CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125557Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Blincyto (blinatumomab)

Date: November 10, 2014
To: File for BLA 125557
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Blincyto conducted by Drs. Gehrke, Chiu and Ricks, and secondary memorandum and labeling provided by Dr. Sheth. I concur with Dr. Sheth's conclusion that Blincyto may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
11/10/2014

MEMORANDUM

Date: November 10, 2014
From: Christopher Sheth, Ph.D.
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
BLA: 125557
Drug: Blincyto (blinatumomab); for continuous intravenous infusion
Indication: Treatment of adults with refractory B-precursor acute lymphoblastic leukemia (ALL)
Applicant: Amgen, Inc.

Blincyto, blinatumomab, is a bispecific construct consisting of single chain variable fragments derived from antibodies directed against CD19 (expressed on B cells (b) (4) (b) (4)) and CD3 B-precursor acute lymphoid leukemia (ALL) is characterized by an overabundance of immature white blood cells (B cell lymphoblasts) in the blood and bone marrow. In the presence of CD19+ cells blinatumomab engages T cells through the CD3 (b) (4) chain of the T cell receptor complex resulting in increases in T cell activation and direct T cell mediated killing of B cells. Based on its mechanism of action, the pharmacologic class assigned to blinatumomab is "bispecific CD19-directed CD3 T-cell engager".

Pharmacology, safety pharmacology, and toxicology studies were conducted in in vitro and in vivo models. Genetic toxicology studies were not conducted or needed according to the ICH S6 guidance. As blinatumomab was shown to bind only to human or chimpanzee B and T lymphocytes, non-terminal repeat-dose general toxicology studies were conducted with blinatumomab administered to chimpanzees (a pharmacologically relevant species) in addition to repeat-dose studies in mice treated with a murine surrogate/analog molecule with similar properties. CNS safety pharmacology assessments and an embryo-fetal developmental toxicology study were also conducted in mice using a surrogate antibody. No dedicated studies of fertility and early embryonic development or prenatal and postnatal development were submitted.

In vitro, blinatumomab binding to CD3+ T cells and CD19+ target cells resulted in the release of cytokines IL-2, TNF-α, and IFN-γ. Infusion of chimpanzees with blinatumomab was associated with T-cell activation and increases in body temperature and heart rate and decreases in blood pressure, in addition to increases in cytokines IL-2, IL-6, and INF- γ. These finding are consistent with the cytokine release syndrome observed in the clinical trial in patients with ALL. Blinatumomab treatment was also associated with an increased risk for neurologic adverse events during clinical trials; however clear signs of CNS toxicity were not detected in animal toxicology and CNS safety pharmacology studies.

Blinatumomab-related toxicities in animals were consistent with the pharmacology of the drug. Infusion with blinatumomab decreased lymphocyte levels including CD19+ and CD20+ B cells

and CD3+/CD4+ and CD3+/CD8+ T cells in chimpanzees. Similar toxicities were observed in multiple mouse studies with the murine analog including treatment-related decreases in total B and T cells and the CD4+ and CD8+ T lymphocyte subsets in the blood, spleen, and lymph nodes. These immunophenotypic findings in mice correlated with microscopic findings of decreased cellularity and decreased germinal center development in the lymph nodes (mandibular, mesenteric, left axillary, and left and right inguinal) and Peyer's patches.

Studies in mice using the surrogate showed no drug-related effects in male or female reproductive organs and did not reveal any embryo-fetal toxicity or teratogenicity. The surrogate molecule crossed the placenta and fetal exposure was determined to occur at pharmacologically active concentrations, suggesting the potential for lymphocyte depletion. (b) (4) Based on the pharmacology of the product and the findings in animals, infants born to pregnant women treated with blinatumomab may be at an increased risk for infection. Therefore, Pregnancy Category C is recommended for blinatumomab. This is also consistent with the Pregnancy Category for other approved products that target B cells (e.g., rituximab, a CD20-directed cytolytic antibody).

The nonclinical studies needed to support product labeling were reviewed by Drs. Brenda Gehrke, Tiffany Ricks, and Brian Chiu. The nonclinical findings are summarized in the "Executive Summary" of the BLA review and reflected in the product label.

Recommendation: I concur with the pharmacology/toxicology reviewers that from a nonclinical perspective, Blincyto may be approved and that no additional nonclinical studies are needed to support approval of Blincyto in patients with ALL.

