APPLICATION NUMBER:

761046Orig1s000

PHARMACOLOGY REVIEW(S)
Comments on BLA 761046 Bezlotoxumab

From: A Jacobs, AD

Date: 4/29/16

1. I concur that there are no nonclinical approval issues.

2. I made some suggestions regarding labeling and they will be addressed as appropriate.
This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ABIGAIL C JACOBS
05/02/2016
PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: BLA 761046
Supporting document/s: SDN # 1
Applicant's letter date: 11/22/2015
CDER stamp date: 11/23/2015

Product: Bezlotoxumab (MK-6072) Injection
Indication: Prevention of Clostridium difficile infection (CDI) recurrence in patients receiving antibiotic therapy for CDI
Applicant: Merck & Co., Inc.
Review Division: Division of Anti-Infective Products
Reviewer: Terry J. Miller, Ph.D.
Supervisor/Team Leader: Wendelyn Schmidt, Ph.D.
Division Director: Sumathi Nambiar, M.D.
Project Manager: Christopher Davi, M.S.

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Bezlotoxumab (MK-6072) is a fully human, IgG1 monoclonal antibody (mAb) directed against *Clostridium difficile* (*C. difficile*) toxin B. Bezlotoxumab reportedly prevents binding of toxin B to its target cells, thereby blocking a cellular cascade of tissue damage and inflammation responsible for many symptoms of *C. difficile* infection (CDI). Merck and Co., Inc., is pursuing marketing approval for bezlotoxumab for injection, solution as a single intravenous (IV) 60 minute infusion of 10 mg/kg, for prophylaxis to prevent CDI recurrence in adult patients (≥ 18 years of age) receiving antibiotic therapy for CDI. Bezlotoxumab is not indicated for the pediatric population; safety and efficacy of bezlotoxumab to patients < 18 years of age has not been established. Bezlotoxumab reportedly exhibits no antimicrobial activity against *C. difficile*, so it is not considered to be stand-alone therapy for CDI. Bezlotoxumab is not currently approved for another indication.

The current BLA contains a large number of in vitro and in vivo pharmacology studies with bezlotoxumab in several animal models of CDI; single and repeat dose toxicity studies in mice administered bezlotoxumab IV for 1 dose, 5 doses (3 day dose interval) with monitoring for 14 days, or 2 doses (14 day dose interval) with monitoring for 21 days; and two tissue cross-reactivity studies with bezlotoxumab against a panel of mouse and human tissues. The 14-day repeat dose toxicology study in mice and both tissue cross-reactivity studies with bezlotoxumab were previously reviewed by Dr. Wendelyn Schmidt (see Dr. Schmidt’s review of IND #12823 in DARRTS 12/13/2007).

The Applicant conducted no genetic toxicology, carcinogenicity, or reproductive and development toxicology studies with bezlotoxumab, as these were not applicable for this particular biologic product. The Applicant has no plans to conduct any additional nonclinical studies with bezlotoxumab and no additional nonclinical studies will be recommended at this time.

Clinical investigation of bezlotoxumab has generated five Phase 1 clinical trials to characterize safety, pharmacokinetics (PK), and immunogenicity of bezlotoxumab, administered alone and in combination with actoxumab (mAb directed against *C. difficile* toxin A). The efficacy of bezlotoxumab administered alone and in combination with actoxumab was evaluated in two Phase 2 trials and two Phase 3 trials, in which 1664 CDI patients were administered a single 10 mg/kg infusion of bezlotoxumab, bezlotoxumab+actoxumab (10 mg/kg of each mAb), or placebo (0.9% sodium chloride). The Applicant determined no safety or efficacy benefit of actoxumab+bezlotoxumab

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over bezlotoxumab alone; therefore, bezlotoxumab was selected by Merck and Co., Inc. as the product for registration.

1.2 Brief Discussion of Nonclinical Findings

Bezlotoxumab is a human IgG1 monoclonal antibody that reportedly binds Clostridium difficile (C. difficile) toxin B with high affinity. Nonclinical pharmacology and microbiology studies indicate therapeutic activity of bezlotoxumab against toxin-mediated, cellular intoxication associated with CDI when administered in combination with actoxumab (mAb directed against C. difficile toxin A). Bezlotoxumab has no direct antimicrobial activity against C. difficile. No safety pharmacology studies were conducted and no safety pharmacology endpoints were directly assessed in toxicology studies conducted with bezlotoxumab in mice. PK properties of bezlotoxumab appear relatively consistent between mice and humans, particularly after a single dose administration. Bezlotoxumab has a fairly long half-life in plasma of 16.8 days after a single dose in mice, similar to 19 days observed in patients. Single and repeat intermittent-dose toxicology studies in mice with IV bezlotoxumab administered for 14 days (5 doses/3 day interval) and 21 days (2 doses/14 day interval), failed to demonstrate any clinical signs or evidence of toxicity, at doses up to 50 and 125 mg/kg respectively, and at approximately 2.5 and 7 times greater exposure than observed in the clinic after a single 10 mg/kg dose. All histopathological evaluations of tissues from bezlotoxumab treated animals were similar to controls. No targets of bezlotoxumab toxicity were identified in any of the animal toxicology studies. Bezlotoxumab does not appear to be immunogenic, however there is potential interference noted in the anti-drug antibody (ADA) assay in the presence of high serum antibody levels. Bezlotoxumab injection sites appeared free of irritation and inflammation. Tissue cross-reactivity studies conducted in vitro in at least 38 mouse and human tissues with bezlotoxumab showed no reactivity (positive staining) of tissue samples. No genotoxicity, reproductive/developmental toxicity, or carcinogenicity studies were conducted with bezlotoxumab.

1.3 Recommendations

1.3.1 Approvability

This application is approvable from a pharmacology/toxicology perspective.

1.3.2 Additional Non Clinical Recommendations

None at this time.

1.3.3 Labeling

Sponsor Suggested labeling: (From Module 1.14 Draft Annotated Labeling of the NDA application submitted in the 11/23/2015 submission). In the Highlights section, under Indications and Usage, bezlotoxumab is described as

Reference ID: 3924459
this phrase should be removed. An alternative EPC, “human monoclonal antibody directed against *Clostridium difficile* toxin B”, is recommended.

The Sponsor’s proposed labeling and my labeling changes (underlined in italics and/or strikethrough) can be found below:

**INDICATIONS AND USAGE** (From Highlights and Prescribing Section)

*TRADEMARK* is a human monoclonal antibody directed against *Clostridium difficile* toxin B (*bezlotoxumab*). It is indicated for the prevention of *Clostridium difficile* infection (CDI) recurrence in patients 18 years or older receiving antibiotic therapy for CDI.

**8 USE IN SPECIFIC POPULATIONS**

**8.1 Pregnancy**

*Risk Summary*

Adequate and well controlled studies with TRADEMARK have not been conducted in pregnant women. *No animal reproductive and development studies have been conducted with bezlotoxumab.*

The background risk of major birth defects and miscarriage for the indicated population is unknown; however, the background risk in the U.S. general population of major birth defects is 2-4% and of miscarriage is 15-20% of clinically recognized pregnancies.

**8.2 Lactation**

*Risk Summary*

*The developmental and health benefits of breastfeeding should be considered along with the mother’s clinical need for TRADEMARK and any potential adverse effects on the breastfed child from TRADEMARK or from the underlying maternal condition.*
13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No studies have been performed to test the potential of bezlotoxumab for carcinogenicity or genotoxicity.

Fertility studies have not been conducted with bezlotoxumab.

(Reviewer's Comment: Subsection 13.1 looks acceptable as written.)

2 Drug Information

2.1 Drug

CAS Registry Number: 1246264-45-8

Generic Name: bezlotoxumab

Code Name: MK-6072, MDX-1388

Chemical Name: anti-(Clostridium difficile Toxin B) Immunoglobulin G1 (human monoclonal γ-chain), disulfide with human monoclonal κ-chain, dimer

Molecular Formula/Molecular Weight: Molecular formula not provided / M.W. 148.2 kDa

Structure or Biochemical Description: MK-6072 is a fully human IgG1 monoclonal antibody raised against, and designed to bind and block the action of, Clostridium difficile Toxin B. MK-6072 belongs to the IgG1/kappa isotype subclass; (Please refer to the BLA chemistry review for additional information on the structure).
Pharmacologic Class: Human monoclonal antibody directed against C. difficile toxin B.

2.2 Relevant INDs, NDAs, BLAs and DMFs
BB-IND 12823

2.3 Drug Formulation
The Applicant states that MK-6072 drug product (DP) is sterile, aqueous, preservative-free solution in vials for single use. Each vial contains 1000 mg of MK-6072, sodium citrate, sodium chloride, diethylenetriamine pentaacetic acid (pentetic acid), and polysorbate 80 at pH 6.0. MK-6072 DP is supplied in a 50 mL vial.

