularly from North America. It is possible that the severity of STEC infection varies among different regions, e.g. due previous exposure/pre-existing immunity.
3) The STEC disease susceptible age group in South America may extend to children younger than 1 year and should be included in a future treatment trial;

In conclusion, if the disease course of STEC infections could be interrupted (i.e. reduce the worsening of STMEs, including enteropathic STMEs) with a therapeutic intervention, it is believed that one could impact on HUS, a life-threatening condition which can currently not be prevented.

3.2 TREATMENT OF SHIGA TOXIN-MEDIATED DISEASE

To date, there is no proven, safe treatment for STEC infections and the prevention of Shiga toxin-mediated complications. Antibiotics do not decrease the risk of HUS and, in fact, may exacerbate the condition by inducing the production and release of Stx and, possibly, other bacterial toxins. It is generally acknowledged that antibiotics (specifically

Deoxyribonucleic Acid (DNA)-damaging antibacterials) and antimotility agents are contra-indicated in case of STEC infection[5].

In a small prospective study, early parenteral volume expansion with isotonic saline during *E. coli* O157:H7 infections was associated with attenuated renal injury failure in children who developed HUS. The authors suggested this approach, which implies few days of hospitalization and careful monitoring, for all children diagnosed with STEC infection in their hospital[46].

Anti-toxin therapeutic strategies to date have included those targeting toxin in the gastrointestinal tract, toxin vaccines (parenteral toxoids, live oral attenuated vector vaccines, and parenteral lipopolysaccharide vaccines), and neutralizing monoclonal antibodies. Synsorb-Pk, which targeted toxins in the feces, was unsuccessful in affecting the outcome of HUS, likely because it was administered too late in the course of the disease and also did not intervene at the systemic level[47]. Several vaccine approaches using toxoids and toxin subunits[48, 49, 50, 51] have been successful in protecting against lethal toxin doses in animal studies. While these results are promising, a directed therapeutic approach for a rare acute clinical condition may have a more acceptable risk/benefit ratio than preventative vaccination of the general public.

Neutralization of toxins by passive immunization in STEC-infected and at risk subjects with monoclonal antibodies offers a realistic approach to abrogation of STEC disease. The challenge in treating this disease is early intervention before the pathological process becomes irreversible. It is not clear whether the kidney damage is due to the direct action of Stx on renal cells, to the induction by Stx of a harmful cytokine response, or to a combination of the effects of Stxs and cytokines (See Section 2.2). However, the stimulation of proinflammatory cytokine by Stx and the cytokine up-regulation of Gb3 receptors by both Stx1 and Stx2 in *in vitro* studies, support the toxin injury model wherein there is an increasing amount of toxin binding to the target tissues such as renal endothelial and epithelial cells in an auto amplification-like manner[52]. This suggests that

early intervention with neutralizing antibodies, even after initiation of the cascade and perhaps before a threshold is reached, may have therapeutic benefit.

Since fecal shedding is prolonged in patients who develop HUS[53], early intervention with neutralizing agents such as antibodies that have a long half life, could impact disease progression. Neutralizing antibodies have been developed by several groups and have demonstrated protection in murine and piglet models of infection and toxaemia when targeting Stx1 B subunit or Stx2 A or B subunits[54, 55, 56]. These studies demonstrated protection at 24 to 48 hours post infection at doses 2.5 and 0.4 mg/kg in mouse and piglet studies, respectively.

If the incidence of STMEs can be reduced by the neutralization of circulating toxins in the presence of monoclonal antibodies, it is expected that this treatment also reduces the incidence or severity of HUS.

The importance of a safe therapeutic approach is paramount in a clinical setting where most STEC infections resolve spontaneously. Monoclonal and polyclonal antibodies against foreign proteins have gained wide acceptance and are considered generally safe. Many of the approved products are chimeric antibodies that demonstrate minimal antibody-associated toxicity when injeted intravenously. There were no serious adverse events (SAEs) in a 5 and subsequent 6 year study when BabyBig, a human botulism immune globulin, was administered intravenously at 50 mg/kg to infants within three days of admission[57]. Palivizumab (Synagis®), a monoclonal antibody, is administered intramuscularly in 5 injections at 15 mg/kg to children for the prevention of Respiratory Syncytial Virus in the Impact-RSV trial. In the clinical trials and post-marketing safety surveillance of >250,000 children, SAEs were non-fatal and limited to hypersensitivity and anaphylaxis in <0.001%[58]. In addition, the safety profile of other therapeutic antibodies administered intravenously which target host antigens is in general acceptable (Baselaga 2000 - C225, Davis 2000 - Rituximab, Kon 1998 - αCD20, Hommes 2006 - αIFNγ)[59, 60, 61, 62]. Reported adverse events were mild to moderate and SAEs either non-existent or minimal for doses ranging from 3 to 10 mg/kg in multiple dose regimens.

Hypersensitivity and anaphylactic reactions for those antibodies have been minimal and manageable.

The activation of complement by monoclonal antibodies has been raised as a potential safety concern. The evidence of the relevance of complement-mediated cytotoxicity with anti-cancer antibodies is currently limited to a few *in vitro* and *in vivo* studies[63, 64]. There is also limited evidence for complement-mediated injury to the host in STEC-associated HUS and the low level, short-lived binding of Stx to the Gb3 expressing endothelium may negate complement activation because of the limited amount of Stx-antibody complexes that would be formed. There was no additional pathology in limited, albeit not controlled, studies using intravenous (IV) pooled gamma globulin for treatment of STEC-induced HUS[65, 66].

3.3 CHIMERIC MONOCLONAL ANTIBODIES

Caprion has developed chimeric antibodies to Stx1 and Stx2. The chimeric monoclonal anti-shiga toxin antibody for Shiga toxin 1 (cαStx1) targets the B subunit of Stx1 whereas chimeric monoclonal anti-shiga toxin antibody for Shiga toxin 2 (cαStx2) binds to the A subunit of Stx2. There is sequence diversity in the Stx2 B-subunit among Stx 2 variants; however, the greater homology in the A-subunit may provide an advantage of better coverage against a broader spectrum of variants. There are no Stx1 variants and therefore, there is homology in the Stx1 B-subunit which is targeted by cαStx1. Efficacy of the antibodies has been demonstrated both singly and in combination in various murine models as well as *in vitro* studies. Safety of single and combination modalities is supported by non-clinical and clinical studies.

3.3.1 Pharmacology

In vivo Studies

Pharmacology studies have been conducted using several mouse models. The cαStx2 antibody provided complete or nearly complete protection of mice from an otherwise lethal infection (Survival time: 6-8 days) with an orally administered *E. coli* strain, B2F1,

which produces Stx2d-activatable, when administered at 0, 24, or 48 h post-infection at doses as low as 0.1 mg/kg with partial protection also evident when antibody was given as late as 72 h post-infection (Table 1).

Table 1 Survival of Male CD-1 Mice Given a Moderate Oral Dose of B2F1 and Low Intravenous Dose of cαStx2

Dose of B2F1	Dose of cαStx2, mg/kg	Survival of mice given cαStx2 at the indicated times post-infection*			
		No Injection	24 h	48 h	72 h
2 x 10^6	0.1	ND	7/8	7/8	3/8
	0.01	ND	4/8	2/8	3/8
	0.001	ND	2/8	2/8	1/8
	0.9 % saline	ND	1/8	1/8	3/8
	0	2/11	ND		

ND-not done
* Final evaluation day = Day 28

The cαStx1 antibody provided complete or nearly complete protection of mice from an otherwise lethal Stx1 toxin injection (Survival time: 48-72 hours) when administered one hour post injection in a dose range from 0.2 – 0.75 mg/kg (Table 2 and Table 3).