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/s/

CHRISTOPHER M SHETH
11/09/2014

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	125557
Supporting document/s:	1
Applicant's letter date:	September 19, 2014
CDER stamp date:	September 19, 2014
Product:	Blinatumomab (Blincyto)
Indication:	Philadelphia chromosome negative relapsed or refractory B-precursor acute lymphoblastic leukemia
Applicant:	Amgen Inc.
Review Division:	Division of Hematology Oncology Toxicology (for Division of Hematology Products)
Reviewers:	Brenda J. Gehrke, Ph.D. Haw-Jyh Chiu, Ph.D. Tiffany K. Ricks, Ph.D.
Acting Team Leader:	Christopher M. Sheth, Ph.D.
Division Director:	John Leighton, Ph.D., DABT Ann Farrell, M.D. (DHP)
Project Manager:	Kristopher Kolibab, Ph.D.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125557 are owned by Amgen Inc. or are data for which Amgen Inc. has obtained a written right of reference. Any information or data necessary for approval of BLA 125557 that Amgen Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 125557.

Reference ID: 3655615

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1 Executive Summary

1.1 Introduction

Blinatumomab (Blincyto) is a bispecific T-cell engager antibody construct that binds to CD19 (expressed on B cells) and CD3 (expressed on T cells). The drug will be administered by a 4-week continuous infusion with a 2-week treatment-free interval between each treatment cycle with a proposed clinical dose of 9 µg/day for Week 1 and 28 µg/day for Weeks 2-4 for the first cycle and 28 µg/day for all subsequent cycles. Nonclinical pharmacology and toxicology studies have been submitted and reviewed to support the approval of blinatumomab for the treatment of adult patients with Philadelphia chromosome negative relapsed or refractory B-precursor acute lymphoblastic leukemia (ALL).

1.2 Brief Discussion of Nonclinical Findings

Blinatumomab bound to B and T lymphocytes from human and chimpanzee peripheral blood mononuclear cells (PBMC) but it did not bind to PBMC from the mouse, rat, beagle dog, squirrel monkey, African Green monkey, cynomolgus monkey, rhesus monkey, and baboon. In vitro, binding of blinatumomab to $CD3^+$ ($CD45RO^+CD8^+$ and $CD4^+$) T cells and $CD19^+$ tumor cell lines resulted in cytokine release (i.e. IL-2, TNF-α, and IFN-γ), T cell proliferation, increased expression of granzyme B, and redirected cytotoxicity of $CD19^+$ target cells. Based on the pharmacology data submitted in the BLA, the Established Pharmacological Class (EPC) of “Bispecific CD19-directed CD3 T-cell engager” was determined to be both clinically meaningful and scientifically valid for blinatumomab.

General toxicology studies included non-terminal repeat-dose studies of intravenous administration of blinatumomab in chimpanzees and repeat-dose studies of subcutaneous and intravenous administration of a murine surrogate in mice. Toxicities observed with both the surrogate and blinatumomab were consistent with the pharmacology of blinatumomab. In mice, the surrogate decreased lymphocytes including total B cells and T cells and the $CD4^+$ and $CD8^+$ T lymphocyte subsets in the blood, spleen, and lymph node. Following daily administration of the surrogate for 4 or 13 weeks in mice, lymphoid tissues were the target organs of toxicity with decreased spleen weights and decreased cellularity or germinal center development observed in the lymph nodes, Peyer’s patches, and spleen. In chimpanzees, infusion with blinatumomab decreased lymphocyte levels including B cells ($CD19^+$ and $CD20^+$) and T cells ($CD3^+/CD4^+$ and $CD3^+/CD8^+$) and increased the expression/levels of T cell activation markers sCD25, CD69, and HLA-DR. Increases in cytokines IL-2, IL-6, and INFγ were also observed following infusion with blinatumomab in chimpanzees, a finding consistent with the cytokine release syndrome observed in the clinical trial in patients with ALL. Other blinatumomab-related effects in chimpanzees included increases in body temperature and heart rate and decreases in blood pressure following infusion of blinatumomab. An ear infection was observed in a chimpanzee; while it is unclear if the infection was related to treatment with blinatumomab, infection was one of the major adverse events observed with blinatumomab in patients with ALL. While

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neurologic adverse events were observed in approximately half of the patients in the clinical trial, clear evidence of CNS toxicities was not observed in the general toxicology or CNS safety pharmacology studies.