The MK-6072 requires dilution into 40 mL of a compatible diluent prior to administration by intravenous infusion to reach a target delivery concentration. Composition of the drug product is show in Table 1 below:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Compendial Grade</th>
<th>Target Amount (mg/vial)</th>
<th>Target Concentration</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK-6072</td>
<td>NA</td>
<td>1000</td>
<td>25 mg/mL</td>
<td>Active ingredient</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>Ph. Eur./USP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Citrate Dihydrate(^b)</td>
<td>Ph. Eur./USP</td>
<td>( (b)(4) )</td>
<td>( (b)(4) )</td>
<td></td>
</tr>
<tr>
<td>Citric Acid Monohydrate(^b)</td>
<td>Ph. Eur./USP</td>
<td>( (b)(4) )</td>
<td>( (b)(4) )</td>
<td></td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td>Ph. Eur./NF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTPA</td>
<td>USP(^c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water for Injection</td>
<td>Ph. Eur./USP</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^b\) DTPA is listed in the USP as pentetic acid. There is no Ph.Eur. monograph for this material.

(Table 1 on page 5 in Module 3.2.P.2 Drug Product)
2.4 Comments on Novel Excipients

There were no novel excipients included in the final clinical formulation of MK-6072. The Applicant reported that all of the compounds listed in Table 1 (above) are introduced during the formulation of bulk MK-6072 drug substance. No excipients are added during the drug product manufacturing process.

2.5 Comments on Impurities/Degradants of Concern

The Applicant reported there no product or process-related impurities or degradation products of concern. The Applicant reported that visible particles may develop during the manufacture of the drug product. The Applicant stated that all vials will be “100% inspected” to ensure the final drug product is free of any visible particles. All other detected impurities were well within the release and stability specifications designated for MK-6072 drug substance and drug product.

The Applicant stated that the specified impurities of MK-6072 include ... The Applicant stated that these impurities meet specified acceptance criteria and were further qualified in the nonclinical toxicology program for MK-6072 (Table 2). Based on the clinical indication, proposed clinical dosing, and ... there does not appear to be any indication that impurities detected in the product will pose any significant toxicological risk to patients.

Table 2. List of Process Related Impurities in MK-6072

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Source</th>
<th>Mean Levels*</th>
<th>Acceptance Criterion</th>
</tr>
</thead>
</table>

* Mean impurity level detected in drug lots generated using the commercial process

2.6 Proposed Clinical Population and Dosing Regimen

The proposed labeling states that bezlotoxumab is indicated for the prevention of *Clostridium difficile* infection (CDI) recurrence in adult patients (≥ 18 years) receiving antibiotic therapy for CDI. Bezlotoxumab has no direct antimicrobial properties. Bezlotoxumab should be administered only during a course of antibiotic therapy for CDI. The labeling states that the proposed clinical use will be a single administration of bezlotoxumab to adult patients at a dose of 10 mg/kg (based on patient body weight) IV over 60 minutes after dilution in 0.9% Sodium Chloride Injection, USP (normal saline) or 5% Dextrose Solution, USP, to a final concentration of 1 to 10 mg/mL.

Bezlotoxumab is not indicated for the pediatric population (< 18 years of age); safety and efficacy of bezlotoxumab to patients < 18 years of age has not been established.
2.7 Regulatory Background

Bezlotoxumab (MK-6072, MDX-1388) was submitted to the Division in IND# 12823 on 11/29/2005. During early nonclinical and clinical development, the Applicant examined the safety and PK of bezlotoxumab alone, actoxumab (GS-CDA1; MDX-066) alone, and the combination of bezlotoxumab+actoxumab, administered in single and repeat intravenous infusions to animals, and a single infusion into healthy volunteers. The Sponsor submitted nonclinical studies to support the first in human trials for the antibody combination and MK-6072 alone, including a two week intravenous toxicity study in mice and two tissue cross-reactivity studies with panels of human and mouse tissues, which were reviewed by Dr. Wendelyn Schmidt (in DARRTS 12/13/2007). Early discussion with the Division also involved recommendations to the Sponsor to consider an additional 14 day toxicology study in a diseased animal model of CDI, an embryo-fetal developmental toxicology assessment, and a sub-chronic repeat dose toxicology study of the antibody combination in a second species. However, with continued development and application of the revised ICH S6 Guidance for Industry “Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals”, no additional nonclinical toxicity studies were ultimately required.² The Applicant was granted fast track status designation on May 12, 2010 for treatment of patients with C.difficile infection (CDI) and/or for the prevention of CDI recurrence. The Applicant was granted a deferral on pediatric assessments until after approval of the BLA in adults on January 5, 2015.

Following a teleconference with the Sponsor on October 28, 2009, the Sponsor was reminded to provide clinical evidence for the contribution of each antibody component of the combination product in humans as recommended in the FDA Guidance on the combination drug product rule.³ Subsequent results of the pivotal Phase 3 trials (Protocols 001 and 002) reportedly demonstrated that bezlotoxumab (MK-6072) reduced the CDI recurrence rate compared to placebo and that the combination of two mAbs (MK-6072 and MDX-066) failed to show a safety or efficacy benefit over MK-6072 alone. MK-6072 was selected as the final product for registration. The Applicant has not proposed a proprietary name for the commercial product at the time of review of their BLA.

3 Studies Submitted

(Note: All of the studies submitted to the BLA that assessed the toxicology of the anti-CDA1 monoclonal antibody (anti-toxin A) were not applicable (N/A) to the current BLA marketing application and therefore were not examined in the review of this BLA.)

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3.1 Studies Reviewed

Toxicology:
1. Exploratory Single-Dose Intravenous Toxicokinetic Study in Mice [non-GLP] (Study No. TT #14-1108).
2. Twenty-one day intermittent-dose intravenous toxicity study (with MK-3415A, MK-6072) in mice [GLP] (Study No. TT #15-1015) [partial review].
3. Twenty-one day intermittent-dose intravenous toxicity study (with MK-3415A, MK-6072) in mice [GLP] (Study No. TT #15-1079) [partial review].

3.2 Studies Not Reviewed

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4 The description of the nonclinical study findings included in this review will pertain only to the control and MK-6072 treatment groups, and will not include any findings from the MK-3415A (combination MK-6072 and MK-3415) dose groups, also tested in this study. MK-3415 is an anti-CDA1 monoclonal antibody and therefore is not relevant to the current BLA marketing application.
3.3 Previous Reviews Referenced
Nonclinical review of BB-IND 12823 (MK-6072 injection), by Dr. Wendelyn Schmidt (in DARRTS 12/13/2007) included review of the following previously submitted studies:
1. A two week intravenous toxicity study in mice with MDX-1388 and GS-CDA1 (MDX-066) [GLP] (Study #WIL552001 / TT v#11-7803).
2. Cross reactivity study of MDX-1388-FITC with normal human tissues [GLP] (Study # IM1208 / TT #117801).
3. Cross Reactivity study of MDX-1388-FITC with normal CD-1 mouse tissues [GLP] (Study # IM1209 / TT #117802).

4 Pharmacology
4.1 Primary Pharmacology
The Pharmacology section included 24 study reports with a combination of studies of bezlotoxumab and/or actoxumab. These reports were not reviewed in full. The primary pharmacology of MK-6072 for this BLA is reviewed by the clinical microbiology review team. In brief, bezlotoxumab (MK-6072) and actoxumab (MDX-1388) are fully human monoclonal antibodies raised against C. difficile toxin B (TcdB) and toxin A (TcdA), respectively. Both antibodies reportedly bind directly to their respective toxins and prevent toxin binding to the target cells, resulting in a blockade of cellular intoxication cascades critical to inflammation and pathogenesis of CDI. In preclinical rodent models of CDI, use of the antibody combination (bezlotoxumab+actoxumab) was required to prevent morbidity and mortality; bezlotoxumab treatment alone in CDI animals provided minimal protection to animals, despite reportedly reducing recurrence rate in CDI patients in phase 3 clinical trials. A summary of the reported key findings from the Applicant’s summary of the submitted pharmacology studies conducted with MK-6072 is included below. (Note: Pharmacology information for actoxumab (MDX-1388) is not included below).
- MK-6072 binds TcdB with high affinity/selectivity in vitro (K_d values of 19 and 370 pM in a 2-site binding model); no cross-reactive binding to TcdA was observed.
- Bezlotoxumab binds 2 homologous binding sites within the N-terminus domain of TcdB, and prevents toxin binding to Vero cells (African green monkey kidney epithelial cell line) in vitro.
- The binding affinity and neutralizing potency of bezlotoxumab to TcdB appears C. difficile strain specific; Toxin B from several ribotype strains (027, 036, 078) is differentially sensitive to toxin binding and neutralization by bezlotoxumab.
- In vitro toxin neutralization assays in Vero, Caco-2 (human colon epithelial cell line) and T-84 (human colon carcinoma cell line) cells administered
bezlotoxumab+actoxumab and toxins TcdA and TcdB (LC$_{90}$) showed dose-dependent, protection against cell death and disruption of cell layer integrity.