Table 2 Survival of Male CD-1 Mice Injected with Stx1 (intraperitoneal) and/or cαStx1 (intravenous)

Dose of Stx1 ng	Dose of cαStx1 mg/kg	Timing of cαStx1 injection relative to Stx1 injection	Survival
250	0 (formulation buffer)	- 1 h	0/5
250	0.5	- 1 h	5/5
250	0.05	- 1 h	0/5
250	0.02	- 1 h	0/5
250	0.005	- 1 h	0/5
250	0 (formulation buffer)	+ 1 h	0/5
250	0.75	+ 1 h	5/5
250	0.5	+ 1 h	3/5
250	0.2	+ 1 h	2/5
250	0.05	+ 1 h	1/5

Table 3 Survival of Female CD-1 Mice Injected with Stx1 (intraperitoneal) and/or cαStx1 (intravenous)

Dose of Stx1, ng	Dose of cαStx1, mg/kg	Timing of cαStx1 injection relative to Stx1 injection	Survival
250	0 (formulation buffer)	- 1 h	0/5
250	0.5	- 1 h	5/5
250	0.05	- 1 h	5/5
250	0.02	- 1 h	0/5
250	0.005	- 1 h	0/5
250	0 (formulation buffer)	+ 1 h	0/5
250	0.75	+ 1 h	4/5
250	0.5	+ 1 h	5/5
250	0.2	+ 1 h	5/5
250	0.05	+ 1 h	1/5

In a pilot dual toxemia/dual anti-toxin study, concomitant administration of cαStx1and cαStx2 provided protection from otherwise lethal concomitant toxin injections of Stx1 and Stx2 (survival time: ~48 hours) when antibodies were administered one hour pre-injection. Neither antibody alone protected when both toxins were present, (no survivors in Group 6 with cαStx1 alone nor in Group 7 with cαStx2 alone, as compared with both antibodies in Group 8 with 4/5 survivors), thus demonstrating that both antibodies are required for protection in the presence of both toxins (Table 4)**.**

Table 4 Survival of Mice Injected with One or Both Toxins and One or Both Antibodies

Grp #	Stx1 dose, ng	Stx2 dose, ng	cαStx1, mg/kg	cαStx2, mg/kg	Timing of antibody dose relative to toxin dose	Survivors
1	250	0	0 (Fb)	0	NA	0
2	0	3	0 (Fb)	0	NA	2
3	250	3	0 (Fb)	0	NA	0
4	250	0	5	0	- 1 h	5
5	0	3	0	5	- 1 h	3
6	250	3	5	0	- 1 h	0
7	250	3	0	5	- 1 h	0
8	250	3	5	5	- 1 h	4
9	0	0	5	5	NA	5

In a model adapted from the *in vivo* toxin neutralization model for evaluating mixed botulinum anti-toxins in mice[67], antibodies and Shiga toxins were co-incubated *in vitro* prior to inoculation into mice. There were only 1/10 survivors in Group 8 with cαStx1 in the presence of both toxins and 2/10 survivors in Group 9 with cαStx2 in the presence of both toxins as compared with 7/10 survivors in Group 10 with cαStx1 and cαStx2 in the presence of both toxins. This study demonstrates that both cαStx1 and cαStx2 are required to protect when both toxins are present (Table 5).

Table 5 Survival of Mice Injected with Pre-incubated Toxin/Anti-toxin Doses

Group #	# male mice	Stx1 dose ng	Stx2 dose ng	caStx1 μg	caStx2 μg	Survival
1	10	250	0	0 (Fb)	0	1/10
2	10	0	5	0	0 (Fb)	0/10
3	10	250	5	0	0 (Fb)	0/10
4	10	250	0	0	400	0/10
5	10	0	5	200	0	0/10
6	10	250	0	200	0	10/10
7	10	0	5	0	400	4/10
8	10	250	5	200	0	1/10
9	10	250	5	0	400	2/10
10	10	250	5	200	400	7/10
11	5-8	0	0	200	400	9/9
12	5	0	0	Fb	Fb	5/5

In vitro Studies

In the *in vitro* cell cytotoxicity assay, it was calculated that 82.8 ng of cαStx2 neutralizes 1 pg of pure Stx2 (1 pg of Stx2 is equivalent to a 50% cytotoxic dose [CD_{50}]) while 26 ng of cαStx1 neutralizes 1 pg of pure Stx1[68]. FACS analysis indicates that Stx2 can bind to Gb3 receptors of human microvascular endothelial cells (hMVEC) in culture either as a pre-formed Stx2/cαStx2 complex or sequentially with cαStx2 binding to Stx2 after binding of the toxin to Gb3.

Conclusions of Pharmacology Studies

We have demonstrated that cαStx1 and cαStx2 protect animals in toxaemia or infection model studies, respectively, at the low doses of ≤0.75 mg/kg. Partial protection was even possible with treatment at 72 hours post infection with cαStx2, illustrating that intervention at this late stage of disease in this model was still possible. Protection was

only possible when both antibodies were administered in a dual toxaemia study, demonstrating that both antibodies contribute to protection against STEC strains producing Stx1 and Stx2. Though the availability of the toxin may be short-lived we have demonstrated in *in vitro* studies that antibody is capable of binding to either free or Gb3 receptor-bound toxin, thus increasing the opportunity for neutralization.

3.3.2 Toxicology

Safety studies conducted with the anti-toxin antibodies have included tissue cross-reactivity, in vitro crossreactivity, studies in healthy mice and marmosets, and studies in mouse models.

In Vivo Studies

Neither cαStx1 nor cαStx2 were associated with any overt or systemic toxicity or histopathological findings when anti-toxins were administered individually or simultaneously at repeat doses up to 60 mg/kg and single dose administration of 30 mg/kg in four acute toxicity studies in healthy CD-1 mice (rodent) and one study in healthy marmosets (primate). No biologically relevant difference in weight, clinical chemistry, hematology or urine parameters were observed between control and test article groups, whether the anti-toxins were administered singly or concomitantly.

In B2F1 (Stx2dact producer)-infected mice, no evidence of gross toxicity was observed in the presence of cαStx2 compared to the control group which was administered B2F1 only. Renal histopathology was equivalent between control and delayed treatment groups in the presence of protection suggesting that initiation of renal injury did not preclude protection nor was there exacerbation of injury with treatment.

The Mean Time to Death (MTD) was evaluated in the pharmacology studies described above to ascertain if the presence of the either or both antibodies accelerated the MTD for those animals not protected by the anti-toxins. The range of MTD of treatment groups in

the Stx1 and Stx2 protection studies (2.7-3.0 and 4.3-4.5, respectively) were similar to those of the control groups for each toxin (2.8-3.0 and 4.6, respectively), suggesting that the presence of antibody did not increase the toxicity of either toxin as defined by MTD (Table 6).