The Applicant conducted embryo-fetal development studies in mice with the murine surrogate of blinatumomab. The surrogate molecule failed to show embryo-fetal toxicity or teratogenicity in mice but did cross the placental barrier. Fetal exposure occurred at pharmacologically active concentrations, suggesting the potential for lymphocyte depletion. There are no reproductive and developmental toxicology studies with blinatumomab, and it is not known if blinatumomab can cause fetal harm. Based on the mechanism of action of B cell depletion and to be consistent with the labels of other B cell targeting agents, the Pharmacology/Toxicology team recommends Pregnancy category C.

1.3 Recommendations

1.3.1 Approvability

Recommended for approval. The nonclinical studies submitted to this BLA provide sufficient information to support the use of blinatumomab for the treatment of adult patients with Philadelphia chromosome negative relapsed or refractory B-precursor ALL.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The content for the labeling of blinatumomab is contained in this review. Based on the pharmacology data submitted in the BLA, the Established Pharmacological Class (EPC) of “Bispecific CD19-directed CD3 T-cell engager” was determined to be both clinically meaningful and scientifically valid for blinatumomab.

The Applicant proposed (b) (4) Since there are no reproductive and developmental toxicology studies with blinatumomab, it is not known whether blinatumomab can cause fetal harm. Approved B cell targeting agents include rituximab, obinutuzumab, and ofatumumab. In approved product labels, these agents caused B cell depletion and immunosuppression in offspring in cynomolgus monkeys. Postmarketing data showed that infants exposed to rituximab in utero experienced B cell depletion for less than 6 months. Based on the mechanism of action of blinatumomab, the Pharmacology/Toxicology team recommends Pregnancy category C.

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2 Drug Information

2.1 Drug

CAS Registry Number: 853426-35-4

Generic Name: Blinatumomab

Code Names: AMG103, MEDI-538, MT103

Chemical Name: Bi-specific T cell Engager (BiTE®) targeting CD19 on B cells and CD3 on T cells

Molecular Formula: [redacted] (b) (4)

Molecular Weight: ~54 kDa

Structure or Biochemical Description:

Blinatumomab is a single-chain bispecific antibody construct consisting of 504 amino acids. The antibody construct was developed by genetic engineering from two distinct murine monoclonal antibodies: HD37, which recognizes the pan-B cell antigen CD19 and L2K-07, which binds the T-cell receptor-associated complex CD3. [redacted] (b) (4)

Figure 1: Derivation of blinatumomab from two murine monoclonal antibodies HD37 (anti-CD19) and L2K-07 (anti-CD3)

(excerpted from Applicant's submission)

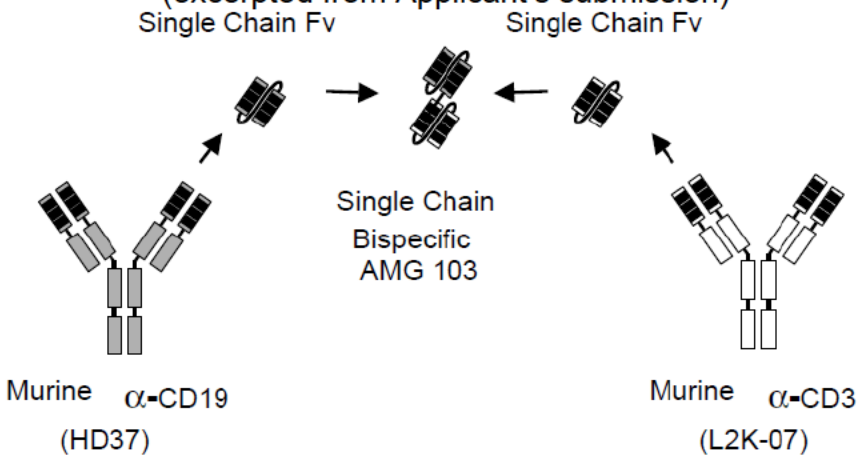

Figure 2: Domain structure of blinatumomab
(excerpted from Applicant's submission)

(b) (4)

Figure 3: Amino acid sequence of blinatumomab (b) (4)
(excerpted from Applicant's submission)

(b) (4)

Pharmacologic Class: Bispecific CD19-directed CD3 T-cell engager

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 100135, IND (b) (4)

2.3 Drug Formulation

The blinatumomab drug product is a sterile, lyophilized powder for reconstitution containing (b) (4) µg blinatumomab per vial. The container for the drug product is a 4 cc Type 1 (b) (4) glass vial (b) (4)

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(b) (4). Blinatumomab is intended for reconstitution with 3 mL of sterile water for injection.