- Bezlotoxumab+actoxumab reduced intestinal loop fluid accumulation, epithelial damage, and inflammation with TcdB+TcdA injection to control levels in an ex vivo model.
- Prophylactic treatment of Swiss mice administered bezlotoxumab+actoxumab (up to 300 mcg each) 1 hour prior to TcdB and TcdA lethal challenge showed a dose-dependent increase in survival (50-100%) up to 72 hours post challenge; bezlotoxumab administered alone 24 hours prior to TcdB challenge did not increase survival.
- Prophylaxis in the hamster model of CDI with bezlotoxumab+actoxumab (administered daily for 4 days with last 2 days coinciding with clindamycin administration and lethal concentrations of C. difficile spores), showed decreased morbidity, decreased C. difficile, and increased survival (up to 40%) at Day 28, compared to 100% mortality in control.
- In the mouse CDI model, prophylaxis and treatment with bezlotoxumab + actoxumab (50 mg/kg each) up to 24 hours before or after spore challenge, respectively, reduced body weight loss, protected against intestinal swelling and hemorrhaging, and prevented mortality observed in controls.
- Bezlotoxumab+actoxumab does not reduce C. difficile colonization in the gut; C. difficile spore burden and toxin concentrations in rodent models of CDI were similar between animals treated with the antibody combination or vehicle.
- In vitro and in vivo efficacy of bezlotoxumab+actoxumab acts independent of Fc-mediated activity; N279Q antibody mutants devoid of effector functions lack Fc receptor binding and showed similar efficacy to wild-type in primary/relapse mouse models of CDI. Similarly, co-administration with 40-fold excess of human IgG or use of transgenic Fc receptor knockout mice also had no significant impact on protection against CDI with bezlotoxumab+actoxumab.

4.2 Secondary Pharmacology

None submitted.

4.3 Safety Pharmacology

None submitted.

5 Pharmacokinetics/ADME/Toxicokinetics

The Pharmacokinetics section included 6 methods validation reports and 2 study reports. These were not reviewed. The Applicant conducted no standard tissue distribution, metabolism, or excretion studies with bezlotoxumab as per current ICH S6(R1) guidance. Merck and Co. validated several immunoassays to quantify levels of C. difficile toxin B, MK-6072 and anti-MK-6072 antibodies. More information can be
found in the Clinical Pharmacology review. Refer to Section 6.2 Repeat-Dose Toxicity in this review document for toxicokinetic information for MK-6072.

(Reviewer’s comment: It is generally considered that large molecules such as monoclonal antibodies are limited to the vascular component and therefore interspecies comparison are made on nominal doses rather than doses normalized to body surface area. It is not known what impact wider distribution of the antibody to extravascular space may have on human equivalent doses).

Method Validation
Merck validated several immunoassays used in the nonclinical development of bezlotoxumab, including enzyme-linked-immunosorbent-assays (ELISA) methods to quantify MK-6072, and anti-MK-6072 antibodies. The list of validated immunoassays used to determine MK-6072 and anti-MK-6072 antibodies is provided in the table 3 below.

Table 3. Bioanalytical Methods and Assay Details

<table>
<thead>
<tr>
<th>Method Description</th>
<th>Study Number</th>
<th>Study Title</th>
<th>Species/ Strain</th>
<th>Validated Assay GLP</th>
<th>Assay purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug assays for GLP toxicity studies</td>
<td>TT 15-1015, TT 15-1079 (BT00114 &amp; BT00116)</td>
<td>Validation Report title: Validation of an Electrochemiluminescence Immunoassay for the Determination of MK-3415 Concentrations in CD-1 Mouse Serum Using Meso Scale Discovery’s Sector Imager 6000 Analyzer. Validation of an Electrochemiluminescence Immunoassay for the Determination of MK-6072 Concentrations in CD-1 Mouse Serum using Meso Scale Discovery’s Sector Imager 6000 Analyzer.</td>
<td>Mouse/CD-1</td>
<td>Yes/Yes</td>
<td>Assay purpose: Determination of drug level in mouse serum Type: ECL</td>
</tr>
</tbody>
</table>

(Table 2.6.4:1 on page 7 of Module 2.6.4 Pharmacokinetic Written Summary)

Tissue Localization of bezlotoxumab in Hamster Model of CDI
The Applicant examined the localization of bezlotoxumab to the site of infection in the gut lumen of hamsters challenged with or without toxigenic *C. difficile*, followed by a single intravenous dose of bezlotoxumab+actoxumab at 50 mg/kg/each (Study No. PD005). From samples of intestinal tissues collected from both infected and uninfected animals at various timepoint after challenge, the Applicant reported the following findings:
• No significant difference in overall antibody levels of bezlotoxumab or actoxumab was observed in intestinal tissues from infected and uninfected hamsters.
• Antibody levels in the GI lumen were significantly greater in infected hamsters, particularly the cecum, and undetectable in healthy animals.
• Significant damage to the gut epithelial barrier in animals infected with C. difficile resulted in immunohistochemical detection of antibodies throughout the mucosal epithelial layer and gut lumen, typically confined only to the mucosal subepithelial space in healthy, uninfected mice with an intact gut barrier.
• Based on these findings and results of an in vitro TER (transepithelial electrical resistance) assay that showed toxin dependent transport of antibodies across a confluent monolayer of Caco-2 cells, it’s suggested that paracellular transport of antibodies across the epithelial gut barrier may actually be toxin-dependent, non-specific leakage of antibodies across a disrupted gut epithelium.

Clinical PK Information for bezlotoxumab (for comparison)
In Module 2.7.2 Summary of Clinical Pharmacology Studies, the Applicant included a summary of clinical PK parameters of a single 10 mg/kg intravenous dose of bezlotoxumab to patients, to which mouse TK data for bezlotoxumab in the submitted toxicology studies are compared in this review.

“Based on the population PK analysis, the geometric mean (%CV) clearance (CL) of bezlotoxumab is 0.317 L/day (40%), with a volume of distribution (Vd) of 7.33 L (16%), and an elimination half-life (t½) of approximately 19 days (28%). In patients who received a single 10 mg/kg IV dose of bezlotoxumab, mean bezlotoxumab AUC_{0-inf} and C_{max} are 53,000 μg.h/mL and 185 μg/mL, respectively.”

6 General Toxicology
The Applicant conducted an exploratory single dose IV toxicity study in mice, several repeat-dose toxicology studies including a 14-day IV toxicity study in mice with MK-6072 (anti-CDB-1 monoclonal antibody) and MK3415 (anti-CDA-1 monoclonal antibody), and two 21-day intermittent-dose IV toxicity studies in mice with MK-6072 and MK-3415A (a combination of MK-6072 and MK3415). A detailed review of the single dose mouse toxicity study and (two) 21-day intermittent dose IV toxicity studies in mice with MK-6072 is provided below.5

The 15-day repeat dose toxicity study in CD-1 mice with MK-6072 (Study No. WIL552001) was previously reviewed by Dr. Wendelyn Schmidt for IND 12823 (see Dr. Schmidt’s Nonclinical Review in DARRTS, 12/13/2007). A brief summary of findings

5 The description of the nonclinical study findings included in this review will pertain only to the control and MK-6072 treatment groups, and will not include any findings from the MK-3415A (combination MK-6072 and MK-3415) dose groups, also tested in this study. MK-3415 is an anti-CDA1 monoclonal antibody and therefore is not applicable (N/A) to the current BLA marketing application.
from Dr. Schmidt’s review for the 15-day repeat-dose toxicology study is also included below.

(Reviewer’s Comment: In Study No. TT #15-1015 described in Section 6.2 Repeat Dose Toxicology below, the Applicant detected a high bioburden level in the vehicle lot that exceeded specification, prompting significant changes to the study design and deviations from the study protocol. These changes made the study report and study findings difficult to follow and interpret. Because of the reported challenges with this study, the Applicant repeated the 21-day intermittent IV mouse toxicology study with MK-6072 (Study No. TT #1501-79). The repeated study with MK-6072 was conducted as planned and is considered pivotal in the nonclinical safety assessment of MK-6072. A review of both studies is included below.)

6.1 Single-Dose Toxicity

The single dose toxicity study in mice was conducted to determine the toxicokinetic profile of MK-6072 when administered as a single intravenous dose to mice.
Study title: Exploratory Single-Dose Intravenous Toxicokinetic Study in Mice (non-GLP)

Study no.: TT #14-1108  
Study report location: EDR in DARRTS  
Conducting laboratory and location: Safety Assessment and Laboratory Animal Resources (SALAR), Merck Research Laboratories, West Point, PA, U.S.A  
Date of study initiation: 12/8/2014  
GLP compliance: No  
QA statement: No  
Drug, lot #, and % purity: MK-6072, Lot #: L-005494597-001C001, 98.9% purity; MK-3415, (N/A).