Table 6 Mean Time to Death of Non-surviving Mice in Controls and Treated Groups

Toxin	Controls MTD	Mab Dose mg/kg	Mab Treatment MTD
Stx1 (Toxemia Study, Stx1-14.0-02)	2.8-3.0	0.75	3.0
		0.5	3.0
		0.2	2.7, 2.75
		0.05	2.8, 3.0
		0.005	2.8, 3.0
Stx2 (Infection Study, GSAW103)	4.6	30	4.3, 4.5
		30 x 2	4.5

*Time in days

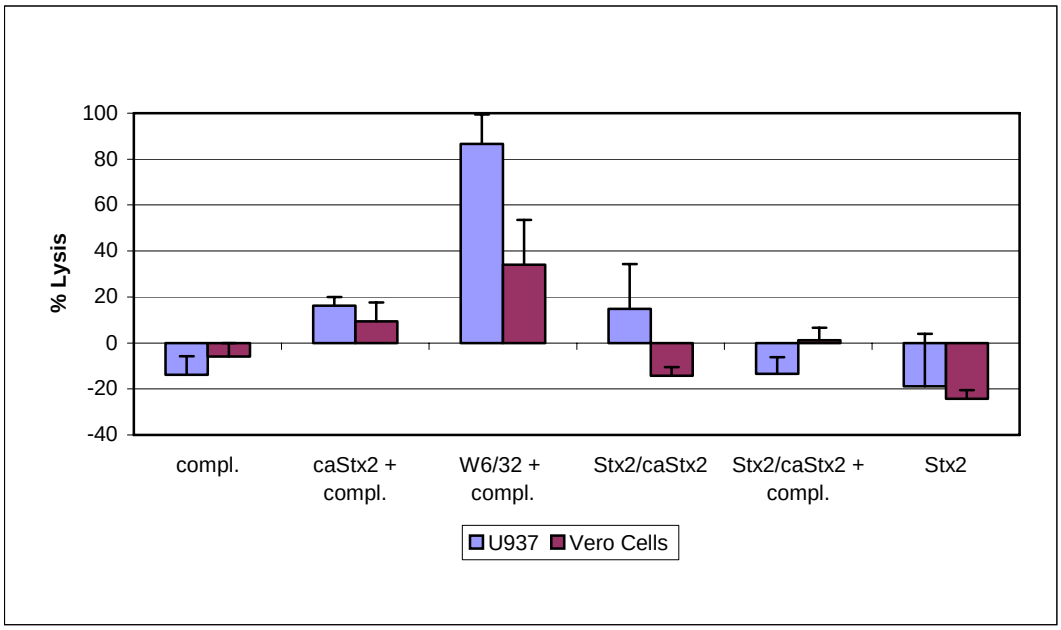
In Vitro Studies

Neither cαStx1 nor cαStx2 indicated tissue cross-reactivity with a panel of 39 different human tissues. ELISA and BiaCore studies have demonstrated that each antibody does not cross-react with the target of the other, supporting the need for both antibodies in neutralizing both toxins.

There was no induction of complement activation in preliminary studies when Vero cells (Green monkey kidney cells) were exposed to cαStx2 alone or in combination with Stx2. Figure 11 below shows that the use of a pre-formed Stx2 toxin/cαStx2 antibody complex does not induce complement lysis of Vero cells. U937, a macrophage-like human cell line, served as the control in that they expressed major histocompatibility complex (MHC) class I antigens and not Gb3 receptors.

Figure 11 Complement Cytotoxicity Assay of U937 and Vero Cells: % Lysis

(duplicate wells)



Conclusions of Toxicology Studies

Toxicology studies demonstrate that cαStx1 and cαStx2, alone or in combination, are safe and well-tolerated in healthy and infected animals, did not exacerbate disease as evidenced by MTD and did not activate complement in a kidney cell culture model.

3.3.3 Clinical Studies

Four Phase I clinical trials involving a total of 50 healthy volunteers have been completed and provide evidence that cαStx1 and cαStx2 are safe to administer, either individually or concomitantly, to humans. No serious adverse events were reported in any of the studies including a trial where 10 volunteers received up to 3 mg/kg of each anti-toxin (for a final dose of 6 mg/kg) in a concomitant manner. The following table summarizes the 4 Phase 1 trials conducted to date:

Table 7 Phase I Studies

Phase	Monoclonal Antibody*	Number of subjects who received the drug per dose	Total number of participants	Safety results
I (Healthy adult volunteers) DMID NIH	cαStx2	0.1 mg/kg (n=3) 1 mg/kg (n=3) 1.5 mg/kg (n=2) 3 mg/kg (n=6) 10 mg/kg (n=3)	N=17	NO SAE REPORTED IN ALL DOSE COHORTS
I (Healthy adult volunteers) DMID NIH	cαStx1	0.1 mg/kg (n=5) 1 mg/kg (n=2)	N=7	NO SAE REPORTED IN ALL DOSE COHORTS
I (Healthy adult volunteers) Study CTP_STX003A CAPRION	cαStx1 and cαStx2, administered individually	cαStx1: 1 mg/kg (n=4) cαStx1: 3 mg/kg (n=4) cαStx2: 1 mg/kg (n=4) cαStx2: 3 mg/kg (n=4)	N=16	NO SAE REPORTED IN ALL DOSE COHORTS
I (Healthy adult volunteers) Study CTP_STX002B CAPRION	cαStx1 and cαStx2, administered concomitantly	cαStx1+ cαStx2: 1 mg/kg of each mAb (n=5) cαStx1+ cαStx2: 3 mg/kg of each mAb (n=5)	N=10	NO SAE REPORTED IN ALL DOSE COHORTS

* The monoclonal antibodies were administered by IV single infusion

As depicted above, two studies were sponsored by the NIH Division of Microbiology and Infectious Disease and one study was published in 2005[69]. The two other studies (26 subjects) were sponsored and recently completed by Caprion and the findings are summarized below:

There were no clinically relevant findings related to the study medication from the safety parameters that were evaluated (i.e. safety laboratory parameters, ECGs, vital signs adverse event reporting, physical examinations).

Pharmacokinetic (PK) parameters for cαStx1 and cαStx2 were well characterized and findings were as expected for monoclonal antibodies. The antibodies appeared to be roughly dose proportional based on the pharmacokinetic parameters maximum observed concentration (C_{max}), area under the concentration-time curve from time zero to the last

non-zero concentration (AUC_{0-T}) and area under the concentration-time curve from time zero to infinity (extrapolated) ($AUC_{0-\infty}$). The time until the maximal concentration, time of maximum observed concentration (T_{max}), appeared to be unchanged between the two doses of each antibody and half-life time ($T_{½el}$) was estimated at approximately 9 days, which is consistent with the long half life expected for monoclonal antibodies. Clearance was low for both cαStx1 and cαStx2 at both doses which is also expected for monoclonal antibodies. Volume of distribution was low as well for both cαStx1 and cαStx2 which is indicative that the monoclonal antibodies are retained within the blood volume.

In conclusion, chimeric monoclonal antibodies cαStx1 and cαStx2, administered individually to healthy male and female adults at doses of 1 mg/kg or 3 mg/kg, are safe and well tolerated. The products tested in these two studies sponsored by Caprion will be used for the future trials. These products are manufactured from mammalian cells (CHO – Chinese Hamster Ovary cells), without animal-derived products, and in compliance with Good Manufacturing Practices and the FDA guidance document: Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (published 2/28/97).

