CATO RESEARCH

Westpark Corporate Center
4364 South Alston Avenue
Durham NC 27713-2220 USA
Phone: 919-361-2286
Fax: 919-361-2290
**www.cato.com**

12 March 2007

Sohail Mosaddegh, Pharm.D., R.Ph.
Advisors and Consultants Staff
Food and Drug Administration
Center for Drug Evaluation and Research
HFD-21, Room 1098
5630 Fishers Lane
Rockville, MD 20852-1734

**Re: Teijin America, Inc.**
**TMA-15**
**Advisory Committee Meeting Briefing Document**

Dear Dr. Mosaddegh:

Reference is made to BB-IND 9773 for TMA-15, a humanized monoclonal antibody being developed for the prevention of HUS in pediatric patients with severe watery diarrhea or bloody diarrhea and confirmed STEC infection. On behalf of the sponsor, Teijin America, Inc. (TAI), Cato Research is submitting a briefing package for the Advisory Committee Meeting on 12 April 2007 for products for the prevention and/or treatment of disease caused by Shiga-toxin producing bacteria.

Per your request on 27 February 2007, Cato Research is submitting 5 paper and 40 electronic copies of this package. This package includes the investigator lists requested as an appendix to the briefing document.

A copy of the TAI Transfer of Regulatory Contact from TAI to CATO is attached. Please direct all communications regarding this package to my attention, and I will keep TAI fully informed. If you have any questions or need additional information, please do not hesitate to contact me at 919-361-2286.

Sincerely,

Lynda Sutton
Chief Operating Officer

# PROVISION FOR REGULATORY CONTACT/ASSISTANCE

For a consideration as provided in the Master Service Agreement, and to the extent shown below, Teijin America, Inc., (TAI) authorizes Cato Research (CATO) to provide professional assistance and to contact and interact with the FDA regarding the IND for the use of TMA-15 to prevent the development or to decrease the incidence and severity of hemolytic uremic syndrome (HUS) and associated sequelae of Shiga-like toxin producing *Escherichia coli* (STEC) infections in patients.

Acceptance of this authorization for contact does not constitute assumption of liability by CATO for assuring the safety and/or efficacy of TMA-15 to TAI or any third party.

TAI requests that the FDA direct all communications and correspondence with regard to the IND for TMA-15 directly to CATO, attention of:

Lynda Sutton
Cato Research
200 Westpark Corporate Center
4364 South Alston Avenue
Durham, North Carolina 27713-2280
Telephone: [919] 361-2286
Fax: [919] 361-2290

IN WITNESS WHEREOF, the parties have executed this Agreement or caused this Agreement to be executed by their duly authorized representatives in duplicate originals on the dates shown below.

This agreement shall be effective on the latter of the signature dates shown below.

| CATO RESEARCH | | TEIJIN AMERICA, INC. | |
|---|---|---|---|
| By: | Lynda Sutton | By: | Yoichi Matsumoto |
| Title: | COO | Title: | President |
| Date: | 9/15/2006 | Date: | 9/12/2006 |

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# ADVISORY COMMITTEE MEETING BRIEFING DOCUMENT

## TEIJIN AMERICA, INC.

## 12 March 2007

**AVAILABLE FOR PUBLIC DISCLOSURE WITHOUT REDACTION**

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | adverse event |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| $AUC_{(0\text{-}inf)}$ | area under the curve from time 0 to infinity |
| $AUC_{(0\text{-}t)}$ | area under the curve from time 0 to time t |
| BD | bloody diarrhea |
| BLA | Biologics License Application |
| BLQ | below the level of quantification |
| BUN | blood urea nitrogen |
| CDC | Centers for Disease Control and Prevention |
| CFR | Code of Federal Regulations |
| $C_{max}$ | maximum concentration |
| CNS | central nervous system |
| CPK | creatine phosphokinase |
| DAB | 3,3-diaminobenzidine tetrahydrochloride |
| EHEC | enterohemorrhagic *Escherichia coli* |
| EIEC | enteroinvasive *Escherichia coli* |
| EPEC | enteropathogenic *Escherichia coli* |
| ETEC | enterotoxigenic *Escherichia coli* |
| FDA | Food and Drug Administration |
| FITC | fluorescein isothyocyanate |
| Gb3 | globotriaosylceramide |
| GLP | Good Laboratory Practices |
| HUS | hemolytic uremic syndrome |
| IC-STAT | immunocard STAT test |
| ICF | informed consent form |
| IgG | immunoglobulin G |
| IND | Investigational New Drug application |
| $\lambda_z$ | terminal elimination rate constant |
| MAb | monoclonal antibody |
| NA | not applicable |
| NOAEL | no observed adverse effect level |
| NOS | not otherwise specified |
| PCR | polymerase chain reaction |
| PEHEC | premier EHEC test |
| PK | pharmacokinetics |
| SAE | serious adverse event |
| SLT | Shiga-like toxin |
| SLT-1 | Shiga-like toxin 1 |
| SLT-2 | Shiga-like toxin 2 (also referred to as Stx2) |
| STEC | Shiga-like toxin-producing *Escherichia coli* |
| Stx | Shiga toxin |
| Stx2 | Shiga toxin type 2 (also referred to as SLT-2) |
| SOC | system organ class |
| $t_{½}$ | half-life |
| TAI | Teijin America, Inc. |
| TBD | to be determined |
| TK | toxicokinetics |
| $T_{max}$ | time to maximum plasma concentration |
| TTP | thrombotic thrombocytopenic purpura |
| WD | watery diarrhea |

# 1.0 OVERVIEW OF EXPERT PANEL MEETING BRIEFING DOCUMENT

Teijin America, Inc. (TAI), headquartered in Princeton, New Jersey, is a subsidiary of Teijin Pharma Ltd., a Japanese pharmaceutical company engaged in the research and development of TMA-15, a biological product for the prevention of hemolytic uremic syndrome (HUS) in pediatric patients with severe watery diarrhea or bloody diarrhea associated with confirmed infection by Shiga-like toxin-producing *Escherichia coli* (STEC). TMA-15 is a recombinant humanized monoclonal antibody (MAb) that binds to the B-subunit of Shiga-like toxin 2 (SLT-2), a member of the Shiga toxin (Stx) family. SLT-2, also referred to as "Stx2", is produced by STEC, and is the presumed causative agent for developing HUS after STEC infection.

The Food and Drug Administration (FDA) has invited TAI to participate in an Advisory Committee Meeting scheduled for 12 April 2007 to discuss the development of treatments for the prevention of HUS. This briefing document is intended to facilitate discussion between TAI and meeting participants by providing summary background information regarding the ongoing development, and problems encountered in designing and conducting clinical studies, of treatments for the prevention of HUS after STEC infection.

This pre-meeting information package is organized as follows:

- Section 2.0 describes the purpose, objectives, and attendees for the 12 April 2007 Advisory Committee Meeting.
- Section 3.0 provides an overview of HUS, STEC infection, and SLT-2, and describes treatment strategies for STEC infection.
- Section 4.0 introduces TMA-15 and the rationale for its development and provides supporting pre-clinical data.
- Section 5.0 provides clinical data TAI has obtained to date during the development of TMA-15.
- Section 6.0 discusses issues faced in designing clinical efficacy studies of a product for preventing HUS after STEC infection.

- Section 7.0 provides a proposed rationale for the approval of a treatment to prevent HUS after STEC infection based on combined animal and human data, and Section 8.0 provides a summary conclusion.

- Appendices provide additional detailed information: Details of stec infection incidence calculations, a draft annotated label, and, per a request from FDA, a list of TMA-15 Principal Investigators.

## 2.0 MEETING PURPOSE

The purpose of TAI's participation in the Advisory Committee Meeting is to present TAI's approach to the development of a treatment for the prevention of HUS after STEC infection, the challenges encountered, and the data obtained to date regarding the safety and efficacy of TMA-15. TAI also wishes to discuss what constitutes a practical package of nonclinical and clinical data needed for approval of a product to prevent HUS after STEC infection.

## 2.1 MEETING OBJECTIVES

The objectives of this meeting are as follows:

- To discuss the clinical and nonclinical issues faced when developing products for the prevention of HUS after STEC infection
- To review the epidemiology and pathophysiology of STEC-induced HUS and its current standard of care
- To summarize the results of studies on the safety and potential efficacy of TMA-15 for the prevention of HUS after STEC infection
- To discuss the role of animal data in the development of products intended to prevent HUS after STEC infection
- To discuss the human database needed to support approval of products intended to prevent HUS after STEC infection

## 2.2 PROPOSED LIST OF ATTENDEES

Teijin America, Inc. will be represented by the following:

- Sheldon Brookman, Ph.D., Executive Director, Clinical Research and Regulatory Affairs
- Ronald Harning, Ph.D., Director, Clinical Research and Regulatory Affairs
- Yoichi Matsumoto, Ph.D., President

- Keiji Komoriya, Ph.D., Vice President
- Hiroaki Sato, Ph.D., Manager

Also attending at the request of TAI:

- William D. Schwieterman, M.D, Consultant
- Eduardo L. López, M.D., Hospital de Niños, Buenos Aires, Argentina
- Joy Cavagnaro, Ph.D. Nonclinical Consultant

On behalf of TAI, Cato Research will be represented by the following:

- Noah Byrd, Ph.D., R.A.C., Regulatory Scientist
- Allen Cato, M.D., Ph.D., President
- Eric Harris, Ph.D., R.A.C., Project Manager
- Kristen Minshew, B.S., Project Coordinator
- Myron Peterson, M.D., Ph.D., F.A.A.P., Director, Medical Affairs
- Lynda Sutton, B.S., Chief Regulatory Officer

# 3.0 INTRODUCTION

## 3.1 HUS

HUS is a serious and life-threatening condition characterized by acute renal impairment, microangiopathic hemolytic anemia, and thrombocytopenia[1,2]. HUS is the leading cause of acute renal failure in children[3]. At present, an effective treatment to reduce the incidence or severity of HUS does not exist and current therapy is largely supportive[4].

Early symptoms of STEC infection include colitis and watery diarrhea, which often progresses to bloody diarrhea (BD). The rate at which STEC infection progresses to HUS is generally reported at approximately 5% of BD cases, but has been reported to be as high as 15%[5, 6]. Figure 1 illustrates the general timeline from STEC infection to development of HUS, including rates of progression.

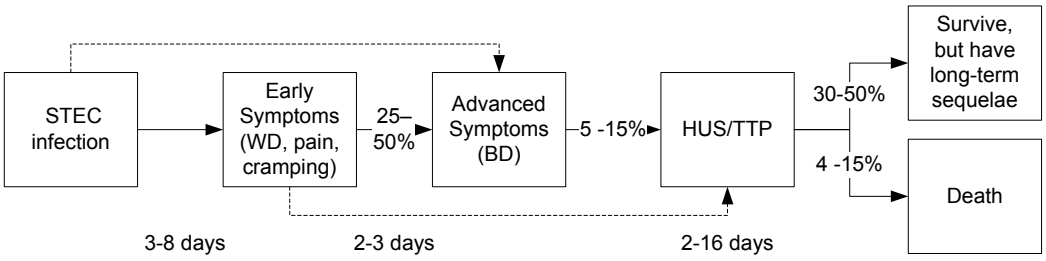


**Figure 1. Timeline and Outcomes of STEC Progression to HUS**
WD = watery diarrhea; BD = bloody diarrhea; HUS = hemolytic uremic syndrome; TTP = thrombotic thrombocytopenic purpura. Dashed lines represent alternative modes of progression.

## 3.2 STEC

The primary cause of HUS is gastrointestinal infection by pathogenic strains of *Escherichia coli* (*E. coli*)[3]. Pathogenic *E. coli* strains usually produce pyogenic infections and diarrhea, and are classified as enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), or enterohemorrhagic (EHEC, STEC). In the United States, *E. coli* of the O157:H7 serotype are the most common cause of STEC infections[7]. *E. coli* O157:H7 was first identified in 1982 as a human pathogen spread by contaminated beef[8].

## 3.3 SLT-2

In addition to *E. coli* O157:H7, a range of other STEC serotypes has been identified; these serotypes are more prevalent in countries other than the United States (e.g. Argentina, Chile, Germany, Great Britain[5,9-11]), but all produce toxins that can cause HUS. The initial event underlying the progression of STEC infection to HUS involves cell injury caused by a Shiga-like toxin (SLT) produced by STEC. Different STEC strains produce different SLTs, i.e., Shiga-like toxin 1 (SLT-1) or Shiga-like toxin 2 (SLT-2) or both[12]. Both SLT-1 and SLT-2 are associated with complications of STEC infections, but SLT-2 appears to be the primary causative agent for developing HUS. SLT-2 is approximately 400-fold more toxic than SLT-1 when injected parenterally in mice, and O157 that produce SLT-2 more frequently cause HUS than O157 that produce SLT-1 only[12-15].

Once SLT-2 enters the bloodstream, it can bind, via its B-subunits, to glycolipid receptors, specifically globotriaosylceramide (Gb3) receptors, including those found on endothelial cells of the glomeruli and arterial cells in the kidney. Once SLT-2 is internalized, the A-subunit separates from the B-subunits and is cleaved into A1 and A2 fragments. The A1-subunit inactivates ribosomes, and thereby induces cellular toxicity. SLT-2 targets endothelial cells and injury to microvascular endothelial cells is the key event underlying the pathogenesis of HUS. This pathophysiological mechanism is illustrated in Figure 2.