Methods

Doses: MK-6072: 50 mg/kg; MK-3415/MK-3415A (N/A)  
Frequency of dosing: Single dose on Study Day 1  
Route of administration: I.V. bolus to tail vein at injection rate of 2 mL/min  
Dose volume: 10 mL/kg  
Formulation/Vehicle: MK-6072 (25 mg/mL), sodium chloride, sodium citrate, DTPA, PS-80, pH 6.0) for injection; diluted into 0.9% sodium chloride (vehicle)  
Species/Strain: Mouse / CRI:CD-1 (ICR)  
Number/Sex/Group: Toxicity Assessment: 30 mice/group, no control  
Age: 10 weeks old  
Weight: Males Only: 31.5-41.6 g  
Satellite groups: None  
Study design: Group housed (n=3/cage) under fluorescent light on a 12 h cycle; food and water ad libitum  
Deviation from study protocol: None reported that affected the integrity of this study.

Dose Justification: Dose levels were based on prior toxicity studies in mice

Observations and Results

Mortality (Once daily):  
All animals treated with MK-6072 and vehicle survived to scheduled euthanization.

Body Weights: Pre-dosing only

Toxicokinetics – blood collected from orbital sinus/vena cava from TK/Immunogenicity animals (Control animals [n=3/sex] at 0, 168 504 hours post dose; MK-6072 treated animals [n=18/sex] at the following timepoints (Note: 2nd dose administered 336 hours after the first dose)  
- Cohort A: 0 and 672 hours post-dose (Study Day 28)  
- Cohort B: 0.167 and 240 hours post-dose (Study Day 10)
Cohort C: 1 and 336 hours post-dose (Study Day 14)  
Cohort D: 1 and 504 hours post-dose (Study Day 21)  
Cohort E: 24 and 168 hours post-dose (Study Day 7)  
Cohort F: 48 and 72 hours post first dose (Study Day 3)

Serum concentrations of MK-6072 was determined using an electrochemiluminescence (ECL) immunoassay on Sector Imager 6000 from Meso Scale Discovery (MSD) (Gaithersburg, MD). This study was supported by validation report BT00116 for MK-6072 (the same validation report for the repeat dose toxicology studies). The toxicokinetic parameters for MK-6072 are presented in Table 4.

### Table 4. Summary Mean (SE) Serum MK-6072 Toxicokinetic Parameters in Male Mice Following Dosing of MK-6072: Study Day 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Dose (mg/kg)</th>
<th>Sex</th>
<th>AUC$_{0-28$ day} $day*mcg/mL</th>
<th>AUC$_{0-\infty}$ day*mcg/mL</th>
<th>C$_{max}$ mcg/mL</th>
<th>t$_{1/2}$ (day)</th>
<th>T$_{max}$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK-6072</td>
<td>50</td>
<td>Male</td>
<td>5540 ± 296</td>
<td>7930 ± ID</td>
<td>734 ±178</td>
<td>16.8 ± ID</td>
<td>0.17 ± NC</td>
</tr>
</tbody>
</table>

NC = Not Calculated  
ID = Insufficient Data

Following intravenous bolus administration of 50 mg/kg MK-6072 to mice, the mean serum concentration profile was biphasic, with mean MK-6072 C$_{max}$ value that was attained 0.17 hours post-dose. Thereafter, mean MK-6072 concentrations eliminated slowly, and were measurable up to 28 days post-dose, with an apparent terminal elimination half-life (t$_{1/2}$) of 16.8 days.

Anti-Drug Antibody Analysis – blood collected (at 0 h pre-dose and on Study Days 7, 14, 21, and 28 days post-dose):  
Results not reported.

Dosing Solution Analysis:  
Results not reported.

(Reviewer’s comments: The study design to determine the toxicokinetic parameters of a single dose injection of MK-6072 to mice was acceptable. No mortality was observed in this study. At 50 mg/kg, the AUC$_{0-\infty}$ and C$_{max}$ values were 7930 mcg*/day/mL and 734 mcg/mL, respectively. The TK profile of MK-6072 is consistent with other monoclonal antibodies, which typically have low clearance and a limited volume of distribution. The terminal elimination half-life (t$_{1/2}$) of MK-6072 in mice administered a single bolus injection was 16.8 hours.)

### 6.2 Repeat-Dose Toxicity

1. **Study title:** (MK-6072 and MK-3415) Twenty-One-Day Intermittent-Dose Intravenous Toxicity Study in Mice
Study no.: TT #15-1015
Study report location: EDR in DARRTS
Conducting laboratory and location: Safety Assessment and Laboratory Animal Resources (SALAR), Merck Research Laboratories, West Point, PA, U.S.A
Date of study initiation: 2/20/2015
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MK-6072, Lot #: L-005494597-001C001, 98.9% purity; MK-3415, (N/A).

Methods

Doses: MK-6072: 125 mg/kg; MK-3415 (N/A)
Frequency of dosing: Once on Dosing Days 1 and 15, total of two doses; staggered dosing – toxicokinetic (TK)/immunogenicity group dosed 5 days after toxicity arm; mice were euthanized on Study Days 11/12 or 21 (4 days after 2nd dose)
Route of administration: I.V. to tail vein, 2 mL/min using a syringe pump
Dose volume: 10 mL/kg
Formulation/Vehicle: MK-6072 (25 mg/mL) sodium chloride, sodium citrate, DTPA, PS-80, pH 6.0) for injection; diluted into 0.9% sodium chloride (vehicle); placebo was the vehicle alone (Lot #: L-005494631-001F001, L-005494631-001F002).
Species/Strain: Mouse / CRI:CD-1(ICR)
Number/Sex/Group: Toxicity Assessment: 10 sex/group including vehicle control
Age: 7 weeks old
Weight: Male: (33.6 – 43.5 g); Female: (24.6 – 32.7 g)
Satellite groups: TK/Immunogenicity: 21/sex/group for MK-6072, 9/sex for control
Study design: Group housed (n=3/cage) under fluorescent light on a 12 h cycle; food and water ad libitum
Deviation from study protocol: On Study Day 4, bioburden level of vehicle used for all animals in main toxicity arm and all male animals in the TK/Immunogenicity arm exceeded specification. Starting Study Day 5, a new

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6 On Study Day 4, the Applicant detected a high bioburden level in the vehicle (batch #L-005494631-001F001) that exceeded specifications. This batch was used for the first dose for all animals in the original Toxicity Arm and all male animals in the original Toxicokinetic/Immunogenicity Arm. On Study Day 5, a new batch of vehicle (batch #L-005494631-001F002) was used to treat all remaining animals, including the replacement animals added to the TK/Immunogenicity arm on Study Day 15. The bioburden level of this new vehicle lot met specifications.
vehicle batch was used in the study. On Study Days 11, and 12, all animals dosed with the affected vehicle were euthanized, and replaced with animals originally included in a separate Clinical Pathology arm in the original study design (dosed with the replacement vehicle lot). Clinical pathology examinations were performed on the Toxicity arm animals at final necropsy. Additional male animals were added to the study to the TK/Immunogenicity arm mid-study and were dosed twice, once each on Study Days 18 and 32 (Days 1 and 15). The description of the study design was updated. According to the Study Director, these changes did not affect the overall integrity of this study.

**Study Design (Revised)**
(Note: This study was originally designed with a Toxicity arm (Groups 1-5), Clinical Pathology arm (Groups 6-10), and TK/Immunogenicity arm (Groups 11-15)).

<table>
<thead>
<tr>
<th>Dose Group</th>
<th>Treatment</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Vehicle Control: 105494631</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>MK-6072 at 125 mg/kg/dose</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>MK-3415A (MK-6072 + MK-3415: 10 mg/kg/dose + 10 mg/kg/dose)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>MK-3415A (MK-6072 + MK-3415: 50 mg/kg/dose + 50 mg/kg/dose)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>MK-3415A (MK-6072 + MK-3415: 125 mg/kg/dose + 125 mg/kg/dose)</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
Dose Justification: Dose levels were based on the maximum feasible dose.

Observations and Results
Mortality (Once daily):
All animals treated with MK-6072 and vehicle survived to scheduled euthanization.

Clinical Signs (Once daily):
No test-item related clinical signs were observed during the study.

Body Weight (Pre-test, Once weekly):
No test article related changes in body weight or body weight gain were observed.

Ophthalmologic Examination (Toxicity arm animals only; slit lamp biomicroscopy and indirect ophthalmoscopy; pre-dose; all animals on Study Day 22, three days after second dose):
There were no test article related ophthalmologic findings.
Hematology (at necropsy; 5 mice/sex/group on Study Days 11/12 and on Study Day 21 (4 days after 2\textsuperscript{nd} dose):
There were no test-article related effects on any hematology parameters.