4. RISK TO BENEFIT ANALYSIS

The risk to benefit ratio for the development of therapeutic antibodies for the treatment of STME is of great importance given the treatment population will primarily be children and the rate of progression to nonreversible events is unpredictable. However, some of the constraints surrounding the development of such treatment are listed below:

1) The fact that a validated surrogate efficacy biological endpoint is not available.
2) Shiga toxin-producing bacterial infections are acute conditions in otherwise healthy children.
3) Other limitations are with respect to the logistics of study conduct:
    a. Pediatric population: difficulties in recruitment, limited blood sampling;
    b. Product administration is via infusion;

Despite the challenges listed above, Caprion believes that the benefits outweigh the risks and justify the development of the two monoclonal antibodies. The following benefits associated with developing this product have been identified:

1) There is no available therapy for preventing this life-threatening condition (i.e. HUS and its related complications) thereby representing a unique treatment for STPB bacterial infection and protection against HUS in high-risk populations.
2) Two monoclonal antibodies are being developed against the two toxins that can be produced by the infecting agent, mostly *E. coli*. This is important since approximately 2/3 of the STEC strains are capable of producing both toxins and both toxins have been associated with HUS.
3) The proof of concept in the mice models has been well established. Protection was demonstrated for both monoclonal antibodies in otherwise lethal animal models. Since the monoclonal antibodies are specifically directed against the toxins, there is a strong probability that a high level of efficacy can be demonstrated provided that the therapy is administered at the right time (i.e. not too late in the disease process).
4) Pre-clinical toxicity studies in infected and healthy animals indicate that the monoclonal antibodies are safe. In addition, the safety of cαStx1 and cαStx2 was established in Phase I trials with healthy volunteers and indicate that the monoclonal antibodies are safe in humans.
5) The two monoclonal antibodies will be administered only once in the early phases of the infection (i.e., in a single infusion). Administration by a single infusion will reduce the likelihood of an undesired immunogenic response. In addition, chimeric antibodies have been used successfully in multiple administrations (such as Palivizumab).

It is recognized that to date there is no safety data available in the target patient population. For this reason, any study aiming at testing the monoclonal antibodies in children will be designed carefully to allow the appropriate evaluation of the safety. For example, the establishment of Independent Data Monitoring Committee (IDMC) meetings at appropriate time points during the course of the disease will be done

concurrently with the agency. An interim safety and efficacy analyses will be planned to decide with the agency and the IDMC whether the study should be continued or not. Specific stopping rules for the trial should also be in place. In addition, during the administration of the products, evaluations of acute hypersensitivity reactions will be performed by the Investigator during and after study drug administration. Evaluations for delayed hypersensitivity reactions will also be performed after product administration.

In conclusion, challenges are associated with the development of this product. The use of monoclonal antibodies against the toxins is a therapeutic approach that can be tested in the target patient population with the appropriate design and efficacy endpoints. Adequate safety measures in the clinical studies should also be in place to protect the participating subjects and to allow for the continued evaluation of the risk to benefit ratio.

5. PROPOSED INDICATION

The chimeric monoclonal antibodies cαStx1 and cαStx2 are being developed by Caprion to prevent the worsening of Shiga toxin-related complications induced by Shiga toxin-producing bacteria (STPB) infection in children as indicated by STME progression. Specifically, these monoclonals are being developed for: Treatment of Shiga-Toxin Producing Bacterial Infections. Caprion has received Orphan indication and fast track status by the FDA for this indication.

The proposed administration schedule is one single dose by IV infusion. The final dose will be determined from the pivotal trial (described below) but should not exceed 3 mg/kg of each monoclonal antibody.

6. PROPOSED PHASE II/III TRIAL

This study design (i.e. a hybrid Phase II/III) is proposed because of the rarity of the patients and because it will require screening approximately 50-100 patients to recruit 1 patient positive for Shiga toxins in the stools. This design does not in any way compromise the safety of the patients however it will maximize the quantity of data

captured by evaluating efficacy on as many randomized patients as possible. The objectives of the first part of the study will be the safety evaluation and dose finding. The objectives of the second part of the study will be safety and efficacy.

6.1 STUDY SYNPOSIS

Protocol Title:

A Safety and Efficacy Phase II/III Study of Chimeric Monoclonal Antibodies to Shiga Toxins 1 (cαStx1) and 2 (cαStx2) Administered Concomitantly to Children with Proven Shiga Toxin-Producing Bacterial (STPB) Infection.

Objectives:

Part A

Primary Objectives:

- To evaluate the safety and tolerability of different intravenous doses of cαStx1 and cαStx2 administered concomitantly in children presenting with early signs of proven STPB infections. Safety indicators will include frequency and severity of treatment-emergent adverse events and clinical and biological indicators such as vital signs, physical exam, ECGs and laboratory parameters, including complement activity evaluations.
- To determine, based on the safety analysis of Part A, the dose to be administered in Part B.

Part B

Primary Objectives:

- To evaluate, in a larger sample, the safety and tolerability of the intravenous dose of cαStx1 and cαStx2 selected from Part A.
- To evaluate the efficacy of cαStx1 and cαStx2 by clinical monitoring of the progression of toxin effect. Toxin effect will be evaluated through the monitoring of STMEs. The primary efficacy endpoint will be the number of subjects who had a STME that progressed during the study up to study Day 14 (as described in Section 3.1). STMEs will be graded according to the STPB Disease Severity and Progression Scale.

Secondary Efficacy Objectives:

- To compare the severity and duration of STMEs between groups.
- To compare the incidence and duration of hospitalization between groups.
- To compare the incidence of Hemolytic Uremic Syndrome (HUS) between groups and in patients defined as "very high risk of developing HUS" based on coagulation parameters [D dimer, tPA, and prothrombin fragment 1+2][8].

Other Objectives:

- To assess the frequency of development of human anti-chimeric antibodies (HACA), including immunoglobulin class and subclass.
- To describe the patterns of resource use in children presenting with STPB symptoms using a pharmacoeconomic instrument.
- To evaluate the pharmacokinetic characteristics of cαStx1 and cαStx2 in a subset of subjects.

Study Design:

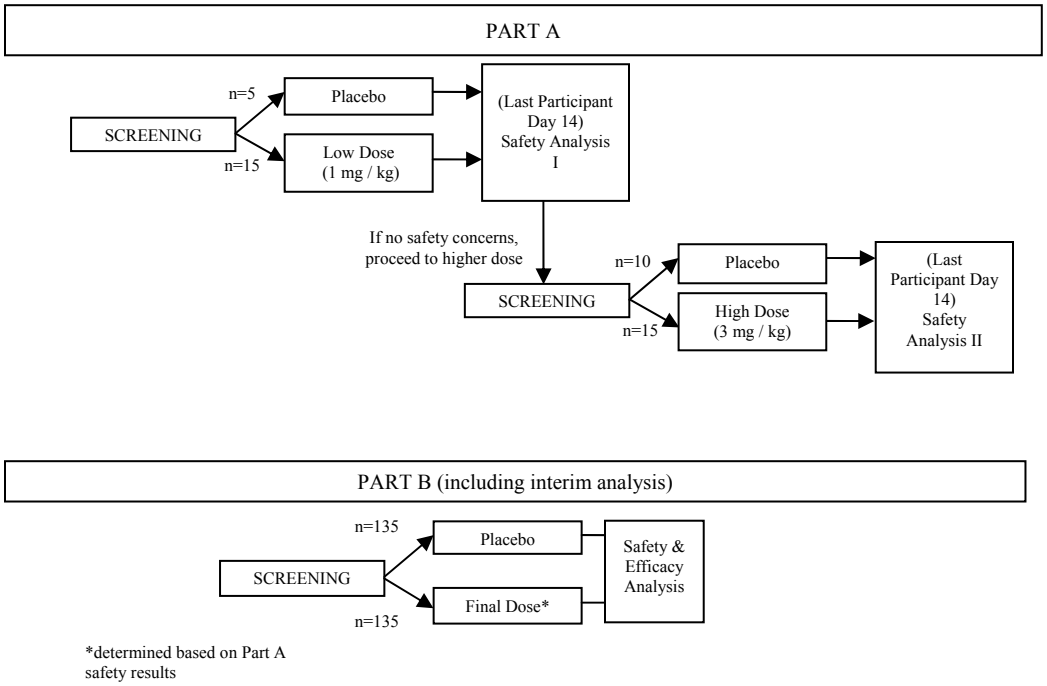
Phase II/III, international, multiple dose, randomized, double blinded, placebo-controlled study. A total of approximately 315 study participants will be randomized.