The intravenous solution stabilizer is supplied as a sterile solution in 10 cc Type 1 (b) (4) glass vials containing 10 mL deliverable product (b) (4)

The drug product is prepared for infusion by aseptically adding the appropriate amount of intravenous solution stabilizer to an infusion bag containing normal saline, and the appropriate volume of reconstituted blinatumomab drug product is then added aseptically to the infusion bag containing the intravenous stabilizer and saline.

The compositions of blinatumomab drug product and the intravenous solution stabilizer are presented in the tables below.

Table 1: Composition of the blinatumomab drug product
(excerpted from Applicant's submission)

Component	Grade	Function	Target concentration before lyophilization	Unit formula per vial[a]
blinatumomab[a]	In house	Active ingredient	(b) (4)	
Citric Acid Monohydrate	USP, PhEur, JP	(b) (4)		
Trehalose Dihydrate	USP, PhEur			
Lysine Hydrochloride	USP, PhEur			
Polysorbate 80	NF, PhEur, JP			
Sodium Hydroxide	NF, PhEur, JP	pH adjustment	As needed for adjustment to pH 7.0	
(b) (4)	NF, PhEur, JP	(b) (4)	C	C

(b) (4)

Table 2: Composition of the intravenous solution stabilizer (10 mL/vial)
(excerpted from Applicant's submission)

Component	Grade	Function	Concentration		Quantity per vial
Citric Acid Monohydrate	PhEur, USP/NF, JP	(b) (4)	(b) (4)		52.5 mg
Lysine Hydrochloride	PhEur, USP/NF				2283.8 mg
Polysorbate 80	PhEur, USP/NF, JP				10 mg
Sodium Hydroxide	PhEur, USP/NF, JP	pH adjustment	N/A	N/A	qs to target pH 7.0
Water For Injection	PhEur, USP	(b) (4)	N/A	N/A	(b) (4)

qs = quantum sufficit

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is adult patients with Philadelphia chromosome negative relapsed or refractory B-precursor ALL. Blinatumomab is to be administered by a 4-week continuous infusion with a 2-week treatment-free interval between each treatment with a proposed clinical dose of 9 µg/day for Week 1 and 28 µg/day for Weeks 2-4 for the first cycle and 28 µg/day for all subsequent cycles.

2.7 Regulatory Background

Meetings held with the FDA include an End of Phase 2 meeting on March 25, 2013 and a pre-BLA meeting on June 23, 2014. Blinatumomab was granted Orphan Drug designation for treatment of patients with ALL in May 2008 and Breakthrough therapy designation was granted to blinatumomab for the treatment of adult patients with Philadelphia chromosome negative relapsed or refractory B-precursor ALL on June 30, 2014. BLA 125557 was submitted on September 19, 2014.

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3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Study Number	Study Title	eCTD Location
103-PCD-007	Determination of the relevant animal species for MT103 toxicology studies.	4.2.1.1.
103-PCD-0040	Species cross-reactivity study of MEDI-538 to peripheral mononuclear cells in African Green Monkey, marmoset, squirrel monkey, mouse and rat using flow cytometry.	4.2.1.1.
103-PCD-0061	MT103-mediated redirected lysis of B cell lymphoma cell lines.	4.2.1.1.
103-PCD-0063	MT103 mediated dose and time dependent expression of granzyme B.	4.2.1.1.
103-PCD-0065	In vitro characterization of binding, cytotoxicity and mode of action.	4.2.1.1.
103-PCD-0066	In vitro characterization of MT103 in human and chimpanzee test systems.	4.2.1.1.
103-PCD-0067	Impact of effector-to-target cell ratio on MT103-mediated redirected lysis and T cell activation.	4.2.1.1.
103-PCD-0076	MT103-mediated redirected lysis of human pediatric B cell acute lymphoblastic leukemia cell lines.	4.2.1.1.
103-PCD-0094	Comparison of the pharmacodynamics effects of MT103 and its murine surrogate muS103new in vitro.	4.2.1.1.
DR-RE-103-001	MuS103new BiTE antibody generation of a murine-like MT103 surrogate molecule with paired affinities.	4.2.1.1.
R20140011	AMG 103-induced T cell-adhesion to endothelial cells and its mitigation by anti-adhesive agents.	4.2.1.2.
R20140012	T cell activation by AMG 103 alters expression of adhesion molecules on endothelial cells.	4.2.1.2.
103-PCD-0078	Evaluation of the effects of muS103new behavior using the primary observation (Irwin) test in the mouse following intravenous (bolus) administration.	4.2.1.3.
103-PCD-0103	Exploratory 7-day continuous intracerebroventricular infusion of muS103new to BALB/c mice.	4.2.1.3.
103-PCD-0071	Influence of dexamethasone and indomethacin on MT103-mediated cell lysis and cytokine release.	4.2.1.4.