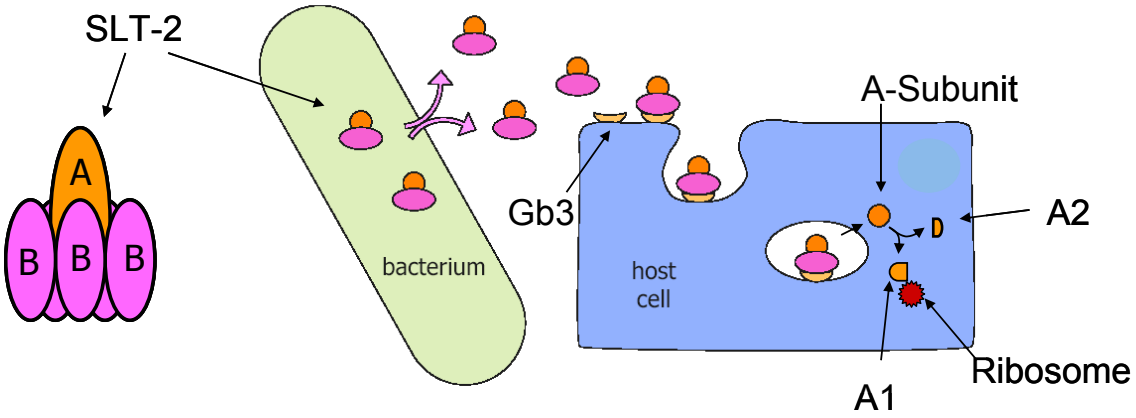


**Figure 2. Mechanism of SLT-2 Cellular Toxicity.**

The cascade of cellular events triggered by SLT-2 ultimately leads to the clinical signs of HUS, including glomerular thrombosis, oliguric renal failure, hemolysis, and thrombocytopenia. Given the mechanism of SLT-2-induced cellular toxicity, treatments that target SLT-2 are key to impeding the progression of HUS after STEC infection.

## 3.4 CURRENT TREATMENT OF STEC INFECTION AND PREVENTION OF HUS

The prognosis for patients with HUS is poor. At present, no effective or approved treatment to reduce the incidence or severity of HUS is available. Treatment for colitis and diarrhea is largely supportive, including oral and intravenous fluid replacements. Treatment of these conditions with antibiotics and antimotility agents remains controversial. There is no evidence that antibiotics improve the course of disease, and treatment with some antibiotics may precipitate kidney complications[16,17]. Even with comprehensive medical care, 5% to 15% of subjects who develop HUS from STEC infection die, while 30% to 50% of the surviving population develops long-term sequelae, such as chronic renal disease, including hypertension, and neuronal complications (e.g., seizures, blindness, paralysis)[18-20].

The development of therapeutics that target and neutralize SLT-2 represents a novel and promising approach for preventing HUS. TAI has taken this approach by developing TMA-15, a humanized monoclonal antibody to SLT-2. Treating STEC infection by eliminating SLT has also been pursued using molecular entities other than MAbs. For instance, Synsorb Pk® (Synsorb Biotech Inc., Calgary Canada), an insoluble inert matrix coupled with trisaccharide receptors that specifically bind and neutralize SLT, was developed as an oral treatment that binds SLT in the gut. This approach was abandoned after equivocal results were obtained in clinical studies, and is somewhat flawed since toxins may exit the gut and enter systemic circulation, thereby limiting the benefits of an agent administered to the gut. However, an intravenously administered MAb (e.g., TMA-15) could neutralize the SLT-2 toxin in the circulatory system, thereby preventing systemic SLT-2-induced complications.

## 3.5 PLAN FOR THE DEVELOPMENT OF PRODUCTS TO PREVENT HUS

The following describes a theoretical approach to the development of therapeutic agents to prevent HUS:

1. Identify key potential molecular targets involved in the pathology of HUS.

2. Once a target is identified, develop a therapeutic product to neutralize its activity.

3. Analyze the protective effect in animal models including dose selection.

4. Determine the safety and pharmacokinetics of the product in predictive animal models.

5. Conduct safety and pharmacokinetics studies in healthy adult human subjects.

6. Conduct studies to determine the efficacy and safety in the target population.

The rare and sporadic nature of STEC infection outbreaks combined with the rarity of HUS development creates considerable challenges to developers of therapeutic agents to prevent HUS. The following sections present an overview of TAI's experience in this developmental approach, and the challenges faced in an effort to make available a product targeted at SLT-2 that can be expected to be safe and effective in patients.

# 4.0 TMA-15

TMA-15 is a recombinant, humanized, monoclonal antibody of the immunoglobulin G (IgG) subclass IgG1, targeted against the B-subunit of SLT-2. This agent is derived from the murine MAb, MuVTm1.1. TMA-15 is being developed by TAI to prevent HUS in pediatric patients with severe watery diarrhea or BD and confirmed STEC infection. Data obtained from various nonclinical studies suggest that TMA-15 exerts a protective effect against the effects of SLT-2 by inhibiting SLT-2 binding and uptake into endothelial cells, thereby preventing the induction of irreversible cellular damage. Thus, it is anticipated that TMA-15 will prevent or reduce SLT-2-related complications of STEC infection (e.g., HUS) in pediatric patients. The following two sections present the results of pre-clinical efficacy and safety studies with TMA-15.

## 4.1 PRE-CLINICAL EFFICACY STUDIES OF TMA-15

TAI has examined the ability of TMA-15 to neutralize the SLT-2 toxin *in vitro,* and the ability of TMA-15 to protect against SLT-2 and STEC *in vivo* using mice injected with SLT-2 or infected with STEC. In addition, TAI has evaluated TMA-15 pharmacokinetics (PK) in mice, and safety and pharmacokinetics in the monkey.

### 4.1.1 *In Vitro* Neutralization of SLT-2 by TMA-15

The ability of TMA-15 to neutralize SLT-2 was assessed *in vitro* using toxin-sensitive cell lines to which culture supernatants from 200 different STEC clinical isolates were applied. Using 80% or greater protection as the endpoint, 10 µg/mL of TMA-15 neutralized 99% (198/200) of the SLT-2-containing culture supernatants (Figure 3), demonstrating that TMA-15 has a broad spectrum of neutralizing activity on SLT-2-producing STEC isolates, and that TMA-15 was maximally effective at 10µg/mL.

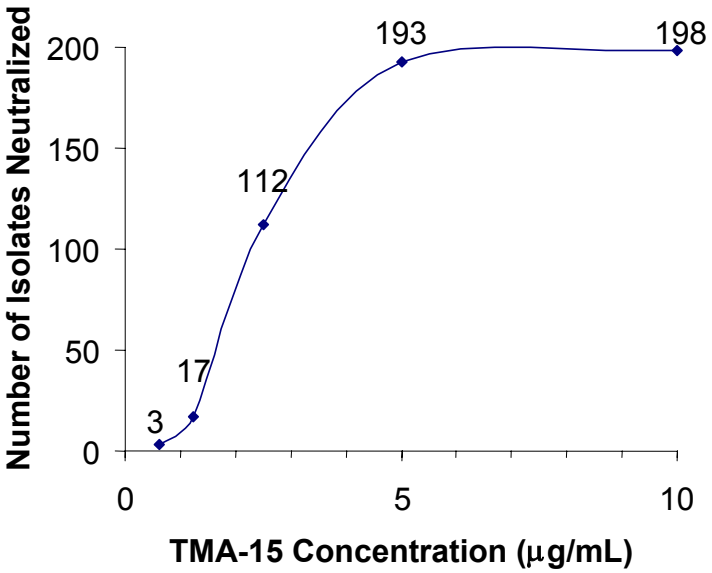


**Figure 3. TMA-15 Concentration-Effect Curve for Neutralization of SLT-2 in STEC Culture Supernatants.**

### 4.1.2 Prevention of Mortality in SLT-2-injected Mice

The ability of TMA-15 to neutralize SLT-2 *in vivo* has been assessed using a SLT-2-injected mouse model. In brief, mice were treated intravenously with different doses of TMA-15 (0.15, 0.31, 0.62, and 1.23 μg) followed 1 hour later by an intraperitoneal injection of 59 ng (10× $LD_{50}$) of SLT-2. TMA-15 increased seven-day survival in a dose-dependent manner as shown in Table 1 below.

**Table 1. Survival of SLT-2-Challenged Mice Treated with TMA-15.**

| Group | | No. of surviving mice / no. of SLT-2-challenged mice |
|---|---|---|
| Antibody | Toxin | |
| TMA-15 (0.15 μg/head) | SLT-2 | 1/10 |
| TMA-15 (0.31 μg/head) | SLT-2 | 1/10 |
| TMA-15 (0.62 μg/head) | SLT-2 | 4/10 |
| TMA-15 (1.23 μg/head) | SLT-2 | 10/10 |

The doses of 0.15, 0.31, 0.62, and 1.23 μg/head of each antibody corresponded to 1.25, 2.5, 5 and 10 times that of SLT-2 on a molar basis, respectively.
Kimura, T., et al. Hybrid Hybridomics. 2002. 21:161-168.

### 4.1.3 Prevention of Mortality in STEC-Infected Mice

The STEC-infected mouse is a well-characterized animal model of STEC infection, in which SLT derived from STEC causes lethality; this model has previously been used to evaluate anti-STEC infection therapy[12-14, 22]. In this model, mice typically die within four to five days after STEC inoculation; therefore, this model represents a far more severe infection than generally encountered in the clinic.

TMA-15 at 0.25 or 2.5 mg/kg was administered intravenously to STEC-infected mice at different time points in relation to STEC-infection, and mortality was monitored for 10 days. TMA-15 completely prevented morbidity when administered prior to STEC infection. Even when administered 24 hours post-infection, TMA-15 at 2.5 mg/kg completely prevented mortality. When TMA-15 was administered 48 hours after STEC infection, however, no animals survived to day 10, even at 2.5 mg/kg (Figure 4).

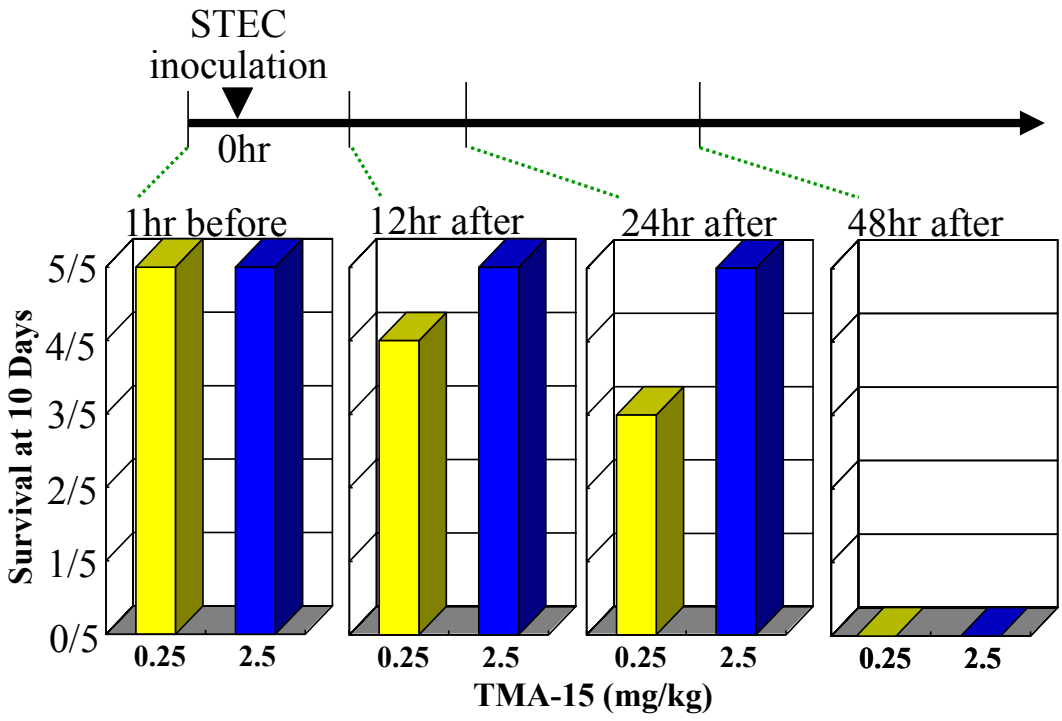


**Figure 4. Survival of Mice Treated with TMA-15 at Different Times After STEC Infection.**
Yamagami, S., et al. J. Infect. Dis. 2001. 184:738-742.

### 4.1.4 Dose-Response of TMA-15 against STEC-induced Mortality in Mice

To determine the dose-response effect for TMA-15, mice were given an oral dose of STEC and injected with 0, 0.25, 0.5, 1.0, or 2.0 mg/kg TMA-15 intravenously 24 hours after STEC inoculation (20 mice per dose level). Mortality was examined for 10 days after inoculation. The results are summarized in Figure 5.

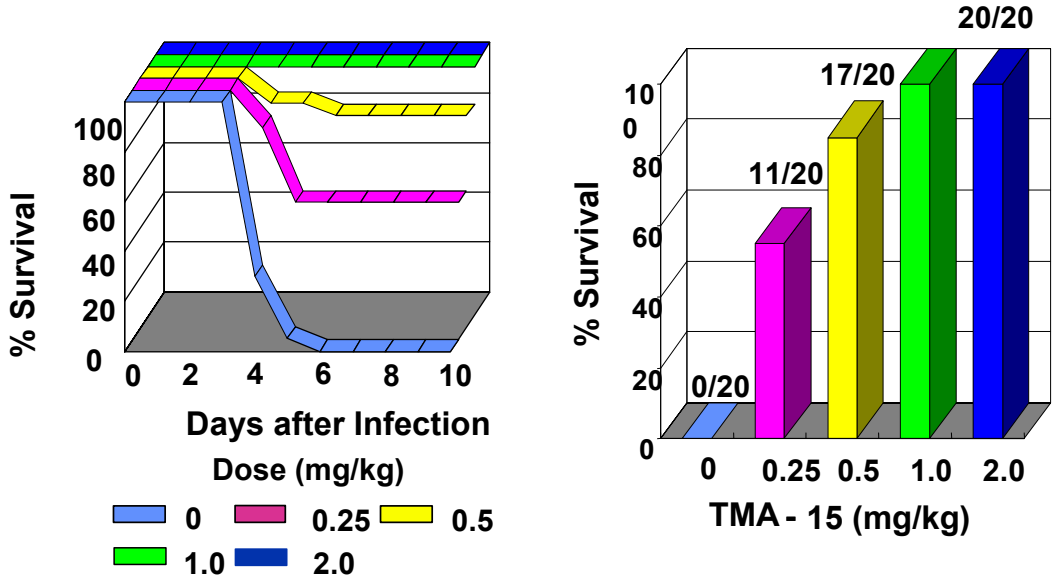


**Figure 5. Dose Response of TMA-15 on STEC-Induced Mortality in Mice.**
Yamagami, S., et al. J. Infect. Dis. 2001. 184:738-742.