Part A will include two cohorts. An initial cohort of 20 participants will be randomized to either placebo or 1 mg/kg per antibody cαStx1 and cαStx2. Following an Independent Data Monitoring Committee (IDMC) review of the safety data once all participants complete Day 14, a second cohort of 25 participants will receive either placebo or 3 mg/kg per antibody cαStx1 and cαStx2. Once all participants in Part A receive treatment and complete follow-up to Day 14, the data will be examined by an IDMC.

In Part B, approximately 270 eligible pediatric participants will be randomized in equal proportions to receive either placebo or cαStx1 and cαStx2 at the final dose selected from Part A.

The following diagram depicts the general study design:

Figure 12 Study Design



6.2 STUDY POPULATION AND RECRUITMENT

The target study population will be children aged between 6 months and 18 years inclusive and presenting with diarrhea (bloody or non-bloody by visual inspection) for no more than 3 consecutive days prior to baseline. Diarrhea is expressed as a change from usual bowel pattern. Change in usual pattern is defined as a change to a looser consistency with an increase of at least 2 additional stools per day.

In order to detect and treat STEC infected patients as early as possible in the disease course, the Biostar OIA® SHIGATOX Assay, a rapid point of care commercial diagnostic test (that has been recently approved by the FDA) will be used to screen for the presence of Shiga toxin (Stx1 and/or Stx2) in stool (see Section 2.3). Compared to the currently available commercial tests, the time to result is much faster, i.e. less than 15 minutes, and can be performed without intervention of a microbiology laboratory.

6.3 METHODS OF EVALUATION

The visit schedule is presented in the table below (Table 8):

Table 8 Visit Schedule

	Day 0*	**Day 1**	**Day 2**	**Day 3**	**Day 5**	**Day 7**	**Day 10**	**Day 14**	**Day 20**	**Day 28**	**Day 42**	**Day 56/ End of Study**	**Month 6**	**Month 12**
Site Visit	x	x	x	x		x		x		x		X		
Telephone Contact					x		x		x		x		x	x

6.3.1 Safety Measures

In addition to adverse event reporting by trained site staff, evaluations of acute hypersensitivity reactions will be performed by the Investigator or delegate at baseline (Day 0), during study drug administration, and 1, 2, 6 and 12 hours after the initial study drug administration. Signs of anaphylaxis will be continuously monitored for 12 hours following study medication administration. These adverse events will be documented in the corresponding section of the Case Report Form (CRF). Sites will be equipped with adrenergics and must make it available for treatment of acute allergic symptoms during administration of study medication. Evaluations for delayed hypersensitivity reactions may include (but not be limited to) one of the following adverse events: cutaneous eruption, arthralgia, or lymphadenopathy occurring more than 24 hours after study drug administration.

Safety evaluations will include standard laboratory tests (biochemistry, hematology, and urinalysis). In addition, in Part A, complement activity (C3, C4 and Ch50) will also be assessed before the infusion and 2 and 14 days after the infusion. This will be done only in Part A due to the amount of blood required for these analyses (approximately 3 mL per time point). ECG evaluations will also be performed in Part A of the study.

A complete physical examination along with vital signs (oral or axillary temperature, respiratory rate, blood pressure and pulse) and hydration assessments will be performed during all site visits for both parts of the study.

IDMC

The IDMC will be responsible for the following:

- To assess the safety and tolerability of two different intravenous doses of the concomitant administration of cαStx1 and cαStx2;
- To determine, based on the results obtained from Part A, the appropriate dose to administer in Part B of the study;
- To determine, based on the results of Part A, the appropriateness of the study design;
- To perform a sample size-estimation in Part B;
- To assure the safeguard of the participating subjects by regular safety reviews of safety data. The IDMC will review the safety data, at a minimum, at the following intervals: when 50 participants have been enrolled and treated and every 100 thereafter. The IDMC will meet at least semi-annually. If at any time during the trial, the number of HUS cases is greater than 10% of the highest expected rate of HUS (i.e. maximum of 30% incidence), the IDMC will convene.

Regulatory approval will be sought prior to resuming study recruitment from Part A to Part B. In addition, the results of the interim analysis in Part B will be provided to the agency for review and approval. The outcome of each IDMC meeting will also be provided to the agency.

Stopping Rules

The IDMC can stop the trial if the investigational drug appears to be causing unexpected harm to participants, or if there is evidence that the risks outweigh the benefits. The trial will be stopped, pending IDMC review and approval to continue, if one participant in Part A (at any dose) or two participants in Part B experience severe anaphylactic reactions.

6.3.2 Efficacy Measures

Efficacy evaluation will be based on the aggregate clinical data obtained from Parts A and B. To assess efficacy, each participant will be examined for presence of STMEs as described in section 3.1. Signs and symptoms will be captured by medical assessments performed by clinical staff and diaries completed by the patient and/or parent.

6.4 STATISTICAL CONSIDERATIONS

6.4.1 Efficacy Statistical Methodology

Efficacy Analyses

The primary efficacy analysis will be a simple comparison of proportions of participants who experience a progression of at least one STME during the study (up to Day 14) as described in Section 3.1.

For the secondary efficacy analyses, binary endpoints, such as occurrence of HUS, will be analyzed as described above for the primary endpoint. Continuous endpoints, such as duration of STMEs, will be analyzed using the Wilcoxon rank-sum test or, for analysis of time to an event when there are losses to follow-up, the logrank test.

Regression modeling will be used to evaluate potential risk factors for the primary and selected secondary endpoints, as well as for complications and/or severity of endpoint events. Factors to be evaluated include biomarkers, clinical symptoms, age, gender, type of strains, and regional variability.

6.4.2 Interim Efficacy Analysis

The IDMC will review the aggregate STME data (i.e. rate, severity, progression) derived from the STPB Disease Severity and Progression Scale in order to assess, prior to initiating Part B of the trial, the appropriateness of the scale, the selection criteria, and sample size. Following the review, the IDMC will recommend going on

to Part B or terminating the trial. If the IDMC recommends continuing, it may recommend changes to the protocol, including, but not limited to, modification of the scale (e.g., scoring, symptom descriptions), the selection criteria, or the sample size required for Part B. Based on the extent of any protocol changes, the IDMC will also recommend either that the Part A data be included in the efficacy evaluation at the end of Part B, or that only Part B data be used and that more subjects be randomized to provide sufficient power for Part B alone.

The IDMC will conduct one unblinded efficacy analysis for the purpose of re-estimating sample size or recommending that the trial be terminated for futility (i.e., lack of efficacy). This analysis will be planned to occur about midway through Part B, when Day 14 data should be available for 50% of the participants to be included in the efficacy analysis. The interim analysis will use the approach of Lan and Trost[70]. The IDMC will evaluate unblinded data both for any STME (the primary endpoint) and for HUS and will recommend that the study continue as planned, continue with an increased sample size, or be terminated due to a very small chance that a statistically significant effect of active treatment will be found. Termination for futility would be made on the basis of the STME analysis, but continuation with an increased study size might occur because of either endpoint (STMEs or HUS). Increased sample size might be recommended if the conditional power for HUS analysis was larger than expected, either because HUS rates were higher than anticipated or because the observed effect of active treatment on HUS was larger than anticipated.