Toxicology

Study Number	Study Title	eCTD Location
103-PCD-0075	Toxicity study by intravenous (bolus) administration to BALB/c mice for 4 weeks followed by a 4-week recovery period	4.2.3.2.
103-PCD-0082	muS103new: Toxicity study by twice-daily subcutaneous administration to BALB/c mice for 13 weeks followed by a 4-week recovery period	4.2.3.2.
103-PCD-0015	Multiple dose study for the bispecific single chain monoclonal antibody MT103 (CD19XCD3) in chimpanzees	4.2.3.2.

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103-PCD-0079	Preliminary study for effects on embryo-fetal development by intravenous route (bolus) in mice	4.2.3.5.
103-PCD-0080	Study for effects on embryo-fetal development by intravenous route (bolus) in mice	4.2.3.5.
103-PCD-0041	Immunopathology Report: Cross-reactivity of MEDI-538 with normal tissues	4.2.3.7.7.

3.2 Studies Not Reviewed

Pharmacology

Study Number	Study Title	eCTD Location
103-PCD-0057	Anti-tumor activity of MT103 in subcutaneous NALM-6 xenograft model in NOD/SCID mice: dose-finding study.	4.2.1.1.
103-PCD-0058	Impact of delayed treatment initiation on anti-tumor activity of MT103 in a subcutaneous NALM-6 xenograft model in NOD/SCID mice.	4.2.1.1.
103-PCD-0059	Specificity of antitumor activity of MT103 and MT102 in a subcutaneous NALM-6 xenograft model in NOD/SCID mice.	4.2.1.1.
103-PCD-0060	Anti-tumor activity of MT103 in a disseminated NALM-6 xenograft model in NOD/SCID mice: dose-finding study.	4.2.1.1.
103-PCD-0097	Efficacy evaluation of AMG 103 BiTE antibody in a Raji xenograft model.	4.2.1.1.
103-PCD-0098	Evaluation of AMG 103 anti-tumor activity in an orthotopic Grant-519 advanced stage xenograft tumor model in NOD/SCID mice.	4.2.1.1.
103-PCD-0099	Efficacy evaluation of AMG 103 BiTE antibody in an SEM_c xenograft model.	4.2.1.1.
R20130026	AMG 103 pharmacology report: Evaluation of the in vivo anti-tumor activity of AMG 103 in a RAJI tumor formation model in female NOD/SCID mice after intravenous bolus administration.	4.2.1.1.
103-PCD-0006	Examination of the influence of MT103 on several cardiovascular parameters and the respiration in anaesthetized beagle dogs following intravenous administration.	4.2.1.3.
103-PCD-0077	Evaluation of the effect of muS103new on respiratory function in the conscious mouse (whole body plethysmography) following intravenous (bolus) administration.	4.2.1.3.

Pharmacokinetics

Study Number	Study Title	eCTD Location
VR-BIA-103-002	Quantification of MT103 in serum or plasma via CD69 activation assay	4.2.2.1.
VR-BIA-103-009	Specific detection of muS103new in serum and plasma with ECL-assay	4.2.2.1.
VR-BIA-103-010	ECL-assay for the detection of anti-muS103new antibodies in murine serum	4.2.2.1.
VR-BIA-103-011	Specific detection of muS103new in CD-1 serum of pregnant mice and fetuses via ECL-assay	4.2.2.1.
103-PCD-0008	Examination of the influence of MT103 on several	4.2.2.2.