Clinical Chemistry (at necropsy; 5 mice/sex/group on Study Days 11/12 and on Study Days 21 (4 days after 2\textsuperscript{nd} dose):
There were no test-article related effects on any serum chemistry parameters.

Toxicokinetics –blood collected from orbital sinus/vena cava from TK/Immunogenicity animals (Control animals \[n=3/\text{sex}\] at 0, 168 504 hours post dose; MK-6072 treated animals \[n=18/\text{sex}\] at the following timepoints (Note: 2\textsuperscript{nd} dose administered 336 hours after the first dose)

Cohort A: 0.167 and 336.17 hours post first dose
Cohort B: 4 and 340 hours post first dose
Cohort C: 7 and 343 hours post first t dose
Cohort D: 24 and 360 hours post first dose
Cohort E: 48, 336 and 384 hours post 1st dose
Cohort F: 0, 168 and 504 hours post first dose

Serum concentrations of MK-6072 was determined using a biotin-streptavidin bridging immunoassay analyzed on a MSD Discovery Sector Imager 6000 (Meso Scale Discovery [MSD], Gaithersburg, MD, USA). The MK-6072 toxicokinetic parameters are presented in Table 5. Serum samples from control animals showed no detectable level of MK-607 (LLQ = 0.04 mcg/mL).

Table 5. Serum Toxicokinetic Parameters of MK-6072 in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>AUC (_{0-336\ hr}) (mcg/mL(\cdot)hr)</th>
<th>AUC (_{0-504\ hr}) (mcg/mL(\cdot)hr)</th>
<th>(C_{\text{max}}) mcg/mL</th>
<th>(T_{\text{max}}) (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK-6072</td>
<td>Female</td>
<td>211,000 ± ID</td>
<td>381000 ± 10600</td>
<td>2710 ± 51.9</td>
<td>336.17</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>168,000 ± ID</td>
<td>331000 ± 22900</td>
<td>2590 ± 137</td>
<td>336.17</td>
</tr>
</tbody>
</table>

ID = Insufficient Data (Reason for ID undefined)

Mean female/male AUC values were 211,000/168,000 mcg/mL\(\cdot\)hr at 336 hours after the first dose, respectively, and 381,000/331,000 mcg/mL\(\cdot\)hr at 504 hours post first dose (7 days after the second dose), respectively. Mean maximal MK-6072 concentrations in female and male mice were observed at 0.167 hr (approximately 10 min after the first dose) or 336.17 hours after the first dose (approximately 10 min after the second dose) over the concentration vs time profile from 0 to 504 hours. Mean serum MK-6072 concentrations at 504 hours were 21-37% of the respective mean \(C_{\text{max}}\) values. There were no clear sex differences in AUC and \(C_{\text{max}}\) values of MK-6072 at all sampling periods. (Note: The validation study [Study No. BT00116 Rev. 1] of the ECL immunoassay for MK-6072 in CD-1 mouse serum indicate the ECL assay to be acceptable for selectivity, accuracy, and reproducibility).
Anti-Drug Antibody Analysis - blood collected from orbital sinus/vena cava, n=6 mice/sex/cohort from TK/Immunogenicity animals (at pre-dose and 504 hours after first dose (7 days after 2nd dose) was administered):
Serum anti-drug antibody (ADA) response to MK-6072 was determined using an electrochemiluminescence assay. No anti-MK-6072 drug antibodies were observed in any serum sample collected pretest, or at 504 hr post-dose in serum from controls and MK-6072 treated mice.

Gross Necropsy (at necropsy; all animals on Study Days 11/12 and 21 (4 days after 2nd dose):
No macroscopic changes attributed to MK-6072 were recorded at necropsy.

Organ Weights (at necropsy; all animals on Study Days 11/12 and 21 (4 days after 2nd dose):
No test article related organ weight changes were detected at necropsy.

Histopathology (at necropsy; all animals on Study Days 11/12 and 21 (4 days after 2nd dose)
Adequate Battery: Yes
Routine Tissues Collected:

<table>
<thead>
<tr>
<th>Adrenal glands*</th>
<th>Large intestine</th>
<th>Seminal vesicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>Liver*</td>
<td>Skeletal muscle (quadriceps)</td>
</tr>
<tr>
<td>Brain*</td>
<td>Lungs</td>
<td>Skin</td>
</tr>
<tr>
<td>Cecum</td>
<td>Lymph nodes mandibular</td>
<td>Spinal cord</td>
</tr>
<tr>
<td>Cervix</td>
<td>Lymph node, mesenteric</td>
<td>Spleen*</td>
</tr>
<tr>
<td>Bone (femur, tibia, femorotibial joint)</td>
<td>Mammary gland</td>
<td>Sternal</td>
</tr>
<tr>
<td>Epididymis</td>
<td>Muscle</td>
<td>Stomach</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Optic nerves</td>
<td>Testes*</td>
</tr>
<tr>
<td>Eyes</td>
<td>Ovaries</td>
<td>Thymus*</td>
</tr>
<tr>
<td>Femur, proximal</td>
<td>Pancreas</td>
<td>Thyroids/parathyroid</td>
</tr>
<tr>
<td>Gall Bladder</td>
<td>Peripheral nerve (sciatic)</td>
<td>Tongue</td>
</tr>
<tr>
<td>GALT</td>
<td>Pituitary</td>
<td>Trachea</td>
</tr>
<tr>
<td>Heart*</td>
<td>Prostate</td>
<td>Ureters</td>
</tr>
<tr>
<td>Harderian gland</td>
<td>Rectum</td>
<td>Urinary bladder</td>
</tr>
<tr>
<td>Kidneys*</td>
<td>Salivary glands</td>
<td>Uterus</td>
</tr>
<tr>
<td>Injection Site</td>
<td>Sciatic nerve</td>
<td>Vagina</td>
</tr>
<tr>
<td>Larynx</td>
<td>Small intestine</td>
<td>Peyer’s patch</td>
</tr>
</tbody>
</table>

*Organ weights collected

Peer Review: yes

Histological Findings: There were no notable treatment-related microscopic changes at the injection site or in any systemic organs evaluated in animals administered MK-6072. All microscopic findings observed in treated animals were considered incidental
changes, as they also occurred in controls, were of low incidence, had no dose relationship in incidence or severity, and/or are common background findings for this species.

**Dosing Solution Analysis:** Dosage forms were prepared on day of dosing. Solutions were within ± 10% nominal concentrations, indicating all dose formulations were accurately prepared. MK-6072 was confirmed to be absent from the vehicle control sample.

(Reviewer’s comments: Despite the lack of any adverse findings in this study, this study should be considered more a proof-of-concept and not a pivotal GLP toxicology study. The Applicant’s redesign of this study and study deviations from the study protocol were confusing and difficult to follow. The Applicant’s changes to the study included: 1) unscheduled euthanization of the Toxicity arm and males in the TK/Immunogenicity arm on Study Days 11/12; 2) change in vehicle lots administered between staggered treatment groups; 3) replacement of toxicity arm animals with animals originally assigned to the clinical pathology group; and 4) addition of a new group of male animals mid-study (Study Day 15) for TK/Immunogenicity assessment. The descriptions of the methodology in the study report often did not match the revised study design tables. Also, considering the challenges encountered in this study, the staggered dosing and euthanization paradigm (while acceptable) were confusing and challenged the consistency and reliability of findings. Control animals in the Toxicity and TK/Immunogenicity arms were administered different vehicle lots. The Applicant’s replacement of only males in the TK/Immunogenicity arm on Study Day 15 and retention of the original female animals in this treatment group dosed on Study Day 1, showed different treatment of the sexes, increased opportunity for variability, and diminished reliability in the TK/Immunogenicity results. Because of the obvious problems with this study, the Applicant repeated this 21-day intermittent toxicity study with MK-6072.

Overall, the findings of this first study showed that two intravenous doses (14 day interval) of MK-6072 (125 mg/kg each dose; slow infusion) to mice to be well tolerated, with no treatment related effects noted on any test parameters (clinical signs, body weight, ophthalmology, clinical pathology, and gross and microscopic pathology). The No Observed Adverse Effect Level (NOAEL) was determined to be 125 mg/kg when administered in two doses (with a 14 day interval between doses). At this NOAEL, the mean female/male AUC values were 211,000/168,000 mcg/mL·hr at 336 hours after the first dose, respectively, and 381,000/331,000 mcg/mL·hr at 504 hours post first dose (7 days after the second dose), respectively. No ADAs against MK-6072 were detected up to 504 hours after the first dose of MK-6072 was administered. As the real impact of these changes on the integrity and reliability of study findings is unknown, caution is advised when interpreting the clinical relevance of nonclinical findings from this study.)