6.4.3 Sample Size Calculation

For estimation of the needed sample size for the primary analysis, we assume an STME progression rate of 30%[71] in placebo recipients and 15% (i.e., a 50% reduction) in participants treated with active drug. For 80% power and a z-test at the one-sided 0.025 significance level, approximately 121 participants per group are needed. To allow for approximately 20% attrition either from ineligibility based on post-screening stool testing or loss to follow-up for the primary endpoint, we plan to

randomize 150 participants into each of the two groups, or a total of 300 participants. Assuming 15 participants in each group will come from Part A of the study, we plan to randomize an additional 135 participants into each group, or a total of 270 participants, in part B. The increased sample size should be adequate to keep the power at or above 80% if there is loss to follow-up and the analysis is therefore a comparison of Kaplan-Meier estimates.

7. SUMMARY

STEC infections are associated with enteropathic and thombotic extra-intestinal manifestations which can lead to the severe life-threatening condition, HUS, especially in children. There is no standard treatment for HUS and there are no predictor factors for the disease. STEC infection and HUS rates are extremely low, thereby qualifying them as orphan diseases.

The challenge to develop a therapeutic intervention in these indications is not only the rarity of patients but the acute condition of the disease. Caprion developed monoclonal antibodies (cαStx1 and cαStx2) against the Shiga toxins 1 and 2, produced by STEC, that have been associated with the complications of STEC disease from enteropathy to renal dysfunction and encephalopathy. Caprion proposes to treat the early stages of STEC disease because early interruption of the Stx-mediated cascade by monoclonal antibodies is likely to have an impact on severity of the illness and associated complications. For this purpose, Shiga toxin-mediated events have been identified and the STPB Disease Severity and Progression Scale has been developed for the evaluation of the progression of STMEs during the acute phases of the disease.

The proof of concept of Caprion's monoclonal antibodies has been demonstrated in animal models and the toxicology studies have indicated that the products are not associated with any overt signs of toxicity. In addition, the monoclonal antibodies were administered to 50 healthy adult volunteers and were found to be generally safe and well tolerated and have not caused allergic or hypersensitivity reactions.

Due to the rarity of patients, a Phase II/III design is proposed for the evaluation of the monoclonal antibodies in children. This design does not in any way compromise the safety of the patients however it will maximize the quantity of data capture by evaluating efficacy on as many randomized patients as possible.

The objectives of the first part of the study are safety and dose finding. In the second part, the objectives are the evaluation of the safety and efficacy of cαStx1 and cαStx2 by clinical monitoring of the progression of toxin effect. Toxin effect will be evaluated through the monitoring of STMEs. The primary efficacy endpoint will be the number of subjects who had a STME that progressed during the study up to study Day 14. STMEs will be graded according to the STPB Disease Severity and Progression Scale.

Caprion's monoclonal antibodies against the Shiga toxins are a therapeutic approach that can be tested in the target patient population with the appropriate design and efficacy endpoints. In addition, adequate safety measures in the definitive clinical study will be in place to protect the participating subjects and to allow for the continued evaluation of the risk to benefit ratio.

8. REFERENCES

[1] Haque QM, Sugiyama A, Iwade Y, Midorikawa Y & Yamauchi T. Diarrheal and Environmental Isolates of *Aeromonas* spp. Produce a Toxin Similar to Shiga-Like Toxin 1. Current Microbiology 32(5):239-245 (1996)

[2] Herold S, Karch H & Schmidt H. Shiga toxin-encoding bacteriophages-genomes in motion. Int. J. Med. Microbiol. 294:115-121 (2004)

[3] Strauch E, Lurz R & Beutin L. Characterization of a Shiga Toxin-Encoding Temperate Bacteriophage of Shigella sonnei. Infect. Immun. 69(12):7588-7595 (2001)

[4] Paton JC & Paton AW. Pathogenesis and Diagnosis of Shiga Toxin-Producing *Escherichia coli* Infections. Clin. Microb. Rev. 11(3):450-479 (1998)

[5] Tarr P, Gordon CA & Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet 365:1073-1086 (2005)

[6] Thorpe CM. Shiga Toxin-Producing Escherichia coli Infection. CID 38(9):1298-1303 (2004)

[7] Bell BP, Griffin PM, Lozano P, Christie DL, Kobayashi JM & Tarr PI. Predictors of Hemolytic Uremic Syndrome in Children During a Large Outbreak of Escherichia coli O157:H7 Infections. Pediatrics 100(1):E12 (1997)

[8] Chandler WL, Jelacic S, Boster DR, Ciol MA, Williams GD, Watkins SL, Igarashi T & Tarr PI. Prothrombotic Coagulation Abnormalities Preceding the Hemolytic-Uremic Syndrome. N. Engl. J. Med. 346:23-32 (2002)

[9] Slutsker L, Ries AA, Greene KD, Wells JG, Hutwagner L & Griffin PM. AIM 126(7): 505-513 (1997)

[10] Ochoa TJ & Cleary TG. Epidemiology and spectrum of disease of *Escherichia coli* 0157. Curr. Opin. Infect. Dis. 16:259-263 (2003)

[11] Elliott EJ & Robins-Browne RM. Hemolytic Uremic Syndrome. Curr. Probl. Adolesc. Health Care, 310-330 (2005)

[12] Garg AX, Moist L, Matsell D, Thiessen-Philbrook HR, Haynes RB, Suri RS, Salvadori M, Ray J & Clark W, for the Walkerton Health Study Investigators. Risk of hypertension and reduced kidney function after acute gastroenteritis from bacteria-contaminated drinking water. CMAJ 173:261-268 (2005)

[13] Garg AX, Marshall J, Salvadori M, Thiessen-Philbrook HR, Macnab J, Suri RS, Haynes RB, Pope J, Clark W, on behalf of the Walkerton Health Study Investigators. A

gradient of acute gastroenteritis was characterized, to assess risk of long-term health sequelae after drinking bacterial-contaminated water. J. Clin. Epid. 59:421-428 (2006)

[14] Andreoli SP, Trachtman H, Acheson DWK, Siegler RL & Obrig TG. Hemolytic uremic syndrome: epidemiology, pathophysiology, and therapy. Ped. Nephrol. 17:293-298 (2002)

[15] Hurley BP, Thorpe CM & Acheson DWK. Shiga Toxin Translocation across Intestinal Epithelial Cells Is Enhanced by Neutrophil Transmigration. Infect. Immun. 69(10):6148-6155 (2001)

[16] Siegler RL, Pysher TJ, Tesh VL & Taylor FB. Response to Single and Divided Doses of Shiga Toxin-1 in a Primate Model of Hemolytic Uremic Syndrome. J. Am. Soc. Nephrol. 12:1458-1467 (2001)

[17] Siegler RL. Postdiarrhoeal Shiga Toxin-Mediated Hemolytic Uremic Syndrome. JAMA 290(10):1379-1381 (2003)

[18] Kamitsuji H, Nonami K, Murakami T, Ishikawa N, Nakayama A & Umeki Y. Elevated tissue factor circulating levels in children with hemolytic uremic syndrome caused by verotoxin-producing E. coli. Clin. Nephrol. 53(5):319-324 (2000)

[19] Nestoridi E, Kushak RI, Duguerre D, Grabowski EF & Ingelfinger JR. Up-regulation of tissue factor activity on human proximal tubular epithelial cells in response to Shiga toxin. Kidney Int. 67(6):2254-2266 (2005)

[20] Nestoridi E, Tsukurov O, Kushak RI, Ingelfinger JR & Grabowski EF. Shiga toxin enhances functional tissue factor on human glomerular endothelial cells: implications for the pathophysiology of hemolytic uremic syndrome. J. Thromb. Haemost. 3(4):752-762 (2005)