The effectiveness of TMA-15 increased in a dose-dependent manner and administration of 1.0 or 2.0 mg/kg TMA-15 completely inhibited the lethal effects of STEC in mice.

### 4.1.5 Serum SLT-2 Levels in STEC-Infected Mice

To examine the relationship between the ability of TMA-15 to protect against STEC infection and the accumulation of SLT-2 in the bloodstream following STEC infection, TAI measured serum SLT-2 concentrations in infected mice at numerous times after STEC infection. Serum SLT-2 levels were below the detection limit (10 pg/mL) until 12 hours after injection. SLT-2 was detectable in the serum of 6 of 10 mice at 24 hours. Peak levels of SLT-2 were observed at

48 hours and SLT-2 was undetectable, in survivors, at 96 hours. These results are summarized in Figure 6.

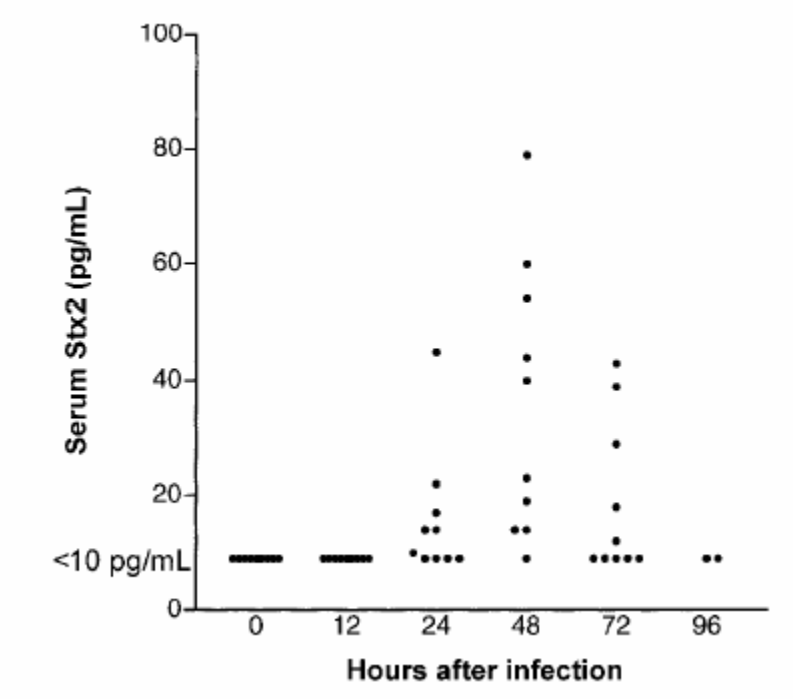


**Figure 6. Serum SLT-2 Concentrations in STEC-Infected Mice.**
Yamagami, S., et al. J. Infect. Dis. 2001. 184:738-742.

In conclusion, in efficacy studies in mouse models, TMA-15 prevents mortality caused by both SLT-2 injection and STEC infection. In the STEC-infected mouse model, TMA-15 is effective even when administered up to 24 hours after STEC inoculation, a time at which SLT-2 has begun accumulating in blood.

### 4.1.6 Pharmacokinetics of TMA-15 in Mouse Models of STEC Infection

TAI investigated the PK of TMA-15 after a single intravenous injection in STEC-inoculated mice. Animals were treated with TMA-15 (1 mg/kg) 24 hours after STEC inoculation and blood samples were collected for determination of TMA-15 serum concentration. Serum TMA-15 reached a maximum concentration of 18.30 μg/mL and remained at an average of 7.54 μg/mL up to 168 hours post-dose. These findings are consistent with the *in vitro* data that TMA-15 is maximally effective at 5 to 10 μg/mL, and also suggest that maintaining this concentration of TMA-15 is key in preventing STEC-induced lethality. These findings also provide the basis for a dose rationale in humans.

## 4.2 PRE-CLINICAL SAFETY STUDIES OF TMA-15

For monoclonal antibody drugs such as TMA-15 whose target antigen or epitope is not present on human or animal tissues (e.g., a bacterial or viral protein), animal toxicology studies may not be relevant for evaluating human safety; this is especially so if no cross-reactivity with human tissues is observed, as is the case for TMA-15 (Section 4.2.1). Nevertheless, a pre-clinical safety study was conducted in a primate (Section 4.2.2).

### 4.2.1 Absence of TMA-15 Cross-reactivity with Human Tissues

Although the anti-SLT-2 antibody-based therapy is designed to bind SLT-2 specifically, and no SLT-2 antigen is expected to be present in normal human tissues, TAI completed a cross-reactivity study to determine whether TMA-15 binds to human tissue. The results are summarized in Table 2 below. TMA-15 does not cross-react with the human tissues examined.

**Table 2. Results of Cross-Reactivity Study of TMA-15 with Normal Human Tissue**

| Tissues | Negative control antibody FITC labeled human $IgG_1$ | | FITC labeled TMA-15 | |
|---|---|---|---|---|
| | 1 μg/mL | 10 μg/mL | 1 μg/mL | 10 μg/mL |
| Positive control slides (SLT-2) | - | - | + | + |
| Positive control slides (SLT-1) | - | - | - | - |
| Normal human tissues: adrenal, blood cells, blood vessels, bone marrow, brain (cerebrum, cerebellum), breast, eye, gastrointestinal tract (colon, esophagus, small intestine, stomach), heart, kidney, liver, lung, lymph node, ovary and fallopian tube, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, spinal cored, spleen, skeletal muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder, uterus (body, cervix) | - | - | - | - |

-:negative, +: positive; FITC = fluorescein isothyocyanate; SLT-1 = Shiga-like toxin 1; SLT-2 = Shiga-like toxin 2.

### 4.2.2 Acute Safety Study of TMA-15 in Monkeys

A single bolus intravenous injection of TMA-15 was administered to Cynomolgus monkeys, consistent with the intended clinical use of TMA-15. No TMA-15-related toxicological changes were noted up to the maximum dose (20 mg/kg), which is approximately 6.7 times the expected human therapeutic dose of 3 mg/kg (see Section 7.1). TMA-15 was well-tolerated, and no mortality, treatment-related clinical signs, or local irritation at the injection site were seen.

TMA-15 toxicokinetics in monkeys were also assessed as part of the monkey acute toxicity study. Serum concentrations of TMA-15 were determined at 2, 48, 144, and 336 hours after dosing; the serum levels of TMA-15 detected after dosing with 2 and 20 mg/kg are shown in Figure 7. Under these experimental conditions, $C_{max}$ and AUC increased in a dose-dependent manner, no gender differences were observed, and no serum TMA-15 was detected in the control group at any time point.

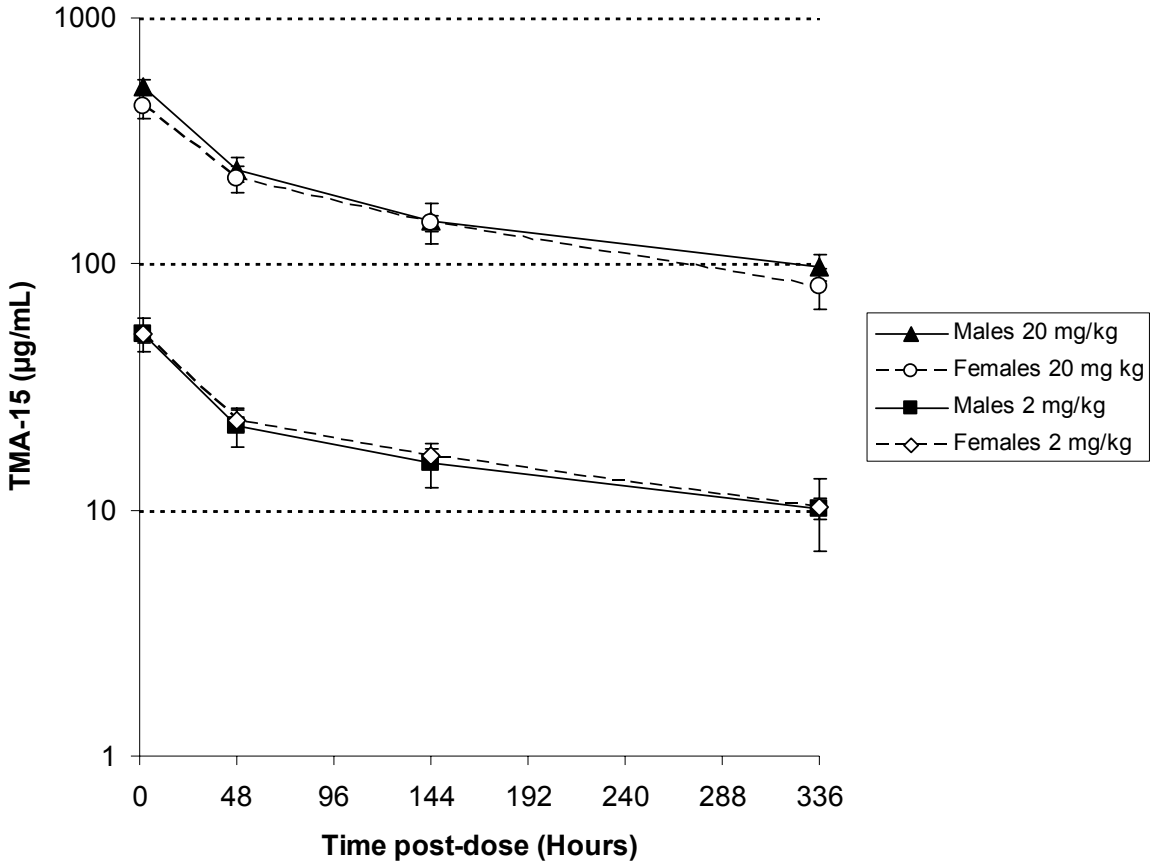


**Figure 7. Mean Serum Concentration-Time Profile of TMA-15 in Monkeys after Single Intravenous Bolus Administration of 2 or 20 mg/kg.**
Data represent the mean and SD from 3 male and 3 female animals.

To assess for potential antigenicity, serum from monkeys necropsied on 14 days after TMA-15 administration was assayed for anti–TMA-15 antibodies during the acclimation period and on Day 14. No anti-TMA-15 antibodies were observed in either sex in either the 2 or 20 mg/kg dose groups at either time point.

## 4.3 PRE-CLINICAL SUMMARY

TMA-15 shows sufficient SLT-2 neutralization activity on *in vitro* cell toxicity at a concentration of 10 µg/mL, and a protective effect on *in vivo* morbidity induced by either SLT-2 or STEC. To date, there are no identified pre-clinical safety concerns with TMA-15, and no evidence that TMA-15 interacts with human tissues.

# 5.0 CLINICAL DEVELOPMENT OF TMA-15 FOR THE PREVENTION OF HUS

Because STEC-infections are infrequent and unpredictable, demonstrating that a product is safe and effective in preventing HUS in STEC-infected patients is very challenging. This section describes TAI's activities to address some of the challenges of obtaining clinical data on the safety, PK, and efficacy of TMA-15.

## 5.1 PHASE 1 CLINICAL STUDY

To assess the safety of TMA-15, TAI conducted study TMA-CL-001, a Phase 1 study in 32 healthy adult volunteers. This study also examined the PK of TMA-15 in an effort to identify doses yielding blood concentrations of TMA-15 that would be expected to effectively neutralize SLT-2. The dose-escalation study assessed four different intravenous doses of TMA-15 (0.1, 0.3, 1.0, and 3.0 mg/kg) compared to placebo. Healthy adults were selected to provide reliable safety and PK data as well as enable an assessment of potential TMA-15 immunogenicity. All 32 subjects completed the study and were included in the safety analyses.

### 5.1.1 Safety of TMA-15 in Healthy Adults

Safety analyses indicated no dose-related trends with respect to vital signs, electrocardiograms, clinical laboratory results, or physical examinations.

No serious adverse events (SAEs) occurred. There were 80 adverse events (AEs) experienced by 22 (69%) of the 32 healthy adult volunteers treated in this trial. Most AEs were mild in severity, and all were considered unrelated to study treatment by the investigator. Two subjects experienced urticarial episodes that occurred between Day 38 and Day 48, but these episodes were considered by the investigator to be unrelated to TMA-15. Headache was the most common treatment-related AE reported.

TMA-15 appeared to be safe and generally well-tolerated up to the highest single intravenous dose (3 mg/kg) in this population of healthy male and female adult subjects.

### 5.1.2 Pharmacokinetics of TMA-15 in Healthy Adults

Blood samples were analyzed by enzyme immunoassay for detecting serum TMA-15. The disposition of TMA-15 after a 30 minute infusion (all dose ranges) followed a biexponential decline, as has been observed with many human and humanized monoclonal immunoglobulins. The mean terminal elimination half-life was reasonably linear across dose groups, ranging from 24.6 days (3.0 mg/kg) to 29.0 days (0.3 mg/kg), which indicates that elimination of TMA-15 is independent of dose over the range of doses examined. Mean maximum observed serum drug concentration ($C_{max}$) values ranged from 2.56 μg/mL (at 0.1 mg/kg) to 71.74 μg/mL (at 3.0 mg/kg) and were also reasonably linear. Within the four cohorts, mean concentrations of TMA-15 appeared to decrease to approximately one-half of the $C_{max}$ by Day 4 after dosing and to approximately one-third of the $C_{max}$ by Day 14 after dosing. It is predicted that circulating levels of TMA-15 for the 1.0- and 3.0-mg/kg cohorts would exceed 10 μg/mL for at least 7 days after dosing. This concentration of TMA-15 has been shown to be effective *in vitro* and *in vivo* in mice. These results are consistent with data obtained with other human and humanized immunoglobulin preparations that have previously been studied.