2. **Study title:** (MK-6072 and MK-3415) Twenty-One-Day Intermittent-Dose Intravenous Toxicity Study in Mice
   Study no.: TT #15-1079
Study report location: EDR in DARRTS
Conducting laboratory and location: Safety Assessment and Laboratory Animal Resources (SALAR), Merck Research Laboratories, West Point, PA, U.S.A.
Date of study initiation: 4/24/2015
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MK-6072, Lot #: L-005494597-001C001, 98.9% purity; MK-3415, (N/A).

Methods

Doses: MK-6072: 125 mg/kg; MK-3415 (N/A)
Frequency of dosing: Once on Dosing Days 1 and 15, total of two doses; staggered dosing – animals in Toxicity arm dosed 5 days after mice in toxicokinetic (TK)/Immunogenicity arm; mice euthanized on Study Day 21 (4 days after 2nd dose)
Route of administration: I.V. to tail vein, 2 mL/min using a syringe pump
Dose volume: 10 mL/kg
Formulation/Vehicle: MK-6072 (25 mg/mL), sodium chloride, sodium citrate, DTPA, PS-80, pH 6.0) for injection; diluted into 0.9% sodium chloride (vehicle); placebo was the vehicle alone.
Species/Strain: Mouse / Crl:CD-1 (ICR)
Number/Sex/Group: Toxicity Assessment: 10 /sex/group including vehicle control
Age: 9 weeks old
Weight: Male: (28.8 – 42 g); Female: (23.1 – 33 g)
Satellite groups: TK/Immunogenicity: 21/sex for MK-6072, 9/sex for Control.
Study design: Group housed (n=3/cage) under fluorescent light on a 12 h cycle; food and water ad libitum
Deviation from study protocol: None reported that affected the integrity of this study.

Dose Justification: Dose levels were based on the maximum feasible dose.

(Reviewer's Comment: The Applicant’s study design included a split study start time; the Toxicity arm of this study was initiated 5 days after the animals in the Toxicokinetic/Immunogenicity arm received their first dose, and were monitored for up to 5 days after the TK/Immunogenicity arm animals were euthanized (Study Day 19) to complete their two week dosing/observation period as intended).

Observations and Results
Mortality (Once daily):
All animals treated with MK-6072 and vehicle survived to scheduled euthanization.

**Clinical Signs (Once daily):**
No test-item related clinical signs were observed during the study.

**Body Weight (Pre-test, Once weekly):**
No test article related changes in body weight or body weight gain different from controls were observed.

**Ophthalmologic Examination (Toxicity arm animals only; slit lamp biomicroscopy and indirect ophthalmoscopy; pre-dose; all animals on Study Day 19), three days after second dose:**
There were no test article related ophthalmologic findings.

**Hematology (at necropsy; 5 mice/sex/group on Study Day 21 (4 days after 2nd dose):**
There were no test-article related effects on any hematology parameters.

**Clinical Chemistry (at necropsy; 5 mice/sex/group on Study Day 21):**
There were no test-article related effects on any serum chemistry parameters.

**Toxicokinetics – blood collected from orbital sinus/vena cava from TK/Immunogenicity animals (Control animals [n=3/sex] at 0, 188 504 hours post dose; MK-6072 treated animals [n=18/sex] at the following timepoints (Note: 2nd dose administered 336 hours after the first dose)**

- **Cohort A:** 0.167 and 336.17 hours post first dose
- **Cohort B:** 4 and 340 hours post first dose
- **Cohort C:** 7 and 343 hours post first t dose
- **Cohort D:** 24 and 360 hours post first dose
- **Cohort E:** 48, 336 and 384 hours post 1st dose
- **Cohort F:** 0, 168 and 504 hours post first dose

Serum concentrations of MK-6072 was determined using a biotin-streptavidin bridging immunoassay analyzed on a MSD Discovery Sector Imager 6000 (Meso Scale Discovery [MSD], Gaithersburg, MD, USA). The MK-6072 toxicokinetic parameters are presented in Table 6. Serum samples from control animals showed no detectable level of MK-607 (LLQ = 0.04 mcg/mL).

**Table 6. Serum Toxicokinetic Parameters of MK-6072 in Mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>AUC$_{0-336}$ hr (mcg/mL*hr)</th>
<th>AUC$_{0-504}$ hr (mcg/mL*hr)</th>
<th>C$_{max}$ mcg/mL</th>
<th>T$_{max}$ (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK-6072</td>
<td>Female</td>
<td>204000 ± ID</td>
<td>370000 ± 11900</td>
<td>2540 ± 101</td>
<td>336.17</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>193000 ± ID</td>
<td>370000 ± 16500</td>
<td>2610 ± 71.3</td>
<td>336.17</td>
</tr>
</tbody>
</table>

ID = Insufficient Data (Reason for ID undefined)
Mean female/male AUC values were 204,000/193,000 mcg/mL•hr at 336 hours after the first dose, respectively, and 370,000/370,000 mcg/mL•hr at 504 hours post first dose (7 days after the second dose), respectively. Mean maximal MK-6072 concentrations in female and male mice were observed at 0.167 hr (approximately 10 min after the first dose) or 336.17 hours after the first dose (approximately 10 min after the second dose) over the concentration vs time profile from 0 to 504 hours. Mean plasma MK-6072 concentrations at 504 hours were 23-53% of the respective mean C\text{max} values. There were no clear sex differences in AUC and C\text{max} values of MK-6072 at all sampling periods. Terminal elimination half-life (t\text{1/2}) of MK-6072 was not determined. (Note: The validation study (Study No. BT00116 Rev.1) of the ECL immunoassay for MK-6072 in CD-1 mouse serum indicate the ECL assay to be acceptable for selectivity, accuracy, and reproducibility).

Anti-Drug Antibody Analysis - blood collected from orbital sinus/vena cava, n=6 mice/sex/cohort from TK/Immunogenicity animals (at pre-dose and 504 hours after first dose (7 days after 2\text{nd} dose) was administered): Serum anti-drug antibody (ADA) response to MK-6072 was determined using an electrochemiluminescence assay. No anti-MK-6072 drug antibodies were observed in any serum sample collected pretest, or at 504 hr post dose in serum from controls and MK-6072 treated mice.

Gross Necropsy (at necropsy; all animals on Study Day 21 (4 days after 2\text{nd} dose)): No macroscopic changes attributed to MK-6072 were recorded at necropsy.

Organ Weights (at necropsy; all animals on Study Day 21 (4 days after 2\text{nd} dose)): No test article related organ weight changes were detected at necropsy.

Histopathology (at necropsy; all animals on Study Day 21 (4 days after 2\text{nd} dose)): Adequate Battery: Yes Routine Tissues Collected:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal glands*</td>
<td>Liver*</td>
<td>Skin</td>
</tr>
<tr>
<td>Aorta</td>
<td>Lungs</td>
<td>Small intestine</td>
</tr>
<tr>
<td>Bone (femur, tibia, femorotibial joint)</td>
<td>Lymph nodes mandibular</td>
<td>Spinal cord</td>
</tr>
<tr>
<td>Brain*</td>
<td>Lymph node, mesenteric</td>
<td>Spleen*</td>
</tr>
<tr>
<td>Cecum</td>
<td>Mammary gland</td>
<td>Sternum</td>
</tr>
<tr>
<td>Cervix</td>
<td>Muscle</td>
<td>Stomach</td>
</tr>
<tr>
<td>Epididymis</td>
<td>Optic nerves</td>
<td>Testes*</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Ovaries</td>
<td>Thymus*</td>
</tr>
<tr>
<td>Eyes</td>
<td>Pancreas</td>
<td>Thyroids/parathyroid</td>
</tr>
<tr>
<td>Gall Bladder</td>
<td>Peripheral nerve (sciatic)</td>
<td>Tongue</td>
</tr>
<tr>
<td>GALT</td>
<td>Pituitary</td>
<td>Trachea</td>
</tr>
<tr>
<td>Heart*</td>
<td>Prostate</td>
<td>Ureters</td>
</tr>
</tbody>
</table>

Reference ID: 3924459
**Histological Findings:**

There were no notable treatment-related microscopic changes at the injection site or in any systemic organs evaluated in animals administered MK-6072. All microscopic findings observed in treated animals were considered incidental changes, as they also occurred in controls, were of low incidence, had no dose relationship in incidence or severity, and/or are common background findings for this species.

**Dosing Solution Analysis:**

Dosage forms were prepared on day of dosing. Solutions were within ± 10% nominal concentrations, indicating all dose formulations were accurately prepared. MK-6072 was confirmed to be absent from the vehicle control sample.