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	cardiovascular parameters and the respiration in anaesthetised Beagle dogs following intravenous administration: Pharmacokinetic analysis	
103-PCD-0024	Pharmacokinetics of MT103 after 2 hr intravenous infusion-evaluation of a dose escalation study and a multi dose study in chimpanzees	4.2.2.2.
103-PCD-0039	MEDI-538 (MT103) intravenous and subcutaneous pharmacokinetic study in rats: PK and clinical chemistry analysis	4.2.2.2.
103-PCD-0062	Single dose bioavailability study of MEDI-538 delivered intravenously or subcutaneously in cynomolgus monkeys	4.2.2.2.
103-PCD-0081	Pharmacokinetic analysis of MT103 and its murine surrogate molecule muS103 following intravenous and subcutaneous bolus administration in BALB/c mice	4.2.2.2.
103-PCD-0090	Pharmacokinetic analysis of muS103new after intravenous, subcutaneous and intraperitoneal bolus administration to BALB/c mice	4.2.2.2.
103-PCD-0017	Pharmacokinetic study of MT103 after single administration to cynomolgus monkeys-pharmacokinetic analysis	4.2.2.3.
103-PCD-0093	Contribution of renal clearance to elimination of MT103 in mice	4.2.2.5.
NSX0011	MT103: Potential suppression of cytochrome P450 activities in cultured human hepatocytes	4.2.2.6.
103-PCD-0102	Pharmacokinetic comparability study of different AMG 103 production processes after a single intravenous bolus injection to BALB/c mice	4.2.2.7.

Toxicology

Study Number	Study Title	eCTD Location
103-PCD-0091	Exploratory three day repeated dose toxicity study of muS103new after different routes of administration to BALB/c mice	4.2.3.2.
103-PCD-0092	muS103new: Exploratory toxicity study of intravenous and subcutaneous administration to BALB/c mice for 5 days	4.2.3.2.
103-PCD-0070	muS103new: Preliminary toxicity study by subcutaneous and intravenous (bolus) administration to BALB/c mouse for 2 weeks	4.2.3.2.
103-PCD-0074	muS103new: Toxicity study by subcutaneous administration to BALB/c mice for 4 weeks followed by a 4 week recovery period	4.2.3.2.
103-PCD-0009	Dose escalation study in chimpanzees for MT103; in vivo and in vitro analysis	4.2.3.2.
103-PCD-0016	Dose escalation study for the bispecific single-chain monoclonal antibody MT103 (CD19xCD3) in a chimpanzee	4.2.3.2.
103-PCD-0044	14-day intravenous injection toxicity and toxicokinetic study with MEDI-538 in rats with a 15-day recovery period	4.2.3.2.
103-PCD-0010	Local tolerance test of MT103 in rabbits after a single intravenous, intramuscular, intra-arterial, paravenous and subcutaneous administration	4.2.3.6.
103-PCD-0089	Development of immunogenicity after multiple subcutaneous and intravenous administrations of muS103new in BALB/c mice	4.2.3.7.1.

Reference ID: 3655615

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

Study title: Determination of the relevant animal species for MT103 toxicology studies

Study No.:	103-PCD-0007 (formerly MT103-PE-003a)
Report Date:	January 6, 2011
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Micromet AG
GLP:	No

The objective of the study was to identify pharmacologically relevant species for toxicity testing of blinatumomab.

Results and Conclusions

- Blinatumomab bound to B ($CD20^+$) and T ($CD4^+$) lymphocytes from the chimpanzee (Pan troglodytes) but not from the SJL mouse, beagle dog, cynomolgus monkey (*Macaca fascicularis*), rhesus monkey (*Macaca mulatta)*, and baboon (*Papio sp.*), using a flow cytometric assay.
- Binding of blinatumomab to B and T lymphocytes from chimpanzees resulted in redirected lysis of B lymphocytes by T lymphocytes in peripheral blood mononuclear cell (PBMC) samples, as measured by a flow cytometric autologous depletion assay.