### 5.1.3 Absence of Immunogenicity in Healthy Adults

To determine whether TMA-15 induced an immune response in humans, TAI analyzed serum levels of anti-TMA-15 antibodies in study TMA-CL-001. Values were below the level of quantification during the observation period (57 days) indicating a low immunogenic potential of TMA-15 in humans.

### 5.1.4 Phase 1 Summary

There were no safety issues identified, the pharmacokinetics were reproducible and comparable to those in animals, and there was no evidence of immunogenicity at doses producing blood concentrations of TMA-15 expected to be effective at neutralizing SLT-2. Therefore, TAI considered it appropriate to begin studies in STEC-infected patients.

## 5.2 PHASE 2 CLINICAL STUDY

To assess safety, PK and preliminary efficacy of TMA-15, TAI conducted study TMA-CL-002, a Phase 2 study in 109 STEC-infected pediatric subjects (1 to 15 years old). In addition, TAI is currently conducting a PK and safety study in infants (0.5 to 1 year old).

### 5.2.1 TMA-CL-002: Multi-center, Double-Blind, Placebo-Controlled Study in Pediatric Patients of the Safety and Pharmacokinetics of Intravenous TMA-15

TMA-CL-002 was a two-part Phase 2 study in pediatric subjects with STEC infection. The first part of this study (Part A) sequentially assessed the safety and PK parameters of two different intravenous doses of TMA-15 (1 and 3 mg/kg) as compared with placebo in 24 pediatric subjects with STEC infection, in a dose-escalating manner. An independent medical review of the preliminary safety data from Part A determined that there were no safety concerns that would prevent the start of Part B of the study. In Part B, safety, PK, and preliminary efficacy of TMA-15 were evaluated in a parallel manner by following the progression of clinical symptoms of STEC infections toward complete HUS in subjects with STEC infection. Part B was conducted in 85 pediatric subjects. Data presented from Study TMA-CL-002 were pooled for analysis and are presented as integrated results from both parts of the study (A and B), unless otherwise indicated.

#### 5.2.1.1 Safety of TMA-15 in Pediatric Patients

In Study TMA CL-002, TAI collected safety data from 109 pediatric subjects (24 from Part A and 85 from Part B), including 35 subjects who received 3 mg/kg of TMA 15 and 38 subjects who received 1 mg/kg of TMA-15. There were few AEs considered to be related to TMA-15 administration; treatment–related AEs were experienced by only four subjects. Three of these were mild, and one was moderate in intensity. The mild AEs were pyrexia (one subject in the 1 mg/kg dose group); headache (one subject in the 3 mg/kg dose group), and erythema (one subject in the 3 mg/kg dose group). The AE classified as moderate (vomiting) occurred in one subject in the placebo group.

**Table 3. Intensity and Frequency of Adverse Events**

| Intensity | Number of subjects with AEs (%; subjects/total subjects) | | | |
|---|---|---|---|---|
| | 1mg/kg (n = 38) | 3 mg/kg (n = 35) | Placebo (n = 36) | Total (n = 109) |
| Mild | 20 (52.6) | 20 (57.1) | 20 (55.6) | 60 (55.0) |
| Moderate | 10 (26.3) | 9 (25.7) | 10 (27.8) | 29 (26.6) |
| Severe | 2 (5.3) | 2 (5.7) | 2 (5.6) | 6 (5.5) |
| Total number of subjects with AEs | | | | 95 (87.2) |

AE = adverse event.

A total of 25 SAEs were observed in 18 subjects. Nine SAEs occurred in the 1 mg/kg treatment group, six SAEs in the 3 mg/kg treatment group, and ten SAEs occurred in subjects administered placebo. Four SAEs were reported related (remotely) to TMA-15 administration including hypokalemia, intussusception, HUS in the 1 mg/kg treatment group, and HUS in the placebo group. Overall, most SAEs were considered unrelated to TMA-15. There were two subject deaths in the study, one in the 1 mg/kg dose group and one in the placebo group, with neither considered related to clinical trial material administration by the investigator. The subject death that occurred in the 1 mg/kg treatment group was determined to be related to *Klebsiella pneumoniae* infection, and the subject death in the placebo group was deemed related to progression of STEC infection. There were no deaths in the 3 mg/kg treatment group.

### 5.2.1.2 Pharmacokinetics of TMA-15 in Pediatric Patients

There was little difference between the PK of TMA-15 in Parts A and B of the study. Mean PK parameters (and standard deviation) for TMA-15 (pooled data, Parts A and B) are summarized in Table 4.

**Table 4. TMA-15 Pharmacokinetic Parameters in TMA-CL-002 (Part A and Part B combined)**

| Dose Group | $C_{max}$ (µg/mL) | $T_{max}$ (days) | $AUC_{(0\text{-}120)}$ (µg*d/mL) | $AUC_{(0\text{-}inf)}$ (µg*d/mL) | $\lambda_z$ (day $^{-1}$) | $t_{½}$ (days) |
|---|---|---|---|---|---|---|
| 1 mg/kg | 19.64 ±4.07 | 0.136 ±0.174 | 330.26 ±94.99 | 342.30 ±105.05 | 0.031 ±0.011 | 24.42 ±6.40 |
| 3 mg/kg | 56.12 ±12.08 | 0.122 ±0.200 | 841.59 ±239.71 | 868.52 ±268.47 | 0.033 ±0.009 | 22.39 ±6.16 |

$AUC_{(0\text{-}inf)}$ = area under the curve from 0 to infinity; $AUC_{(0\text{-}120)}$ = area under the curve from 0 to 120 days; $C_{max}$ = maximum concentration; $\lambda_z$ = terminal elimination rate constant; $T_{max}$ = time to maximum concentration; $t_{½}$ = half-life.

The disposition of TMA-15 after an infusion of 1 or 3 mg/kg appeared to follow a biexponential decline. The duration of the predicted effective concentration

(10 µg/mL based on preclinical studies) was approximately four days in the 1 mg/kg dose group, and at least 22 days in the 3 mg/kg dose group.

As in the TMA-CL-001 study, the $\lambda_z$ observed with both doses of TMA-15 indicated that elimination of TMA-15 was dose-independent over the doses used in this study. As in the Phase 1 assessment, the primary measures of exposure, $C_{max,}$ and $AUC_{(0\text{-}inf)}$ increased with dose, but dose-proportionality was not formally demonstrated nor refuted in the present study. These data are consistent with other human and humanized immunoglobulin preparations that have previously been studied. Mean TMA-15 serum concentrations over time (Parts A and B of the study; pooled data) are indicated by dose group (Figure 8).

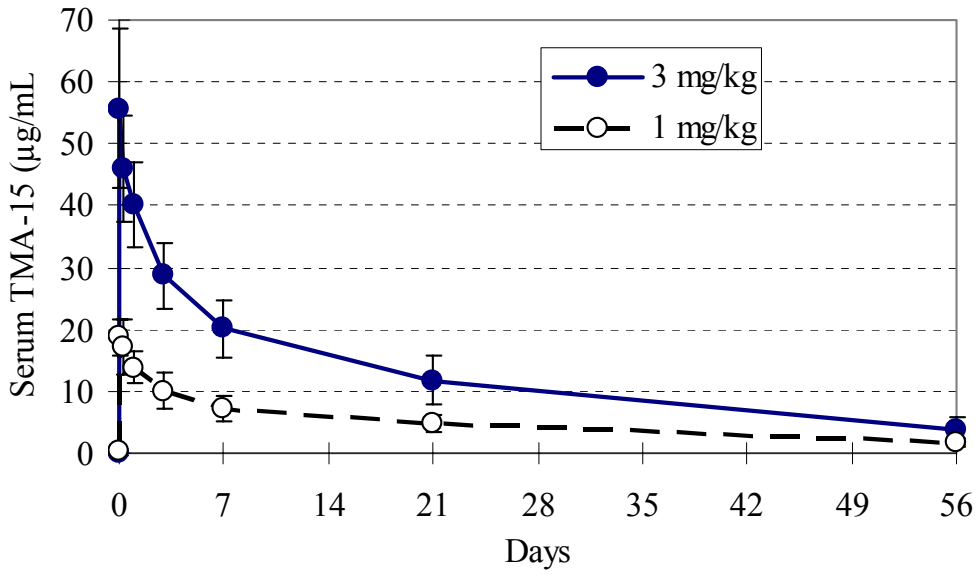


**Figure 8. Mean TMA-15 Serum Concentrations (Parts A and B combined).**

### 5.2.1.3 Comparative Pharmacokinetic Data

TAI compared PK data from the subject populations in Studies TMA-CL-001 and TMA-CL-002. Because the health status and age of these subject populations was notably different, the possibility remained that PK parameters across these groups would also be significantly different. Adult and pediatric PK data are compared below in Figure 9, and in Table 5 that follows.

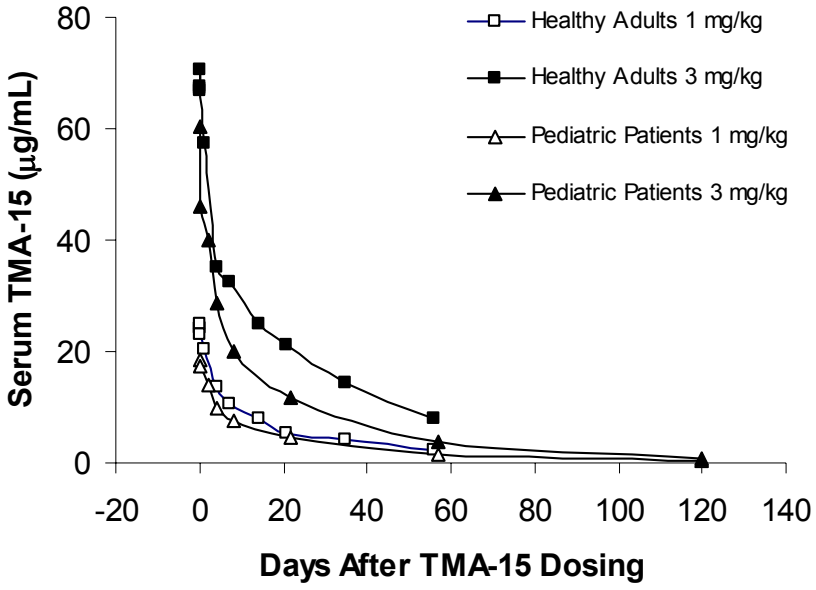


**Figure 9. Comparative Pharmacokinetics of TMA-15: Healthy Adult Volunteers vs. Pediatric Subjects.**

**Table 5. Summary of Comparative TMA-15 Pharmacokinetic Parameters**

| PK Parameters | Healthy Adult Volunteers Phase 1 | | Pediatric subjects Phase 2 | |
|---|---|---|---|---|
| | 1 mg/kg (n = 6) | 3 mg/kg (n = 6) | 1 mg/kg (n = 37) | 3 mg/kg (n = 35) |
| $T_{max}$ (days) | 0.091 ±0.084 | 0.133 ±0.157 | 0.136 ±0.174 | 0.122 ±0.200 |
| $C_{max}$ (µg/mL) | 25.49 ±2.35 | 71.74 ±12.52 | 19.64 ±4.07 | 56.12 ±12.08 |
| $AUC_{(0\text{-inf})}$ (µg*d/mL) | 480.00 ±127.08 | 1,437.50 ±286.92 | 342.30 ±105.05 | 868.52 ±268.47 |
| $t_{1/2}$ (days) | 26.6 ±4.7 | 24.6 ±5.0 | 24.42 ±6.40 | 22.39 ±6.16 |

$AUC_{(0\text{-inf})}$ = area under the curve from time 0 to infinity; $C_{max}$ = maximum concentration; PK = pharmacokinetic; $T_{max}$ = time to maximum concentration; $t_{1/2}$ = half-life.

Similar half-life ($t_{½}$) values were observed in STEC-positive pediatric subjects and healthy adults (Table 5; mean and standard deviation). A comparison of mean PK data obtained in TMA-CL-001 and TMA-CL-002 indicates that systemic exposure to TMA-15 was slightly lower in pediatric subjects when compared with healthy adults.

#### 5.2.1.4 Evaluation of potential predictive HUS markers

In an attempt to identify measures other than HUS that might be useful for assessing efficacy, TAI conducted a preliminary assessment in a subpopulation of subjects in Study TMA-CL-002, specifically those that were confirmed as positive for SLT-1/2 or SLT-2 (19 subjects in the 1 mg/kg group, 17 subjects in the 3mg/kg group, and 25 subjects in the placebo group). Several parameters were assessed for changes from baseline (Day 0): plasma and urine thrombomodulin (TM), hemoglobin, platelet count, serum creatinine, and vital signs after TMA-15 administration.

None of the parameters measured showed statistically-significant changes or trends, therefore, none are suitable as potential surrogate efficacy measures.

### 5.2.2 TMA-CL-INF: A Multicenter, Double Blind, Placebo Controlled, Safety and Pharmacokinetic Study of a Single Intravenous Dose of TMA-15 in Infants Between 6 and 12 Months of Age.

Because STEC-infected patients at risk of developing HUS can be as young as six months of age, TAI is currently conducting an additional clinical study, TMA-CL-INF, in which patients from 0.5 to 1 year of age will receive 3 mg/kg TMA-15. Study TMA-CL-INF is designed as a double-blind, placebo-controlled study in pediatric patients to assess the safety and PK of a single intravenous dose of 3 mg/kg TMA-15 in patients aged 0.5 to 1 year of age.