(Reviewer’s comments: Two intravenous doses (14 day interval) of MK-6072 (125 mg/kg each dose; slow infusion) to mice showed the test article to be well tolerated, with no treatment related effects noted on any test parameters (clinical signs, body weight, ophthalmology, clinical pathology, and gross and microscopic pathology). The No Observed Adverse Effect Level (NOAEL) was determined to be 125 mg/kg when administered in two doses with a 14 day interval between doses. At this NOAEL, the mean female/male AUC values were 204,000/193,000 mcg/mL hr at 336 hours after the first dose, respectively, and 370,000/370,000 mcg/mL hr at 504 hours post first dose (7 days after the second dose), respectively. Corresponding mean $C_{max}$ values in female and male mice over the concentration vs time profile from 0 to 504 hours were 2540/2610 mcg/mL, respectively, at 336.17 hours (10 minutes after the second dose). No ADAs against MK-6072 were detected up to 504 hours after the first dose of MK-6072 was administered. Terminal elimination half-life ($t_{1/2}$) of MK-6072 with repeat dosing was not determined.

The overall study design was sufficient to evaluate the repeat-dose toxicity (2 doses) of MK-6072 in mice. Optimally, this study should have been designed to include additional dose levels of MK-6072 to establish a dose-response to any observed toxicity. However, the single maximum feasible dose of 125 mg/kg bezlotoxumab (absent any toxicity findings) in mice is approximately 12.5 times greater than the maximum human clinical dose of 10 mg/kg on a straight mg/kg comparison basis, and approximately 7 times greater AUC exposure and 14 times greater $C_{max}$ values in mice relative to patients administered a single 10 mg/kg IV injection of bezlotoxumab.)
3. **Study title:** A two week intravenous toxicity study in mice with MK-6072 (MDX-1388) and GS-CDA1 (MDX-066); (Study No. 552001; GLP; 2005). *(From the Nonclinical Review of IND 12823 by Dr. Wendelyn Schmidt, in DARRTS 12/13/2007)*

A GLP, repeat-dose toxicology study was conducted to assess the general toxicity and toxicokinetic parameters of MK-6072 (1, 10, 50 mg/kg) administered as a bolus injection (via tail vein) to adult CD-1 mice (n=15/sex/dose) on Days 0, 3, 6, 8, and 13. In this study, mice were euthanized on Day 1 (5/sex/dose) and on Day 14 (10/sex/dose). Study parameters included assessments of mortality and clinical signs (twice daily); body weight (dosing days); food consumption (weekly); and clinical pathology, gross necropsy, organ weights, and histopathology (Days 1 and 14). Overall, five intravenous injections (3-day interval) of MK-6072 (up to 50 mg/kg/dose) to male and female mice appeared to be well tolerated, with no treatment related effects noted on any test parameters. The No Observed Adverse Effect Level (NOAEL) was determined to be 50 mg/kg in this study, with a mean serum concentration for both sexes of 758 mcg/mL on Day 1 and 962 mcg/mL on Day 14. The NOAEL dose of 50 mg/kg bezlotoxumab in mice is 5 times greater than the recommended single clinical dose of 10 mg/kg on a straight mg/kg comparison; mean serum concentrations at this dose in mice are approximately 4 times greater than detected in patients. As stated by Dr. Schmidt, the “design of the toxicokinetic component of this study does not allow a differentiation between serum accumulation and a long half-life.” Anti-drug antibodies were not measured in this study.

7 **Genetic Toxicology**

None submitted.

8 **Carcinogenicity**

None submitted.

9 **Reproductive and Developmental Toxicology**

None submitted.

10 **Special Toxicology Studies**

The Applicant conducted two in vitro tissue cross-reactivity studies with MK-6072 using a panel of human and mouse tissues; these studies were previously reviewed by Dr. Schmidt.

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7 The description of the nonclinical study findings included in this review will pertain only to the control and MK-6072 treatment groups, and will not include any findings from the combination test groups of MK-6072 and GS-CDA1 (MDX-066). MDX-066 is an anti-CDA1 monoclonal antibody and therefore is not relevant to the current BLA marketing application.
Wendelyn Schmidt for IND 12823 (see Dr. Schmidt’s Nonclinical Review in DARRTS, 12/13/2007). A brief summary of findings from Dr. Schmidt’s review for these tissue cross-reactivity studies with MK-6072 is included below. The in vitro tissue cross-reactivity studies conducted with the anti-CDA1 monoclonal antibody were not examined during the review of this BLA.

1) Cross-Reactivity Study of MDX-1388-FITC with Normal Human Tissues (Study No. TT #117801 / IM1208; GLP; 2005)
   - **Study Design:** Cryosections containing 38 human tissue samples (obtained from at least 3 separate donors) listed in Table 5 were evaluated for tissue cross-reactivity using an indirect immunoperoxidase staining method with fluoresceinated MDX-1388 monoclonal antibody at 2 concentrations (2 and 20 µg/mL). The fluoresceinated human IgG1 antibody (HuIgG1κ-FITC) antibody was used as the negative antibody control. *C. difficile* toxin B fragment UV resin spot slides served as the positive control. The human hypercalcemia of malignancy peptide (PTHrP[1-34]) UV activated resin spot slides served as the negative control.

   **Table 7. Human Donor Tissue Analyzed for Tissue Cross-Reactivity with MDX-1388**

<table>
<thead>
<tr>
<th>Adrenal glands</th>
<th>Lung</th>
<th>Spinal cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood vessels</td>
<td>Lymph Node</td>
<td>Spleen</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Ovary</td>
<td>Stomach</td>
</tr>
<tr>
<td>Brain (cerebrum)</td>
<td>Ovary (fallopian tube)</td>
<td>Striated muscle (skeletal)</td>
</tr>
<tr>
<td>Brain (cerebellum)</td>
<td>Pancreas</td>
<td>Testis</td>
</tr>
<tr>
<td>Breast (mammary)</td>
<td>Parathyroid</td>
<td>Thymus gland</td>
</tr>
<tr>
<td>Colon (large intestine)</td>
<td>Peripheral Nerve</td>
<td>Thyroid</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Pituitary</td>
<td>Tonsil</td>
</tr>
<tr>
<td>Eyes</td>
<td>Placenta</td>
<td>Ureter</td>
</tr>
<tr>
<td>Gall Bladder</td>
<td>Prostate</td>
<td>Urinary Bladder</td>
</tr>
<tr>
<td>Heart</td>
<td>Salivary Gland</td>
<td>Uterus - body</td>
</tr>
<tr>
<td>Kidney</td>
<td>Skin</td>
<td>Uterus - cervix</td>
</tr>
<tr>
<td>Liver</td>
<td>Small intestine</td>
<td></td>
</tr>
</tbody>
</table>

   - **Results:** The fluorescein-labeled MDX-1388 antibody reacted strongly with the control *C. difficile* spots and did not specifically react with the negative control material, PTHrP[1-34]. The negative antibody control did not react with either the *C. difficile* spots or the PTHrP[1-34] spots. Overall, there was no reactivity (positive staining) noted with any of the human tissues tested.
   - **Conclusions:** There was no cross-reactivity with human tissues with MK-1388 at both antibody concentrations of bezlotoxumab tested. This study was found adequate.

2) Cross-Reactivity Study of MDX-1388-FITC with Normal CD-1 Mouse Tissues (Study No. TT #117802 / IM1209; GLP; 2005)
   - **Study Design:** Cryosections containing 37 CD-1 mouse tissue samples (obtained from at least 2 mice) listed in Table 6 were evaluated for tissue cross-
reactivity using an indirect immunoperoxidase staining method with fluoresceinated MDX-1388 monoclonal antibody at 2 concentrations (2 and 20 µg/mL). The fluoresceinated human IgG1 antibody (HuIgG1κ-FITC) antibody was used as the negative antibody control. *C. difficile* toxin B fragment UV resin spot slides served as the positive control. The human hypercalcemia of malignancy peptide (PTHrP[1-34]) UV activated resin spot slides served as the negative control.

**Table 8. CD-1 Mouse Tissue Analyzed for Tissue Cross-Reactivity with MDX-1388**

<table>
<thead>
<tr>
<th>Adrenal glands</th>
<th>Lung</th>
<th>Spinal cord</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Bone marrow</td>
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<td>Stomach</td>
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<td>Brain (cerebrum)</td>
<td>Oviduct (fallopian tube)</td>
<td>Striated muscle (skeletal)</td>
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<td>Testis</td>
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<td>Small intestine</td>
<td></td>
</tr>
</tbody>
</table>

- **Results:** The fluorescein-labeled MDX-1388 antibody reacted strongly with the control *C. difficile* spots and did not react with the negative control material, PTHrP[1-34]. The negative antibody control did not react with either the *C. difficile* spots or the PTHrP[1-34] spots. Overall, there was no reactivity (positive staining) noted with any of the mouse tissues tested.

- **Conclusions:** There was no cross-reactivity with CD-1 mouse tissues. Thus, the mouse is likely an appropriate model for toxicity studies with MDX-1388. This study was found adequate.