[21] Schapiro FB, Lingwood C, Furuya W & Grinstein S. pH independent retrograde targeting of glycolipids to the Golgi complex. Am J Physiology 274 (Cell Physiology 43):C319-332 (1998)

[22] Kurioka T, Yunou Y & Kita E. Enhancement of Susceptibility to Shiga Toxin-Producing Escherichia Coli O157:H7 by Protein Calorie Malnutrition in Mice. Infect. Immun. 66(4):1726-1734 (1998)

[23] Stratton JD & Warwicker P. Successful treatment of factor H-related haemolytic uraemic syndrome. Nephrol. Dial. Transplant 17:684-685 (2002)

[24] Klein EJ, Stapp JR, Clausen CR, Boster DR, Wells JG, Qin X, Swerdlow DL & Tarr PI. Shiga toxin-producing Escherichia coli in children with diarrhea: A prospective point-of-care study. J. Pediatr. 141(2):172-177 (2002)

[25] Elliott EJ, Robins-Browne RM, O'Loughlin EV, Bennett-Wood V, Bourke J, Henning P, Hogg GG, Knight J, Powell H, Redmond D and Contributors to the Australian Paediatric Surveillance Unit. Nationwide study of haemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. Arch. Dis. Child. 85:125-131 (2001)

[26] Yamada Y, Fujii J, Murasato Y, Nakamura T, Hayashida Y, Kinoshita Y, Yutsudo T, Matsumoto T & Yoshida S-I. Brainstem Mechanisms of Autonomic Dysfunction in Encephalopathy-Associated Shiga Toxin 2 Intoxication. Ann. of Neur. 45:716-723 (1999)

[27] Garcia A, Bosques CJ, Wishnok JS, Feng Y, Karalius BJ, Butterton JR, Schauer DB, Rogers AB & Fox JG. Renal Injury Is a Consistent Finding in Dutch Belted Rabbits Experimentally Infected with Enterohemorrhagic *Escherichia coli*. JID 193:1125-1134 (2006)

[28] Wadolkowski EA, Sung LM, Burris JA, Samuel JE & O'Brien AD. Acute renal tubular necrosis and death of mice orally infected with Escherichia coli strains that produce Shiga-like toxin type II. Infect. Immun. 58(12):3959–3965 (1990)

[29] Tesh VL, Burris JA, Owens JW, Gordon VM, Wadolkowski EA, O'Brien AD & Samuel JE. Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. Infect. Immun. 61(8):3392-3402 (1993)

[30] Nakagawa I, Nakata M, Yamamura T, Wakisaka S, Kawabata S & Hamada S. Infection and pathogenesis of a murine strain of *Escherichia coli* with genetically introduced Shiga toxin type I operon in conventional mice. Microbial. Pathogenesis 33:63-72 (2002)

[31] Taylor FB, Tesh VL, DeBault L, Li A, Chang ACK, Kosanke SD, Pysher TJ & Siegler RL. Characterization of the Baboon Responses to Shiga-Like Toxin. Descriptive Study of a New Primate Model of Toxic Responses to Stx-1. AJP 154(4):1285-1299 (1999)

[32] Keepers TR, Psotka MA, Gross LK & Obrig TG. A Murine Model of HUS: Shiga Toxin with Lipopolysaccharide Mimics the Renal Damage and Physiologic Response of Human Disease. J. Am. Soc. Nephrol. 17(12):3404-3414 (2006)

[33] Yamamoto ET, Mizuno M, Nishikawa K, Miyazawa S, Zhang L, Matsuo S & Natori Y. Shiga Toxin 1 Causes Direct Renal Injury in Rats. Infect. Immun. 73(11):7099-7106 (2005)

[34] Kehl SC. Role of the Laboratory in the Diagnosis of Enterohemorrhagic *Escherichia coli* Infections. J. Clin. Microbiol. 40(8):2711-2715 (2002)

[35] Park C. Rapid Detection (<20 minutes) of Shiga-Toxin Producing Escherichia coli Directly from Human Stools, Broth Cultures, and Agar Plates by Optical Immunoassay (Innerness Medical-Biostar Inc. OIA SHIGATOX). Poster presented at the 6th International Symposium on Shiga Toxin (Verocytotoxin) – Producing Escherichia coli Infections (VTEC 2006), Melbourne, Australia, 29 October to 1 November 2006

[36] Frenzen PD, Drake A, Angulo FJ. The Emerging Infections Program Foodnet Working Group. Econonic Cost of Illness Due to Escherichia coli O157 Infections in the United States. J. Food. Prot. 68(12):2623-2630 (2005)

[37] Frenzen PD. Foodborne Illness Cost Calculator: STEC O157 (in 2005). USDA Economic Research Service. http//www.ers.usda.gov/data/foodborne illness/ecoli.Intro.asp (March 31, 2006)

[38] Mead PS, Slutsker L, Dietz V, McCraig LF, Bresee JS, Shapiro C, Griffin PM & Tauxe RV. Food-Related Illness and Death in the United States. Emer. Infect. Dis. 5(5):607-625 (1999)

[39] Rangel JM, Sparling PH, Crowe C, Griffin PM & Swerdlow DL. Epidemiology of Escherichia coli O157:H7 Outbreaks, United States, 1982-2002. Emer. Infect. Dis. 11(4):603-609 (2005)

[40] Buzby J, Economics of Foodborne Disease: *E. coli* (2002.) USDA Economic Research Service. http://www.ers.usda.gov/Briefing/FoodborneDisease/ecoli.htm (April 21, 2003)

[41] Lopez EL, Devoto S, Fayad A, CAnepa C, Morrow AL, Cleary TG. Association between severity of gastrointestinal prodrome and long-term prognosis in classic hemolytic-uremic syndrome. J Pediatrics 120(2 Pt 1):210-5 (1992)

[42] Bitzan M & Riviere M. Clinical Proxy Markers for Shiga Toxin Diseases. Poster presented at the American Society for Microbiology Biodefense Research Meeting (ASM BioDefense 2006), Washington, DC, USA, 15-18 February 2006

[43] Bitzan M & Riviere M. Towards a Quantitative Disease Score for Shiga Toxin Producing Escherichia coli Infections Poster presented at the International Conference on Emerging Infections Disease (ICEID 2006), Atlanta, GA, USA, 19-22 March 2006

[44] Bitzan M, Mehran M, Repas K, Prado V& Riviere M. Towards a Quantitative Disease Scale for Shiga Toxin Producing Escherichia coli Infections. Poster presented at the 6th International Symposium on Shiga Toxin (Verocytotoxin) – Producing Escherichia coli Infections (VTEC 2006), Melbourne, Australia, 29 October to 1 November 2006

[45] Wong DL & Baker C. Pain in Children: Comparison of Assessment Scales. Pediatr Nurs.14:9-17 (1988)

[46] Ake JA, Jelacic S, Ciol MA, Watkins SL, Murray KF, Christie DL, Klein EJ & Tarr PI. Relative Nephroprotection During *Escherichia coli* O157:H7 Infections: Association With Intravenous Volume Expansion. Pediatrics 115(6):673-680 (2005)

[47] Trachtman H, Cnaan A, Christen E, et al. Effect of an Oral Shiga Toxin-Binding Agent on Diarrhea-Associated Hemolytic Uremic Syndrome in Children: A Randomized Controlled Trial. JAMA 290(10):1337-1344 (2003)