### 5.2.3 Phase 2 Summary

The Phase 2 study in pediatric patients found that TMA-15, at doses that produce blood concentrations expected to be effective (10 μg/mL), has no significant safety concerns.

This study also confirmed the difficulty of enrolling large numbers of patients at risk for HUS, and the low incidence of HUS in such patients. Given the seriousness of HUS and the absence of any approved or effective treatment, TAI believes it is appropriate to consider innovative development pathways which would expedite the development and approval of this critically-needed agent.

# 6.0 CLINICAL EFFICACY STUDY CHALLENGES

There are a number of challenges to studying treatments designed to prevent HUS related to STEC infection which are outlined in detail below. Due to the sporadic nature of STEC infection, relatively low incidence, and seasonality of HUS, a number of issues complicate subject enrollment and evaluation of clinical benefit.

## 6.1 CHALLENGES FACED IN CONDUCTING PHASE 2/3 STUDIES

Since the overall incidence of HUS is low in STEC-infected patients, and the occurrence of BD precedes the onset of HUS in most cases, TAI attempted to enrich the HUS-susceptible patient population by focusing initially on patients with bloody diarrhea and confirmed STEC infection.

Because the time and location of STEC outbreaks are unpredictable, it is suitable to identify study sites in locations where STEC infection frequency is likely to be higher and more predictable. TAI decided to conduct a multi-center Phase 2 study with sites in Argentina, Canada, and the United States. Canadian and U.S. sites fell far short of estimated enrollment of STEC-infected patients (8 cases from 5 sites within a season). In contrast, Argentinean sites were able to enroll a reasonable number of patients (101 cases from 7 sites in 13 months).

Due to the potentially rapid progression of STEC infection to HUS, quick detection of SLT or *E. coli* O157 in stool specimens is particularly important to determine if it appropriate to use a treatment to prevent HUS. Currently available diagnostic measures for STEC, beyond the traditional time-consuming culturing methods, include Premier EHEC Test [PEHEC] and Immunocard STAT test [IC-STAT]. Using stool samples, PEHEC detects the presence of SLT toxin (either SLT-1 or SLT-2) within 4 to 6 hours, and IC-STAT detects the *E. coli* O157 serotype within 20 minutes. Although both direct stool testing and culture testing are applicable for these tests, stool culturing adds a significant time burden (16 to 24 hours) in the identification of STEC patients, without significant improvement of detection. A polymerase chain reaction (PCR) test for the SLT-2 gene is also available, but requires approximately 24 hours. Current rapid diagnostic tools and time requirements are outlined in Table 6.

**Table 6. Overview of Current Diagnostic Tools for STEC**

| Name | Vendor | Detect | Time Requirements | | Methods |
|---|---|---|---|---|---|
| | | | Direct Stool | Culture | |
| Immunocard STAT | Meridian | O157 | 20 minutes | 16 to 24 hours | Immuno-chromatography |
| Premier EHEC | Meridian | SLT | 4 to 6 hours | 20 to 24 | ELISA |
| PCR | NA | SLT-1 SLT-2 | | 24 to 30 hours | PCR |

SLT = Shiga-like toxin; EHEC = Enterohemorrhagic *Escherichia coli*; ELISA = Enzyme-Linked Immunosorbent Assay; NA = not applicable; PCR = polymerase chain reaction.

## 6.2 HUS SEASONALITY AND DISEASE INCIDENCE

TAI's experience during the conduct of study TMA-CL-002 demonstrates the difficulty of enrolling HUS patients including in Argentina, an area with one of the highest incidence of STEC-related HUS in the world, and a relatively predictable seasonal occurrence. In the United States, most cases of HUS are associated with spontaneous STEC outbreaks, and only a small percentage of STEC-infected patients (5% to 15%[5-6, 24]) develop HUS. In the United States, the incidence of reported STEC cases has been dropping for several years: approximately 8 per 100,000 reported for 1995 and 1997[2, 5], 1.5 per 100,000 reported in 2001[25], 1.1 per 100,000 reported in 2003[26], 0.9 per 100,000 reported in 2004[27], and 1.06 per 100,000 reported in 2005[28]. A recent exception occurred in 2006 where two separate outbreaks produced a total of 270 reported STEC cases in 29 states[29, 30] compared to an average of 21 cases per year from 2003-2005.

One widely-cited report estimates 73,000 cases of STEC infection occur annually in the U.S.[31]. However, this estimate may be misleading because it includes a number of extrapolations and does not reflect the number of cases that would be appropriate for treatment or could be practically recruited for a clinical trial. A detailed discussion of this report and refuting calculations can be found in Appendix A.

Recent outbreaks might also make clinical studies appear feasible, but such outbreaks are identified relatively late, and conducting a clinical study using outbreak patients is impractical, from an operational perspective. The timeline for one recent outbreak is summarized in Figure 10.

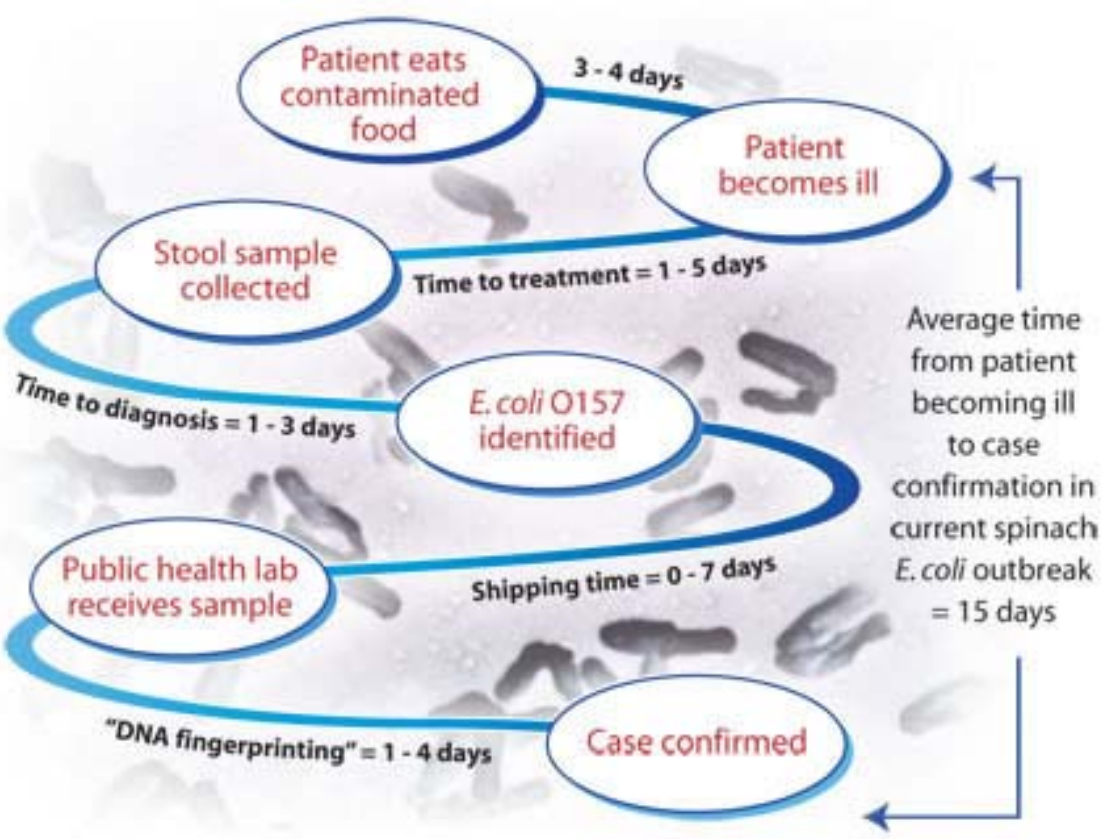


**Figure 10. STEC Outbreak Identification Timeline**
Adapted from http://www.cdc.gov/ecoli/reportingtimeline.htm

## 6.3 SAMPLE SIZE CONSIDERATIONS

Attempting to conduct a Phase 3 efficacy trial in Argentina, where STEC infections are more prevalent and predictable than sporadic outbreaks observed in the U.S., is also impractical. TAI has calculated the estimated sample size needed to conduct a pivotal Phase 3 clinical study in order to show a clinically meaningful and statistically significant reduction of the incidence of HUS in confirmed STEC-positive patients. Table 7 displays the sample size calculations based on an assumed rate of HUS progression of 5% and different assumed levels of efficacy of TMA-15.

**Table 7.Sample Size Estimates for a Single Definitive Study of the Efficacy of TMA-15**

| Presumed Rate of HUS in STEC-Positive Patients (Range from Published Literature) | Hypothetical Efficacy of TMA-15 (%) | Sample Size Estimates‡ | | | |
|---|---|---|---|---|---|
| | | Number Needed to Be Screened† (for Two Treatment Arms) | Number Needed to Be Enrolled (for Two Treatment Arms) | Projected Number of HUS Cases | |
| | | Watery Diarrhea or Bloody Diarrhea | Bloody Diarrhea and STEC Positive | TMA Treated | Placebo Treated |
| 5%* | 50 | 27,919 | 2,424 | 32 | 65 |
| | 75 | 10,366 | 900 | 6 | 25 |
| | 90 | 6,335 | 550 | 2 | 16 |

HUS = hemolytic uremic syndrome; STEC = Shiga-like toxin producing *E. coli.*

* HUS incidence in Study TMA-CL-002 was also approximately 5%.

† Number is based on experience with Part B of TMA-CL-002 (979 patients screened to obtain 85 subjects treated within 72 hours of onset of BD).

‡ Sample size needed to achieve 90% power at $\alpha = 0.05$; to be conservative, sample size estimate does not assume continuity correction.

Table 7 presents a number of scenarios for a potential clinical trial of TMA-15, but TAI believes the most realistic scenario (shaded) is an incidence of HUS in patients with BD of approximately 5% (consistent with numerous published estimates, as well as TAI's recent clinical experience from Study TMA-CL-002), and efficacy of TMA-15 as high as 75%. In this scenario, the number of STEC-positive subjects required for 90% power at $\alpha = 0.05$ is estimated to be 900, which is expected to require screening of more than 10,000 patients. Even if this incidence of HUS is twice as high as expected (i.e., is 10%), obtaining the number of subjects needed to show efficacy would require screening almost 5,000 patients.

To summarize, it is impractical to consider conducting a conventional definitive clinical study of the efficacy of TMA-15, however TAI believes that the nonclinical and clinical data from the existing development plan may provide sufficient information to permit safe and appropriate use of a treatment to prevent HUS.

## 6.4 ABSENCE OF PREDICTIVE SURROGATE MARKERS FOR HUS

The identification of a biomarker(s) of HUS would be particularly useful for the following:

1. To predict the progression of STEC infection to HUS

2. To establish the appropriate timing for TMA-15 administration

3. To provide a potential surrogate endpoint(s) to assess efficacy

Studying potential biomarkers in infant and pediatric patients is restricted due to the ethical limitations of frequent blood sampling. Possible surrogate markers of progression to HUS have been reported in the literature, including the proinflammatory cytokine, tumor necrosis factor (TNF)-α, and D-dimer, a byproduct of clot breakdown (and a fibrin-related marker).

According to published reports, elevated serum TNF-α can be detected in subjects with diarrhea-associated HUS, but the changes in levels of serum TNF-α are inconsistent, with only approximately 20% of HUS patients demonstrating detectable elevations of TNF-α[32]. Therefore, marked elevation of TNF-α in serum is not a reliable predictor of HUS.

D-dimer is considered to be a sensitive measure of intravascular fibrinolysis, and has been investigated as a marker of excess thrombin activity. Elevated plasma D-dimer concentrations may be characteristic of STEC-induced HUS and may precede the development of renal injury in STEC-infected pediatric patients[33]. Higher than normal levels of D-dimer may arise from a certain grade of fibrin lysis at its deposition sites (previously formed thrombi) in the acute phase of the disease[34, 35], but D-dimer levels may also fall within the reference range in patients with HUS[36]. Therefore, because changes are not consistent in cases of HUS, D-dimer, like TNF-α, cannot be considered a reliable predictor of HUS.

Despite the limitation of blood sampling, TAI analyzed some possible biomarkers for progression to HUS in Study TMA-CL-002 including urine and plasma thrombomodulin, hemoglobin, platelet count, serum creatinine, and vital signs; however, these potential markers were not predictive of the onset of HUS.

Based on a review of the literature and the data obtained from Study TMA-CL-002 (Section 5.2.1.4), no early predictive marker exists which can be utilized to identify which patients will develop HUS.

## 6.5 SUMMARY OF PIVOTAL CLINICAL STUDY CHALLENGES

TAI believes that the difficulty in identifying, screening, and enrolling an adequate number of patients makes conducting a definitive randomized, placebo-controlled study to evaluate the efficacy of TMA-15 for the prevention of HUS in STEC-positive patients unfeasible because of:

a. the infrequency and unpredictability of STEC outbreaks,
b. the relatively low overall incidence of STEC infection,
c. the low rate at which HUS develops after STEC infection,
d. the ethical limitations of obtaining frequent and adequate amounts of blood from infant and pediatric patients, and
e. the necessity of delivering TMA-15 to STEC-infected patients in a timely fashion, prior to the onset of HUS.

## 7.0 DATA REQUIRED TO ESTABLISH THE RISK/BENEFIT OF A TREATMENT TO PREVENT HUS

TMA-15 has been clearly shown, both *in vitro* and *in vivo* in animal models, to neutralize the pathogenic toxin (SLT-2) primarily responsible for a serious, untreated complication of STEC infection (HUS). In addition, no safety issues have arisen at doses of TMA-15 that produce SLT-2-neutralizing concentrations *in vitro* and *in vivo*.