**11 Integrated Summary and Safety Evaluation**

Bezlotoxumab (MK-6072) is a human IgG1 monoclonal antibody that binds *Clostridium difficile* (*C. difficile*) toxin B with high affinity, and reportedly prevents the typical cellular intoxication cascade responsible for tissue damage and inflammation characteristic of *C. difficile* infection (CDI) in the clinic. Pharmacology studies conducted in rodent models of CDI required administration of both bezlotoxumab and actoxumab (CDA1, human mAb against *C. difficile* toxin A) to protect against morbidity and mortality associated with CDI, with no apparent, direct antimicrobial activity against the bacterium. Bezlotoxumab reportedly distributes to the infection site in the gut lumen of hamsters with CDI at higher levels than in healthy hamsters, possibly due to toxin-mediated disruption of gut epithelium. However, the mechanism by which circulating IgG antibodies can cross the gut epithelium and directly neutralize *C. difficile* toxins in the gut lumen remains unclear. Both in vitro and in vivo studies conducted with bezlotoxumab indicate biologic efficacy independent of Fc-mediated activity.
Safety pharmacology studies were not conducted with bezlotoxumab and no safety pharmacology endpoints were assessed in the submitted repeat dose toxicology studies. No specific information was obtained on any neurological or cardiovascular effects, including blood pressure or any respiratory or renal effects in animals administered bezlotoxumab. However, the submitted repeat dose toxicology studies conducted with IV bezlotoxumab in mice (14 and 21 day intermittent dosing toxicology studies) showed the antibody to be well tolerated at doses 5 and 12 times the clinical dose on a body weight comparison, respectively, with no clinical signs and no treatment related effects on any test parameters, including histopathology of tissues. To note, bezlotoxumab reportedly had no effect on QTc interval in healthy patients and patients with CDI enrolled in Phase 1 and Phase 3 clinical trials with bezlotoxumab.

Toxicokinetic properties of bezlotoxumab were limited to an exploratory, single-dose toxicokinetic study in mice, and one pivotal 21-day intermittent-dose IV toxicity study in mouse. After a single bolus dose of 50 mg/kg bezlotoxumab to mice (approximately 5 times the human dose), mean $C_{\text{max}}$ and AUC values were 734 mcg/mL and 132960 mcg*hr/mL, approximately 4 and 2.5 times greater than observed in patients after a single 10 mg/kg IV dose in the clinic. Similarly, two-doses of bezlotoxumab (125 mg/kg) injected into mice with a 14-day interval between doses showed mean $C_{\text{max}}$ and AUC values of approximately 2575 mcg/mL and 370,000 mcg*hr/mL, respectively; or approximately 14 and 7 times greater than observed in patients after a single 10 mg/kg IV dose. In both cases, $T_{\text{max}}$ was determined to be 10 minutes after administration, with a mean terminal elimination half-life ($t_{1/2}$) in mice of 16.8 days, compared to approximately 19 days in humans after a single IV injection of bezlotoxumab. There were no reported sex differences in AUC and $C_{\text{max}}$ values for IV bezlotoxumab in mice. In general, The TK profile of bezlotoxumab appears consistent with other monoclonal antibodies, which typically have low clearance and a limited volume of distribution.

No serum anti-drug antibody (ADA) was detected in 21-day toxicity study in mice, administered two doses of bezlotoxumab (125 mg/kg), with a 14-day interval between doses. Based on these results, it would appear that the fully human monoclonal antibody, bezlotoxumab, has low immunogenicity potential, particularly when administered intravenously to mice. However, in the Clinical Overview section for bezlotoxumab (page 30), the Applicant indicated in clinical testing, that high serum concentrations of bezlotoxumab above the drug tolerance limit [DTL] (7.5 mcg/mL) can interfere with the ADA assay. In the presence of high levels of serum bezlotoxumab, there is a potential for false negative ADA results. Therefore, with high serum levels of bezlotoxumab present in serum samples collected from mice for the ADA assay, there is a potential for assay interference, making the results of these analyses inconclusive at best. To note, negative results were reportedly obtained in the ADA assay for patients in Phase 3 trials at serum concentrations of bezlotoxumab below the DTL, and there were no immunogenicity related adverse findings. This might suggest an overall low immunogenicity potential for bezlotoxumab in patients when administered as recommended.
Single and intermittent injection regimens of bezlotoxumab administered intravenously to mice at a dose and exposure level many times greater than the recommended single clinical dose, appeared to be well tolerated, with no clinical signs and no adverse effects reported on body weight, ophthalmologic examination, clinical pathology, and histopathological evaluation of animal tissues. No erythema or inflammation, evidence of a local site reaction or immunogenic stimulatory response, was observed at the injection site in mice. The submitted repeat dose toxicology studies conducted with IV bezlotoxumab in mice (14 and 21 day intermittent dosing toxicology studies) identified no targets of toxicity at doses 5 and 12 times the clinical dose on a body weight comparison, respectively; the No-Observed-Adverse-Effect-Level (NOAEL) was determined to be the maximum dose tested in each study. PK exposure comparison between the pivotal 21-day intermittent dose toxicology study in mice and clinical PK parameters obtained after a single 10 mg/kg dose of bezlotoxumab to patients indicated that the animals were adequately dosed, with C_{max} and AUC levels approximately 14 and 7 times greater than observed in the clinic. Although this toxicology study was originally designed to profile the combined toxicity of bezlotoxumab + actoxumab, the Applicant included a single cohort of animals administered a high concentration of IV bezlotoxumab (125 mg/kg) that showed no evidence of toxicity. In addition, treatment of mice with 5 total doses (3-day interval between doses) of bezlotoxumab at lower doses similarly showed no toxicity at doses of 1, 10, and 50 mg/kg (or approximately 0.1, 1, and 5 times the recommended clinical dose).

Tissue cross-reactivity (TCR) studies performed in at least 38 mouse and human tissues with bezlotoxumab (2 and 20 mcg/mL) showed no reactivity (positive staining) of tissue samples. The assays were adequately conducted and positive and negative controls produced expected results. Although no tissue cross-reactivity with bezlotoxumab was observed, it’s unclear if these concentrations were sufficiently high enough to be relevant to clinical exposures.

The Applicant conducted no genetic toxicology, reproductive and developmental toxicology, or carcinogenicity studies with bezlotoxumab. Since bezlotoxumab is a monoclonal antibody directed against a foreign antigen target, the range and type of genotoxicity, reproductive/developmental toxicity, and carcinogenicity studies routinely conducted for pharmaceuticals are not applicable as per ICH Guidance S6(R1)\(^8\). It should be noted that gross and microscopic evaluation of tissues in the repeat dose toxicology studies in mice conducted with bezlotoxumab showed no specific histopathological findings in any of the reproductive organs of male and female mice at the highest doses of bezlotoxumab tested. Testes organ weights in male mice treated with bezlotoxumab were similar to controls. Although fertility effects were not assessed

\(^8\) Guidance ICH S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.


Reference ID: 3924459
in this Application, the lack of an endogenous target for bezlotoxumab, combined with the absence of any histopathological findings or positive tissue cross-reactivity in any reproductive or related tissues with bezlotoxumab, indicates no specific risk to patient fertility.

The calculated margin of safety for bezlotoxumab based on AUC exposure values at the NOAEL dose determined from the GLP exploratory single dose toxicology study in mice, and the pivotal 21-day intermittent dose toxicology study in mice are shown in Table 9 below. This table does not take into account any species extrapolations based on AUC, which may be more appropriate if the antibody distributes outside of the vascular compartment.

Table 9. Margins of Safety Based on AUC Exposures at Animal NOAEL

<table>
<thead>
<tr>
<th>Study No.</th>
<th>Nonclinical Study</th>
<th>Route of Admin</th>
<th>NOAEL (mg/kg)</th>
<th>No. of Doses</th>
<th>AUC (mcg·hr/mL)</th>
<th>Margin of Safety(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT #14-1108</td>
<td>Single dose toxicity in mice</td>
<td>IV bolus</td>
<td>50</td>
<td>1</td>
<td>132960(^a)</td>
<td>2.5 x</td>
</tr>
<tr>
<td>TT #15-1079</td>
<td>21-day repeat dose toxicity in mice</td>
<td>IV bolus</td>
<td>125</td>
<td>2</td>
<td>370000(^b)</td>
<td>7 x</td>
</tr>
</tbody>
</table>

\(^a\): AUC\(_{0-672h}\) value (males only) with a single IV injection of bezlotoxumab (50 mg/kg)
\(^b\): AUC\(_{0-504h}\) value (both sexes) with 2 injections (14-day interval) of IV bezlotoxumab (125 mg/kg)
\(^c\): Margin of Safety = AUC in animals at NOAEL dose ÷ AUC in humans after single 10 mg/kg clinical dose

Recommendations: From the pharmacology/toxicology perspective, bezlotoxumab (BLA 761046) is approvable.

12 Appendix/Attachments

None.
This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TERRY J MILLER
04/29/2016

WENDELYN J SCHMIDT
04/29/2016

I concur with Dr. Miller's assessment on both the interpretation and completeness of the data submitted to this NDA.