[48] Smith MJ, Teel LD, Carvalho HM, Melton-Celsa AR & O'Brien AD. Development of a hybrid Shiga holotoxoid vaccine to elicit heterologous protection against Shiga toxins types 1 and 2. Vaccine 24(19):4122-4129 (2006)

[49] Suzaki Y, Ami Y, Nagata N, Naito S, Kato H, Taneichi M, Takahashi M, Komiya T, Satoh S, Gondaira F, Sugiyama J, Nakano Y, Mori M, Komuro K & Uchida T. Protection of Monkeys against Shiga Toxin Induced by Shiga Toxin-Liposome Conjugates. International Archives of Allergy and Immunology 127:294-298 (2002)

[50] Bielaszewska M, Clarke I, Karmali MA and Petric M. Localization of Intravenously Administered Verocytotoxins (Shiga-Like Toxins) 1 and 2 in Rabbits Immunized with Homologous and Heterologous Toxoids and Toxin Subunits. Infect. Immun. 65(7):2509-2516 (1997)

[51] Marcato P, Mulvey G, Read RJ, Vander Helm K, Nation PN & Armstrong GD. Immunoprophylactic Potential of Cloned Shiga Toxin 2 B Subunit. JID 183(3):435-443 (2001)

[52] van de Kar NCAJ, Monnens LAH, Karmali MA & van Hinsbergh VWM. Tumor Necrosis Factor and Interleukin-1 Induce Expression of the Verocytotoxin Receptor Globotriaosylceramide on Human Endothelial Cells: Implications for the Pathogenesis of the Hemolytic Uremic Syndrome. Blood 80(11):2755-2764 (1992)

[53] Karch H, Russmann H, Schmidt H, Schwarzkopf A & Heesemann J. Long-Term Shedding and Clonal Turnover of Enterohemorrhagic Escherichia coli O157 in Diarrheal Diseases. J. Clin. Microbiol. 33(6):1602-1605 (1995)

[54] Mukherjee J, Chios K, Fishwild D, Hudson D, O'Donnell S, Rich SM, Donohue-Rolfe A & Tzipori S. Production and Characterization of Protective Human Antibodies against Shiga Toxin 1. Infect. Immun. 70(10):5896-5899 (2002)

[55] Yamagami S, Motoki M, Kimura T, Izumi H, Takeda T, Katsuura Y and Matsumoto Y. Efficacy of Postinfection Treatment with Anti–Shiga Toxin (Stx) 2 Humanized Monoclonal Antibody TMA-15 in Mice Lethally Challenged with Stx-Producing *Escherichia coli.* JID 184:738–742 (2001)

[56] Sheoran AS, Chapman-Bonofiglios S, Harvey BR, Mukherjee J, Georgiou G, Donohue-Rolfe A & Tzipori S. Human Antibody against Shiga Toxin 2 Adminsitered to Piglets after the Onset of Diarrhea Due to a *Escherichia coli* O157:H7 Prevents Fatal Systemic Complications. Infect. Immun. 73(8):4607-4613 (2005)

[57] Arnon SS, Schechter R, Maslanka SE, Jewell NP & Hatheway CL. Human Botulism Immune Globulin for the Treatment of Infant Botulism. N. Engl. N. Med. 354:462-471 (2006)

[58] Connor EM, McClain JB, Sorrentino M, Hirsch RL, Top, Jr, FH. Safety Surveillance in Children Treated with Palivizumab (PLV). Journal of Perinatology (2002) 22, Abstracts from AAP-Section on Perinatal Pediatrics.

[59] Baselga J, Pfister D, Cooper MR, Cohen R, Burtness B, Bos M, D'Andrea G, Seidman A, Norton L, Gunnett K, Falcey J, Anderson V, Waksal H & Mendelsohn J. Phase I Studies of Anti-Epidermal Growth Factor Receptor Chimeric Antibody C225 Alone and in Combination With Cisplatin. J. Clin. Oncol. 18(4):904-914 (2000)

[60] Davis TA, Grillo-Lopez AJ, White CA, McLaughlin P, Czuczman MS, Link BK, Maloney DG, Weaver RL, Rosenberg J & Levy R. Rituximab Anti-CD20 Monoclonal Antibody Therapy in Non-Hodgkins Lymphoma: Safety and Efficacy of Re-Treatment. J. Clin. Oncol. 18(17):3135-3143 (2000)

[61] Kon OM, Sihra BS, Compton CH, Leonard TB, Kay AB & Barnes NC. Randomised, dose-ranging, placebo-controlled study of chimeric antibody to CD4 (keliximab) in chronic severe asthma. Lancet 352(9134):1109-13 (1998)

[62] Hommes DW, Mikhajlova TL, Stoinov S, Stimac D, Vucelic B, Lonovics J, Zakuciova M, D'Haens G, Van Asche G, Ba S, Lee S & Pearce T. Fontolizumab, a humanised anti-interferon γ antibody, demonstrates safety and clinical activity in patients with moderate to severe Crohn's disease. Gut 55:1131-1137 (2006)

[63] Idusogie EE, Presta LG, Gazzano-Santoro H Totpal K, Wong PY, Ultsch M, Meng YG & Mulkerrin MG.. Mapping of the C1q Binding Site on Rituxan, a Chimeric Antibody with a Human IgG1 Fc. J Immunol. 164: 4178-4184 (2000)

[64] Imai M, Landen C, Ohta R, Cheung N-KV & Tomlinson S. Complement-Mediated Mechanisms in Anti-GD2 Monoclonal Antibody Therapy of Murine Metastatic Cancer. Cancer Research 65: 10562-10568 (2005)

[65] Robson WLM, Fick GH, Jadavji T & Leung AKC The use of intravenous gammaglobulin in the treatment of typical hemolytic uremic syndrome. Pediatric Nephrology 5:289-292 (1991)

[66] Bitzan M, Karch H, Altrogge H, Strehlau J & Blaker F. Hemolytic-uremic syndrome associated with a variant Shiga-like cytotoxin of Escherichia coli O111. Pediatr Infect Dis J. 2:128-32 (1988)

[67] Razai A, Garcia-Rodriguez C, Lou J, Geren IN, Forsyth CM, Robles Y, Tsai R, Smith TJ, Smith LA, Siegel RW, Feldhaus M & Marks JD. Molecular Evolution of Antibody Affinity for Sensitive Detection of Botulinum Neurotoxin Type A. J. Mol. Biol. 351(1):158-169 (2005)

[68] Edwards AC, Melton-Celsa AR, Arbuthnott K, Stinson JR, Schmitt CK, Wong HC & O'Brien AD. Escherichia coli O157:H7 and Other Shiga Toxin-Producing E. coli Strains. (ed. Kaper JB & O'Brien AD) 388-392; Washington D.C.; (1998)

[69] Dowling TC, Chavaillaz PA, Young DG, Melton-Celsa A, O'Brien A, Thuning-Roberson C, Edelman R & Tacket CO. Phase I Safety and Pharmacokinetic Study of Chimeric Murine-Human Monoclonal Antibody cαStx2 Administered Intravenously to Healthy Adult Volunteers. Anti. Agents and Chem. 49:1808-1812 (2005)

[70] Lan KKG & Trost DC. Estimation of parameters and sample size re-estimation. Proceedings of the American Statistical Association, 48-51 (1997)

[71] Blanco JE, Blanco M, Alonso MP, Mora A, Dahbi G, Coira MA & Blanco J. Serotypes, Virulence Genes, and Intimin Types of Shiga Toxin (Verotoxin)-Producing *Escherichia coli* Isolates from Human Patients: Prevalence in Lugo, Spain from 1992 through 1999. J. Clin. Microbiol. 42(1):311-319 (2004)