TMA-15 has demonstrated a favorable safety profile in a Phase 1 study in healthy adult volunteers and in a Phase 2 study in STEC-infected pediatric patients at doses that produce SLT-2-neutralizing concentrations *in vitro* and *in vivo*. TAI believes that data from completed pre-clinical and clinical studies combined with positive results from proposed safety and efficacy studies in additional STEC-infected animal models may be sufficient to meet the standard of approval for products addressing unmet needs for which there are significant operational and ethical barriers to conducting conventional clinical efficacy studies.

TMA-15 is intended to prevent the occurrence of HUS after STEC infection and TAI proposes to collect additional human safety data in support of approval for this indication. However, as detailed above, it is not practical to carry out statistically significant clinical efficacy studies of the reduction in the incidence (or severity) of HUS. Nonetheless, there are compelling data on the clinical safety of TMA-15, and these data are supported by robust pre-clinical safety and efficacy investigations. Therefore, TAI believes that for a treatment to prevent HUS after STEC infection, it is appropriate to rely on a combination of human and animal data for evidence of efficacy to support approval, and to provide information that would be needed for prescribing information.

## 7.1 DOSE RATIONALE

A definitive clinical dose-response study in infant and pediatric patients is not practical, but a safe and effective dose can be selected based on understanding the pathophysiology of HUS and the mechanism of action of the treatment, and the pharmacodynamics of the treatment.

TMA-15 effectively neutralizes SLT-2 at 10 μg/mL *in vitro* and prevents morbidity *in vivo*. Administration of 1 mg/kg TMA-15 to human subjects yielded

blood concentrations of TMA-15 that remained above 10 µg/mL for an average of three days, but 3 mg/kg yielded blood concentrations of TMA-15 that remained above 10 µg/mL for an average of 21 days. Given that HUS can develop as late as eight days after the onset of BD it seems both reasonable and prudent to maintain the concentration of TMA-15 above 10 µg/mL for longer than eight days, that is, to use 3 mg/kg as the therapeutic dose, a dose that is devoid of any identified safety concerns in monkeys and humans to date.

## 7.2 THERAPEUTIC TIME WINDOW RATIONALE

The sporadic nature and unpredictability of STEC outbreaks, coupled with the infrequent but rapid progression to HUS, compound the difficulty of identifying exposed patients within an effective treatment window. However, regarding dose selection, a therapeutic time window can currently only be determined based on understanding the disease pathophysiology, treatment mechanism of action and PK.

Bloody diarrhea is a known risk factor for the development of HUS, and HUS develops 3 to 5 days (and possibly longer) after the onset of BD. Since the sequelae of STEC infection appear to result from SLT-2 that enters the bloodstream, TMA-15 should be administered provided there is a reasonable possibility of SLT-2 accumulation in the blood. Data from mouse models clearly demonstrate that TMA-15 can prevent mortality caused by SLT-2 up to 24 hours after infection (shown in Figure 4). The therapeutic time window for TMA-15 in STEC-infected mice (up to approximately 24 hours post infection in this severe model) corresponds to the time at which SLT-2 enters the bloodstream (Figure 6).

These data suggest that TMA-15 should be administered at a dose that can provide protective serum concentrations prior to or as early as possible after the onset of BD.

## 7.3 ADDITIONAL ANIMAL EFFICACY AND SAFETY DATA

Due to the significant hurdles barring the conduct of pivotal clinical efficacy studies as discussed above, TAI proposes that parallel efficacy, PK and safety studies in multiple non-human animals might be useful for confirming the conclusions reached on the basis of the available clinical data. TAI is designing additional studies in STEC-infected gnotobiotic piglets and STEC-infected mice.

Although the models described above have certain limitations, data from these studies should prove useful in further establishing the robust safety and efficacy of TMA-15 in the prevention of HUS.

### 7.3.1 Proposed Confirmatory Efficacy Studies in Piglet Model of STEC Infection

To parallel efficacy and safety studies conducted in the mouse model of STEC infection, TAI proposes to use the STEC-infected gnotobiotic piglet model. The STEC-infected piglet model is a well-characterized animal model of STEC infection, and the efficacy of antibody therapy using this model has already been described[37]. Planned piglet efficacy studies, including safety assessments, will include the following evaluations.

- Determination of an effective dose of TMA-15
- Determination of a therapeutic time window for TMA-15
- Determination of the pharmacokinetic profile of TMA-15 at an effective dose, which will be administered within the therapeutic time window after STEC infection

### 7.3.2 Additional Safety Studies in Animals

During the clinical development program for TMA-15, there have been no identifiable safety concerns related to TMA-15 administration. In addition, anti-TMA-15 antibody levels were below the level of quantification during the observation period for up to four months post-dose, indicating a low immunogenic potential of TMA-15. Therefore, consistent with the results from pre-clinical cross-reactivity studies, TMA-15 appears to be safe and well-tolerated in humans.

SLT-2, the specific antigen of TMA-15, is not naturally present in normal animals or humans, and FDA has requested that Teijin obtain additional safety data in animals in the presence of SLT-2 (i.e., STEC-infected animals). Therefore TAI is planning to use the mouse model of STEC infection to investigate the safety of TMA-15 at supra-therapeutic doses.

#### 7.3.2.1 Proposed Safety Studies in STEC-Infected Mice

The study of TMA-15 in STEC-infected mice is planned which may include doses that are three and ten times higher than the effective dose observed in this model to address higher-dose safety. A variety of assessments are planned which will include hematology, blood chemistry, bone marrow examination as well as gross pathology and histopathology. Although TMA-15 will be used as single-dose drug in clinical, repeat-dose study of TMA-15 in STEC-infected mice is also planned.

In addition to additional studies in mice, the safety of TMA-15 will also be evaluated in the gnotobiotic piglet model described above. TAI believes that demonstrating the safety of TMA-15 in three non-human species (i.e. mouse, pig and monkey) will provide a suitable basis for consideration of approval in lieu of a large safety database in humans.

## 7.4 SAFETY DATABASE IN HUMANS FOR PREVENTION OF HUS

The accumulation of a conventional safety database (e.g., 1500 treated subjects) in a clinical study of STEC-infected patients is particularly difficult due to the rarity and sporadic nature of STEC infections, the lack of predictive markers of HUS, and the limited time window for the diagnosis of STEC infection and prevention of HUS. To date, TAI has collected TMA-15 human safety data from 24 healthy adult volunteers administered TMA-15 at doses of 0.1 to 3 mg/kg (six subjects received 1 mg/kg TMA-15 and six received 3 mg/kg TMA-15) and 73 pediatric patients (including 38 subjects who received 1 mg/kg TMA-15 and 35 subjects who received 3 mg/kg TMA-15). In addition to the TMA-CL-INF study, TAI anticipates having a clinical safety database that will include over 200 subjects (volunteers and patients) exposed to single doses of TMA-15 (primarily at the 3 mg/kg dose level).

### 7.4.1 Safety Databases for Biologics and Drugs Approved for Comparable Rare Indications

The nature of many indications prevents the practical or ethical collection of late phase clinical data. A number of sponsors have faced a situation similar to that faced by TAI where a needed therapy was developed, but there were obstacles to obtaining the usual standard of data from adequate and well-controlled clinical

efficacy studies. FDA has approved a number of products on the basis of the available pre-clinical and limited or no clinical efficacy data, favorable risk/benefit profile, and unmet and serious clinical need. Table 8 highlights several therapeutic agents including Cyanokit®, which was recently approved for marketing by the FDA via Title 21 of the Code of Federal Regulations (CFR), Part 314, Subpart I ("the Animal Rule"). The following sections provide further details of the data collected for each product.

## Table 8. Examples of Approved Compounds with Small Human Databases

| Trade Name (Drug/ Biologic) | Indication | Type of Therapy | Date of Approval | Safety Database | | Pivotal Study | | |
|---|---|---|---|---|---|---|---|---|
| | | | | No. Healthy Vol. | No. Patients | No. Patients | Controlled (Y/N) | Endpoints; (p-value) |
| **BabyBIG® (Biologic)** | Treatment of Infant Botulism (age 1 year or less) | IV- Ig | 10/23/03 | 0 | 422 | **Study 1 (RCT):**<br><br>n= 129<br>(C= 64;<br>Tx= 65);<br><br>*[n-122<br>(C = 63;<br>Tx = 59)]<br><br>*confirmed botulism | Y, placebo | • Length of Hospital Stay (p< 0.0001) [Primary Efficacy Variable]<br>• Length of ICU Stay (p< 0.01) [Secondary Efficacy Variable]<br>• Duration of Mechanical Ventilation (p< 0.001) [Secondary Efficacy Variable]<br>• Length of tube feedings (p< 0.001) [Secondary Efficacy Variable]<br>• Mean number of AEs per patients (p< 0.05) [Secondary Efficacy Variable] |
| | | | | | | **Study 2 (OLS):**<br><br>n= 293<br><br>[*n=134]<br><br>*confirmed botulism | N | • Length of hospital stay |
| **Crofab™ (Biologic)** | Treatment of envenomation by North American pit vipers | IV- Venom specific Fab fragment of Ig- | 10/11/00 | 0 | 42 | **Study 1:**<br><br>n = 11 | N | • Efficacy Score (ES); no change/ decrease in ES considered indicative of efficacy (no statistical analysis)<br>• Investigator's Clinical Assessment (ICA); suggested clinical efficacy; (no statistical analysis) |
| | | | | | | **Clinical Study 2:**<br><br>n= 31 | N | • Efficacy Score (ES) (p< 0.001) |
| **CyanoKit® (Drug)** | Treatment of cyanide poisoning | Injection | 12/15/06 (Animal Rule) | 136 | 69 | **N/A** | | • Approval via animal rule. Efficacy demonstrated in animal models only |
| **Digifab™ (Biologic)** | Treatment of Digoxin toxicity and overdose | IV- Digoxin immune Fab | 8/31/01 | 6 | 15 | n= 15 | N<br>(Tx compared to existing literature regarding Digibind) | • Serum free Digoxin concentrations; levels undetectable after drug administration (no statistics reported) |

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**Table 8 Examples of Approved Compounds with Small Human Databases** (continued)

| Trade Name (Drug/ Biologic) | Indication | Type of Therapy | Date of Approval | Safety Database | | Pivotal Study | | |
|---|---|---|---|---|---|---|---|---|
| | | | | No. Healthy Vol. | No. Patients | No. Patients | Controlled (Y/N) | Endpoints; (p-value) |
| **NovoSeven® (Biologic)** | Treatment of bleeding episodes in hemophilia | Recombinant Coagulation Factor VIIa | 3/24/99 | 0 | 93 | n= 35 (dose 1)<br>n= 43 (dose 2) | N | • Subjective evaluation of efficacy by investigator (no statistics reported) |

C= control group; Tx= Treatment group

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#### 7.4.1.1 BabyBIG®

BabyBIG®, Botulism Immune Globulin Intravenous (Human) (BIG-IV), indicated for the treatment of patients below one year of age with infant botulism (toxin type A or B), received marketing approval from the FDA in October 2003. At the time of marketing approval, the safety and efficacy of BabyBIG had been tested in one randomized, double-blind, placebo-controlled clinical trial (RCT) (129 patients; 122 with laboratory-confirmed infant botulism) and in three open-label studies (OLS, n = 144 patients). Safety data for an additional 147 patients was submitted in a safety update.

#### 7.4.1.2 CroFab™

CroFab™, Crotalidae Polyvalent Immune Fab (Ovine), indicated for the treatment of envenomation by four species of North American pit vipers, received marketing approval from the FDA in October 2000. Two clinical trials using CroFab had been conducted at the time of approval. Both trials were prospectively defined, open-label, multi-center trials conducted in patients 11 years of age or older with minimal or moderate North American crotalid envenomation that showed evidence of progression. The safety and efficacy of CroFab was demonstrated in 11 patients in the first study, and an additional 31 patients in the second study.

#### 7.4.1.3 Cyanokit®

Cyanokit®, indicated for the treatment of cyanide poisoning, received marketing approval from the FDA in December 2006. Due to the infeasibility of conducting clinical efficacy studies in humans, this drug was approved via "the Animal Rule". At the time of marketing approval, safety data had been collected from 139 healthy volunteers and 69 patients exposed to smoke inhalation from fires.

#### 7.4.1.4 DigiFab™

DigiFab™ [Digoxin Immune Fab (Ovine)], indicated for life threatening or potentially life threatening digoxin toxicity or overdose, received marketing approval from the FDA in August 2001. At the time of marketing approval, two clinical trials had been conducted with DigiFab. In the first clinical trial, a randomized and controlled study of DigiFab pharmacokinetics was carried out in

16 healthy adults, six of whom received DigiFab. In a second clinical trial, a single center, open label, randomized, parallel design study examined the efficacy and safety of DigiFab in patients presenting with life-threatening digoxin toxicity. At the time of marketing approval, the safety of DigiFab had been assessed in 6 healthy adults and 15 patients.

#### 7.4.1.5 Novoseven®

Novoseven®, Coagulation factor VIIa (Recombinant), indicated for the treatment of bleeding episodes in hemophilia A or B patients with inhibitors to factor VIII or factor IX, received marketing approval from the FDA in March 1999. At the time of marketing approval, two clinical trials had been conducted with Novoseven; however, data from the initial open-protocol study was insufficient for statistical analysis of safety and efficacy. In a second clinical trial, a double blind, randomized trial was conducted and a total of 78 patients received one of two doses of Novoseven.

## 8.0 SUMMARY

It is not feasible to obtain definitive efficacy data from clinical studies, which rely solely on sporadically-occurring STEC infection and HUS cases, because of the reasons described above. Approvability under "the Animal Rule" is also uncertain because of the relative lack of precedent.

TAI has constructed what it believes to be a reasonable approach to studying a treatment for the prevention of HUS after STEC infection. By combining predictive pre-clinical safety and efficacy data with safety and suggestive, though not statistically-significant, efficacy data in humans, TAI believes that sufficient product labeling can be developed to allow the appropriate use of TMA-15 for the prevention of HUS. Additional information to confirm efficacy or safety in special populations could be continually defined in Phase 4 studies while this product is available to treat the unmet need in vulnerable populations, including during STEC outbreaks.

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# APPENDICES

# APPENDIX A

# DETAILS OF STEC INFECTION INCIDENCE CALCULATIONS

**Incidence Estimated by the CDC**

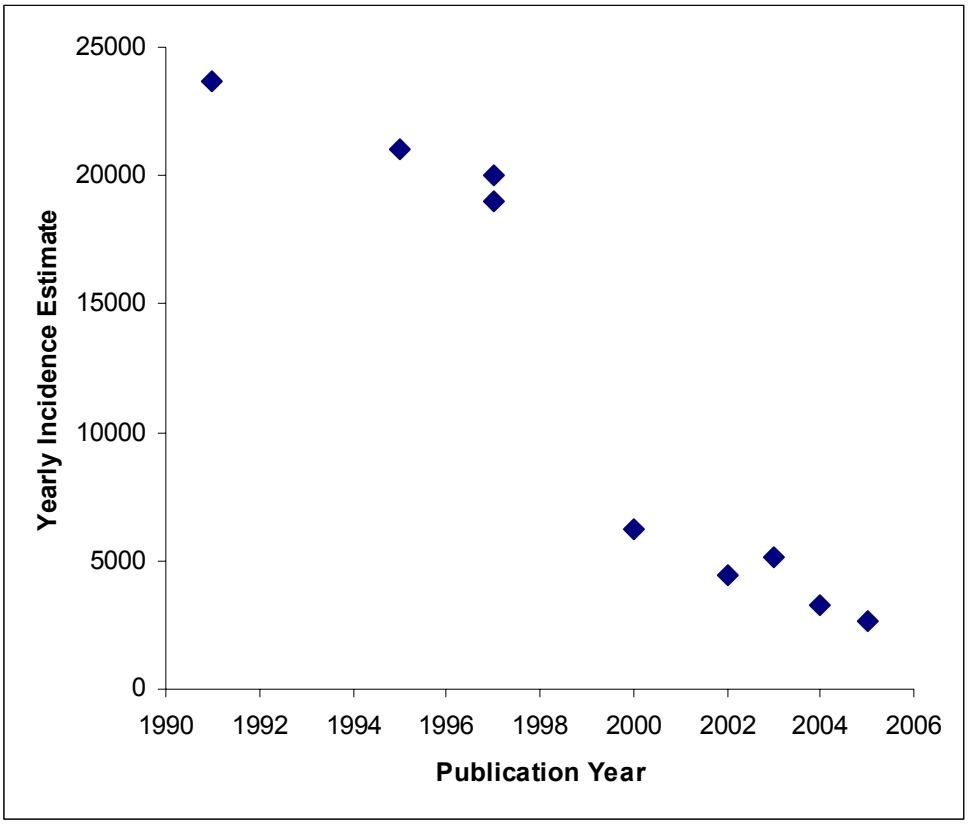
In 1999, the CDC estimated the number of cases of *E. coli* O157:H7 infection each year in the United States to be 73,480 cases[31]. Other recent estimates of the annual incidence of illnesses due to STEC in the United States have been approximately on the order of several thousand. It is important to understand the definition of the CDC estimate before assuming that this is the number of patients available for a clinical study.

The CDC estimate is intended to provide the total number of STEC infections in the United States, and is the result of applying an adjustment factor to the reported surveillance data from 1996 to 1997 in order to reflect the known underreporting of STEC infections. While this may represent an upper estimate of the total cases of STEC infection in the United States at that time, this is not the same number that should be used to estimate the available patient population for a future clinical study. The estimate is based on outdated surveillance data that is higher than recently reported rates, and the number is multiplied by an adjustment factor, a 20-fold increase, in an effort to account for the underreporting of the disease. The surveillance data and the adjustment factor are both overestimates of the incidence of STEC infection, and most STEC infections are not severe enough to even present to a physician and would not be available for a clinical study.

The surveillance data used for the estimate was obtained from the reported cases through active surveillance systems from 1996 to 1997. During this period, there were 3,674 reported cases of STEC infection. In order to obtain the final estimate, the number of reported cases was multiplied by an adjustment factor based on a publication by Hedberg et al[38]. Hedberg estimated, based on population, physician, and laboratory surveys, that for every reported case of STEC infection, there are 13-27 cases that go unreported. The 20-fold increase used for the CDC estimate was the midpoint of the Hedberg et al. adjustment factor for underreporting. Each of the adjustments used to determine an estimated number of total illnesses is also a reason for which the unreported cases would not be available for study. Patients with the disease that do not present to a physician, are not recognized as STEC infection, or were not reported to the surveillance systems, would not be available for clinical study.

**Published Surveillance Data.**

The estimated and reported rates of STEC infections in the United States have dropped steadily and dramatically over time. The 2004 reported incidence of STEC infections was below the 2010 national target of 1.0 cases per 100,000[27]. Figure 11 illustrates published estimates of the total number of STEC infections from sources other than the CDC estimate. These estimates show the rapid decline in STEC incidence, but are also estimates of all STEC infections and still do not represent the available patient population for study for the same reasons as previously mentioned. If only a fraction of these patients are diagnosed or present to physicians, the total number quoted is not available for study.




Graph based on data in Griffen and Tauxe, 1991; Boyce et al, 1995; Mahon et al., 1997; Hedberg et al., 1997; Healthy People 2010, 2nd Ed, 2000; Vugia et al., 2002; Vugia et al., 2003; Vugia et al., 2004; Vugia et al., 2005

**Figure 11. Estimated Yearly Incidence of *E. coli* O157:H7 Infections Over Time from Published Data.**

In addition to a steadily declining incidence of reported STEC infections, the surveillance data also include patients with compatible clinical illness, but without a culture confirmation of STEC infection. This further limits the number of patients available for a clinical study.

**Adjustment factors used in the CDC calculation**

The adjustment factors used in the CDC estimate provide an upper estimate of the total *E. coli* infections, and show the massive underreporting of the disease, but it does not reflect the target patient population for TMA-15 or the patient population available for clinical studies. A description of the mathematics underlying the adjustment factors is a useful tool because each adjustment assumption also illustrates the difficulties with studying this population.

The adjustment factors come from a publication by Hedberg et al.[38], in which six different mathematical steps were used to obtain estimates for the overall incidence of *E. coli* O157:H7 infection cases. Beginning with the actual reported incidence of cases of *E. coli* infection in five states (2.9 per 100,000 across all states), the surveillance data was then adjusted to account for underreporting by factoring the results of physician, population, and laboratory surveys about the presentation and diagnosis of the disease. Six different adjustments were used to reach the final estimated incidence. Three examples of the adjustments are listed below.

Example 1. To account for the differences in physician practice, the surveillance number was divided by the percentage of physicians who cultured the last patient they saw with diarrhea (79%)

Example 2. To account for underreporting by patients, the surveillance number was then divided by an estimate of the percentage of people with BD who see a physician (28%, based on a population survey). It is not clear from the article whether this adjustment is pertaining to patients with BD or STEC-positive patients with BD and consequently may be of questionable relevance.

Example 3. To account for the fact that not all cases result in BD, the surveillance number was then divided by the percentage

*E. coli* O157:H7 cases overall that result in BD (a high and a low estimate, 25% and 50%).

The resulting adjusted overall incidences in the Hedberg et al. publication (39.6 and 79.2 per 100,000) are an average of 20 times the actual reported incidence at the time (2.9 per 100,000). The reason that this derived factor is required to estimate the incidence of the disease is the same reason that prohibits one from identifying the entire available patient population for study:

- If an estimated 28% of cases of BD present to physicians, then this is the percentage of total cases that are available for a clinical study.

- If only 25% to 50% of cases of STEC infection progress to BD, then these are the cases available for clinical study and appropriate for treatment.

In summary, TAI believes that the incidence of cases of BD associated with STEC infection currently available for clinical study in the United States is not 73,480. This number relies on outdated data, is adjusted fallaciously because it includes patients who do not present to physicians, and includes patients who do not exhibit BD or STEC infection.

TAI believes that a more meaningful estimate of the incidence of cases available for clinical study is the reported number of cases of STEC infection with advanced symptoms (n=2664) in the year 2004[27]. Given the very low incidence of HUS, this relative lack of available subjects for a randomized, placebo-controlled clinical trial makes the conduct of such a study unfeasible.

# APPENDIX B

# DRAFT ANNOTATED LABEL

## Annotated Draft Labeling Text

The following is the proposed text of the intravenous TMA-15 labeling. The annotation information is not in final format (i.e., that for a Biologics License Application [BLA]), but will be updated once the BLA is prepared, and will include a column with references to specific BLA sections. For the purposes of preparing for the advisory committee meeting, the annotation is listed in one column only and refers to the actual study that supports each statement.

| | | Annotation |
|---|---|---|
| | | **Supporting Study** |
| | **INDICATION AND USAGE**<br>TMA-15 is indicated for the prevention of hemolytic uremic syndrome (HUS) in pediatric patients with Shiga-like toxin- producing *Escherichia coli* (STEC) infection confirmed using an approved diagnostic. | |
| | **DOSAGE AND ADMINISTRATION**<br>TMA-15 should be administered as a single-dose intravenous (i.v.) infusion of 3 mg/kg. TMA-15 is supplied in 10 mL single-use ampoules containing 5 mL of TMA-15 at a concentration of 4 mg/mL. Before infusion, TMA-15 should be diluted in saline to a final volume of 100 mL. An additional volume of solution should be prepared to allow for priming of the i.v. tubing, and the diluted drug solution should be administered over a one-hour period. Patients weighing less than 10 kg should be administered 10 mL/kg of the TMA-15 infusion solution over a one-hour period.<br><br>The patient should be monitored for possible hypersensitivity reactions for the duration of the infusion and for 8 hours after the infusion. Epinephrine, diphenhydramine, and glucocorticoids should be available for treatment in the unlikely event of acute anaphylactic or other allergic reactions. | TMA-CL-002 |
| | **DOSAGE FORMS AND STRENGTHS**<br>TMA-15 is supplied in 10 mL single-use ampoules containing 5 mL of 4 mg/mL TMA-15 in an aqueous buffer solution. The solution is clear, colorless, and sterile. TMA-15 is to be diluted in saline to a final volume of 100 mL before infusion. An additional volume of solution should be prepared to allow for priming of the i.v. tubing, and the diluted drug solution should be administered over a one-hour period. Patients weighing less than 10 kg should be administered 10 mL/kg of the TMA-15 infusion solution over a one-hour period. | |
| | **CONTRAINDICATIONS**<br>TMA-15 should not be administered to individuals with a prior history of severe reaction to other humanized monoclonal antibody (MAb) treatments or to gamma-globulin preparations. TMA-15 should not be administered to patients with a known hypersensitivity to | |

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| | | **Supporting Study** |
| | TMA-15 or any of its components. | |
| | **WARNINGS AND PRECAUTIONS**<br>No cases of anaphylaxis or severe hypersensitivity reaction have been reported following exposure to TMA-15; however, other MAb treatments have rarely been associated with anaphylactoid or anaphylactic reactions (e.g., fever, urticarial rashes, nausea, dyspnea, anaphylactic shock). Human experience with TMA-15 is limited, and patients should be closely monitored for hypersensitivity reactions during treatment.<br><br>TMA-15 is supplied in single-use ampoules, and any unused portions of the drug product should be discarded.<br><br>TMA-15 is intended for intravenous infusion only and should only be used under the direction of a physician who is familiar with this type of product. | TMA-CL-001, TMA-CL-002 |
| | **ADVERSE REACTIONS**<br>In a randomized, double blind, placebo-controlled, dose-escalating, clinical safety study in healthy adult volunteers, TMA-15 was administered intravenously at a dose range of 0.1 to 3 mg/kg. These doses were within the range prescribed for other approved humanized MAb treatments. Each subject was specifically monitored for possible hypersensitivity reactions throughout the infusion and up to 8 hours after the infusion. The administration of TMA-15 was well tolerated and was not associated with treatment-related hypersensitivity reactions in healthy adult volunteers.<br><br>In a multicenter, randomized, double blind, placebo-controlled, parallel-group Phase 2 study in 109 pediatric patients, two doses of TMA-15 (1 mg/kg and 3 mg/kg) were also well tolerated. There were few AEs considered to be related to TMA-15 administration; treatment–related AEs were experienced by only four subjects. Three of these were mild, and one was moderate in intensity. The mild AEs were pyrexia (one subject in the 1 mg/kg dose group); headache (one subject in the 3 mg/kg dose group), and erythema (one subject in the 3 mg/kg dose group). The AE classified as moderate (vomiting) occurred in one subject in the placebo group. Table 1. | TMA-CL-001<br><br>TMA-CL-002 |

**Table 1. Intensity and Frequency of Adverse Events**

| Intensity | Number of subjects with AEs (%; subjects/total subjects) | | | |
|---|---|---|---|---|
| | 1mg/kg (n = 38) | 3 mg/kg (n = 35) | Placebo (n = 36) | Total (n = 109) |
| Mild | 20 (52.6) | 20 (57.1) | 20 (55.6) | 60 (55.0) |
| Moderate | 10 (26.3) | 9 (25.7) | 10 (27.8) | 29 (26.6) |
| Severe | 2 (5.3) | 2 (5.7) | 2 (5.6) | 6 (5.5) |
| Total number of subjects with AEs | | | | 95 (87.2) |

AE = adverse event.

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| | | **Supporting Study** |
| | ***Immunogenicity***<br>To evaluate the immunogenicity of TMA-15, enzyme-linked immunosorbent assays (ELISAs) were validated and performed. Levels of anti–TMA-15 antibodies were assessed at baseline (before TMA-15 infusion) and again at 22 and 57 days after TMA-15 administration (healthy volunteers). An additional assessment was done 4 months after administration in pediatrics.<br>The levels of anti–TMA 15 antibody in serum were below the limit of detection (1.672 µg/mL) in all subjects evaluated (up to 4 months after administration). | TMA-CL-001<br><br>TMA-CL-002 |
| | **DRUG INTERACTIONS**<br>Studies investigating the interaction between TMA-15 and other medications have not been conducted. | |
| | **USE IN SPECIFIC POPULATIONS**<br>TMA-15 was developed for use in pediatric patients. In a multicenter, randomized, double blind, placebo-controlled Phase 2 study (TMA-CL-002), the safety, PK, and preliminary efficacy of 1 mg/kg and 3 mg/kg TMA-15 were studied in 109 pediatric patients (1 to 15 years old) with STEC infection and bloody diarrhea.<br>*[NOTE: A description of the results of TMA-CL-INF, A Multicenter, Double Blind, Placebo Controlled, Safety and Pharmacokinetic Study of a Single Intravenous Dose of TMA-15 in Infants Between 6 and 12 Months of Age, will be added once those data are available]*<br><br>The safety, but not the efficacy, of this drug has been tested in adult volunteers. There are no available data on the safety and efficacy of TMA-15 in the elderly or in patients with preexisting renal or hepatic disease. | TMA-CL-002<br><br>TMA-CL-INF |
| | **OVERDOSAGE**<br>There is no clinical experience with overdose or multiple-doses of TMA-15. | |
| | **DESCRIPTION**<br>TMA-15 is a recombinant humanized MAb of subclass IgG1 kappa directed against SLT-2, a toxin associated with the development of HUS, produced from a mouse myeloma cell line, 9A11. The molecular weight of TMA-15 is 147 kDa. TMA-15 was originally derived from mouse MAb, MuVTm1.1. Using standard molecular biology technique, the gene encoding TMA-15 in MuVTm1.1 expressing hybridoma was subcloned into appropriate vectors and transfected into the myeloma cell to obtain TMA-15 expressing cell. | |

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| | | **Supporting Study** |
| | **CLINICAL PHARMACOLOGY:**<br>***Mechanism of Action:***<br>TMA-15 neutralizes the toxicity of SLT-2. TMA-15 binds to the B-subunit of SLT-2 that is the binding unit to its glicolipid receptor expressed on the cell membrane. TMA-15 prevents SLT-2 from binding to the receptor, and as a result entry of SLT-2 into target cells and subsequent pathologic effects of SLT-2 are inhibited.<br><br>***Pharmacokinetics:***<br>The pharmacokinetic (PK) properties of TMA-15 were assessed in healthy adult volunteers and pediatric patients.<br><br>In healthy adult volunteers, the disposition of TMA-15 following 0.1-, 0.3-, 1.0-, and 3.0-mg/kg doses appeared to follow a biexponential decline. The mean terminal elimination half-life was reasonably linear across dose groups, ranging from 24.6 days (3.0 mg/kg) to 29.0 days (0.3 mg/kg), which indicates that elimination of TMA-15 is independent of dose over the range of doses examined in the study. Mean maximum observed serum drug concentration ($C_{max}$) values ranged from 2.56 µg/mL (at 0.1 mg/kg) to 71.74 µg/mL (at 3.0 mg/kg) and were also reasonably linear. Within the four cohorts, mean concentrations of TMA-15 appeared to decrease to approximately one-half of the $C_{max}$ by Day 4 after dosing and to approximately one-third of the $C_{max}$ by Day 14 after dosing. It is predicted that circulating levels of TMA-15 for the 1.0- and 3.0-mg/kg cohorts would exceed 10 µg/mL for at least 7 days after dosing. This concentration of TMA-15 has been shown to be effective in SLT-2 neutralization studies conducted *in vitro*.<br><br>In a placebo-controlled PK and safety study, two different doses of TMA-15 were evaluated in 109 pediatric subjects with bloody diarrhea and either the presence of *E. coli* O157 or SLT-2 in the stool or both. Doses of 1 mg/kg and 3 mg/kg produced a serum TMA-15 $C_{max}$ of approximately 20 µg/mL and 56 µg/mL, respectively, and stayed above 10 µg/mL for approximately 4 days and 22 days, respectively. The terminal elimination half-life of TMA-15 was 24.4 days at 1 mg/kg and 22.4 days at 3 mg/kg, suggesting linear PK. The $C_{max}$ and area under the drug concentration time curve from time 0 to infinity [$AUC_{(0\text{-inf})}$] increased proportionally with dose also suggesting linear PK (Table 2). | TMA-CL-001<br><br>TMA-CL-002 |

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| | | **Supporting Study** |

**Table 2. TMA-15 Pharmacokinetic Parameters: Pediatric Subject Population**

| Dose Group | $C_{max}$ (µg/mL) | $T_{max}$ (days) | $AUC_{(0-120)}$ (µg·d/mL) | $AUC_{(0-inf)}$ (µg·d/mL) | $\lambda_z$ ($Day^{-1}$) | $t_{½}$ (days) |
|---|---|---|---|---|---|---|
| 1 mg/kg | 19.64 ±4.07 | 0.136 ±0.174 | 330.26 ±94.99 | 342.30 ±105.05 | 0.031 ±0.011 | 24.42 ±6.40 |
| 3 mg/kg | 56.12 ±12.08 | 0.122 ±0.200 | 841.59 ±239.71 | 868.52 ±268.47 | 0.033 ±0.009 | 22.39 ±6.16 |

$AUC_{(0-120)}$ = area under the drug concentration-time curve from time 0 to time 120; $AUC_{(0-inf)}$ = area under the drug concentration-time curve from time 0 to infinity; Cmax = maximum observed serum drug concentration; λz = terminal elimination rate constant; Tmax = time of maximum serum drug concentration; t½ = elimination half-life.

Table 3 illustrates mean TMA-15 serum concentrations and 95% confidence interval (CI) 21 days after administration for both doses (1 mg/kg and 3 mg/kg). In the 3 mg/kg group, serum TMA-15 level was 11.7 ± 4.0 µg/mL (95% CI: 13.1~10.3). Previous data have demonstrated that 10 µg/mL TMA-15 effectively neutralizes SLT-2 *in vitro*.

**Table 3. Serum concentration of TMA-15 at 21 day post administration**

| | Serum concentration of TMA-15 at 21 day post administration (µg/mL) | |
|---|---|---|
| | **1 mg/kg** | **3 mg/kg** |
| Mean ± SD | 4.7 ± 1.4 | 11.7 ± 4.0 |
| Upper Limit of 95% CI | 5.1 | 13.1 |
| Lower Limit of 95% CI | 4.2 | 10.3 |

**NONCLINICAL TOXICOLOGY**

***Pharmacology (in vitro)***

TMA-15 neutralized SLT-2 produced by 198 out of 200 clinical isolates of STEC at the concentration of 10 µg/mL

TMA-PH-018

***Pharmacology (in vivo)***

In murine models of both SLT-2 injection and STEC inoculation, TMA-15 prevented SLT-2 induced lethality (100% 7-day survival at a TMA-15 dose of 250 µg/kg) and reduced renal damage induced by SLT-2 as monitored by blood urea nitrogen, serum creatinine, and histology.

TMA-PH-004, TMA-PH-015, TMA-PH-017

**Pharmacokinetics**

The PK properties of TMA-15 were measured in cynomolgus monkeys after a single 2-mg/kg intravenous dose. These data showed a maximum TMA-15 concentration of 10 to 15 µg/mL at Day 7, with an estimated half-life of 12 days.

TMA-ME-001

***Toxicology***

Toxicology studies with TMA-15 indicated that a single intravenous administration of up to 20 mg/kg TMA-15 was generally well tolerated in cynomolgus monkeys when assessed for up to 14 days. Clinical observations and pathology data revealed no adverse effects related to TMA-15 exposure.

TMA-TX-001

TMA-CL-001, TMA-CL-002

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| | | **Supporting Study** |
| | A TMA-15 human immunochemistry cross-reactivity study revealed no detectable specific binding of TMA-15 to any of the tested human tissues at TMA-15 concentrations of 1 and 10 μg/mL. | TMA-TX-004 |
| | **CLINICAL STUDIES**<br>Two clinical studies have been conducted with TMA-15.<br><br>An initial Phase 1, randomized, double blind, placebo-controlled, single-dose, dose-escalating study (TMA-CL-001) was designed to assess the safety and PK of four doses of TMA-15 (0.1, 0.3, 1.0, and 3.0 mg/kg administered intravenously) and placebo in 32 healthy, adult volunteers. Safety data collected from 32 healthy volunteers, including 6 subjects who received 3 mg/kg, revealed no significant safety issues.<br><br>In a multicenter, randomized, double blind, placebo-controlled Phase 2 study (TMA-CL-002), the safety, PK, and preliminary efficacy of TMA-15 were studied in pediatric patients. In part A of the study, the safety and PK of two doses of TMA-15 (1 and 3 mg/kg administered intravenously) were examined in 24 pediatric patients with STEC infection. In Part B of the study, the safety, PK, and preliminary efficacy of TMA-15 were evaluated in 85 pediatric subjects by following the progression of clinical symptoms of STEC infection toward complete HUS in subjects with evidence of *E. coli* O157:H7 or STEC infection. Safety data were collected from 109 pediatric patients, including 35 patients who received 3 mg/kg of TMA-15 and 38 patients who received 1 mg/kg TMA-15. No treatment-related safety issues were evident.<br><br>*[NOTE: A description of the results of TMA-CL-INF, A Multicenter, Double Blind, Placebo Controlled, Safety and Pharmacokinetic Study of a Single Intravenous Dose of TMA-15 in Infants Between 6 and 12 Months of Age, will be added once those data are available]* | TMA-CL-001<br><br>TMA-CL-002<br><br>TMA-CL-INF |

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| | | **Supporting Study** |
| | **HOW SUPPLIED/STORAGE HANDLING**<br>TMA-15 is a recombinant humanized MAb. The drug product is supplied in 10-mL single-use ampoules containing 5 mL of 4 mg/mL TMA-15 in an aqueous buffer solution containing 20 mM sodium citrate, 60 mM sodium chloride, 4% w/v sucrose, and 0.01% w/v polysorbate 80, pH 6.0. The solution is clear, colorless, and sterile. TMA 15 is to be diluted in saline to a final volume of 100 mL before infusion. An additional volume of solution should be prepared to allow for priming of the i.v. tubing. The diluted drug solution (100 mL) should be administered over a one-hour period. Patients weighing less than 10 kg should be administered 10 mL/kg of the TMA-15 infusion solution over the one hour period. The drug product must be stored at 2 to 8°C (35.6 to 46.4°F) (do not freeze) and protected from bright light. Discard any unused portion after 24 hours post preparation. | |

# APPENDIX C

# TMA-15 PRINCIPAL INVESTIGATORS

# TMA-15 Principal Investigators

| Study | Investigator Name |
|---|---|
| TMA-CL-001 (PI) | James C. Kisicki, M.D.<br>MDS Pharma Services<br>621 Rose Street<br>Lincoln, NE 68502 |
| TMA-CL-002 (PI),<br>TMA-CL-INF (PI),<br>TMA-CL-EPI (PI) | Eduardo L. Lopez, M.D.<br>Hospital de Niños "Dr. Ricardo Gutiérrez"<br>Buenos Aires, Argentina |
| TMA-CL-002 | Raquel Bianchi, M.D.<br>Hospital Heller<br>Neuquén, Argentina |
| TMA-CL-002 | Daniel E. Allende, M.D.<br>Hospital Provincial Neuquén<br>Neuquén, Argentina |
| TMA-CL-002,<br>TMA-CL-EPI | Roberto Santoro, M.D.<br>Hospital Interzonal Especializado Materno Infantil<br>Mar del Plata, Argentina |
| TMA-CL-002,<br>TMA-CL-EPI | Silvia González Ayala, M.D.<br>Hospital de Niños "Sor María Ludovica"<br>La Plata, Argentina |
| TMA-CL-002,<br>TMA-CL-INF,<br>TMA-CL-EPI | Gustavo Ezcurra, M.D.<br>Hospital Materno Infantil "San Roque"<br>Parana, Argentina<br><br>Hospital de Niños Orland Alassia<br>Mendoza 4151 (3000), Santa Fe<br>Argentina |
| TMA-CL-002,<br>TMA-CL-INF,<br>TMA-CL-EPI | Eduardo Gabriel Glatstein, M.D.<br>Hospital de Niños de la Santísima Trinidad<br>Córdoba, Argentina |
| TMA-CL-002,<br>TMA-CL-INF,<br>TMA-CL-EPI | Eduardo Teplitz, M.D.<br>Hospital Italiano Regional del Sur, Bahía Blanca<br>Provincia de Buenos Aires, Argentina |
| TMA-CL-002 | Miguel Tregnaghi, M.D.<br>Hospital Infantil de Córdoba<br>Argentina |

| Study | Investigator Name |
|---|---|
| TMA-CL-002 | Verna Yiu, M.D.<br>University of Alberta Hospitals<br>Edmonton, Alberta, Canada |
| TMA-CL-002 | Paul Goodyer, M.D.<br>The Montreal Children's Hospital<br>Montreal, Quebec, Canada |
| TMA-CL-002 | Julian Midgley, B.M., B.Ch., M.R.C.P.(UK)<br>Alberta Children's Hospital<br>Calgary, Alberta, Canada |
| TMA-CL-002 | Tom Blydt-Hansen, M.D., F.R.C.P.C., F.A.A.P.<br>Winnipeg Children's Hospital<br>Winnipeg, Manitoba, Canada |
| TMA-CL-002 | Gabrielle Weiler, M.D.<br>Children's Hospital of Eastern Ontario<br>Ottawa, Ontario, Canada |
| TMA-CL-002 | Douglas Ford, M.D.<br>The Children's Hospital<br>Denver, Colorado, USA |