Study title: Species cross-reactivity study of MEDI-538 to peripheral mononuclear cells in African Green monkey, marmoset, squirrel monkey, mouse and rat using flow cytometry

Study No.:	103-PCD-0040
Report Date:	June 1, 2006
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	(b) (4)
GLP:	No

The objective of the study was to evaluate the cross-reactivity of blinatumomab to PBMCs from the African Green monkey (*Cercopithecus aethiops*), squirrel monkey (*Saimiri sciureu*), rat (*Rattus norvegicus*; F344), mouse (*Mus musculus*, ND4 Swiss Webster), and human, using a flow cytometric assay.

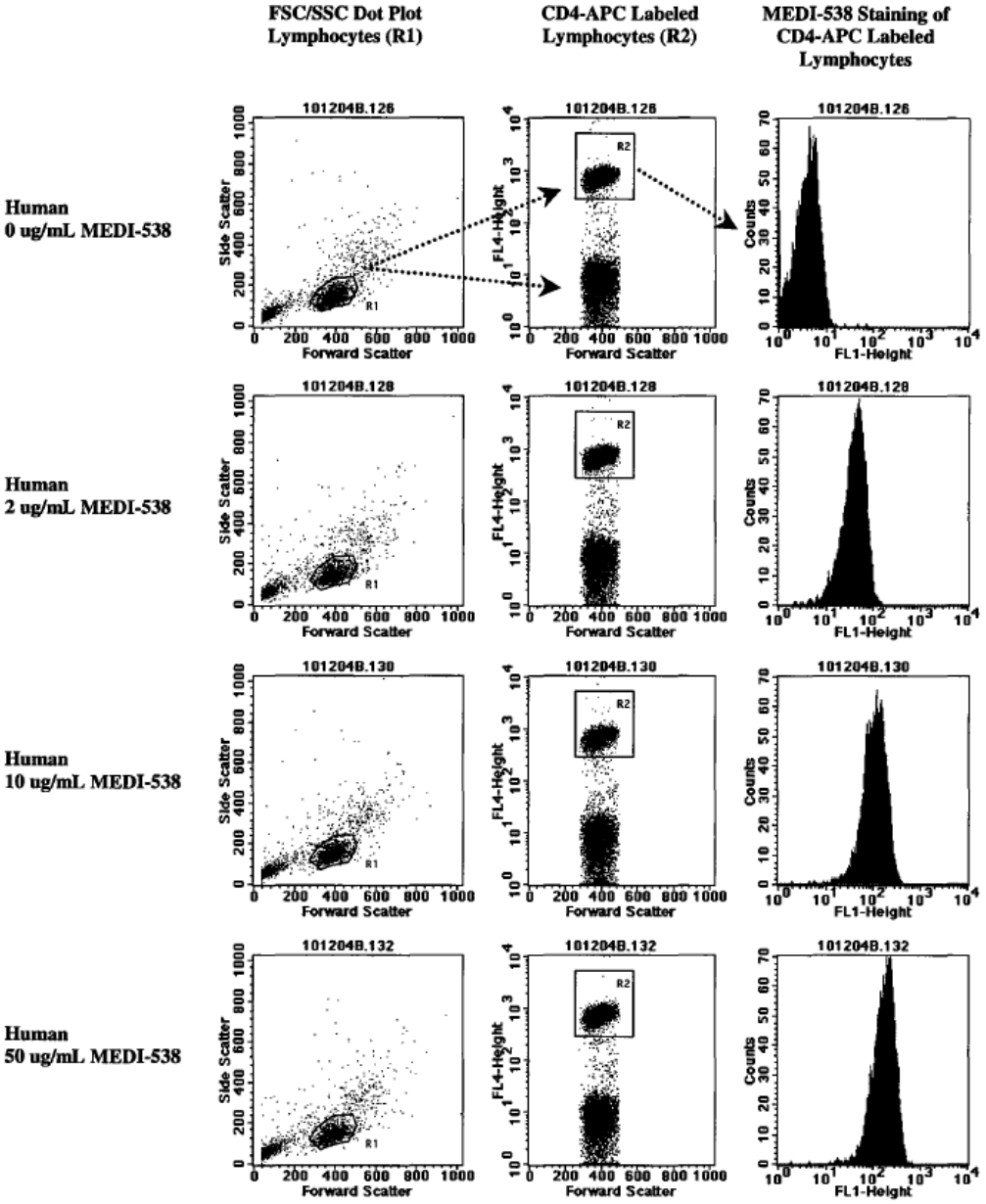
Reference ID: 3655615

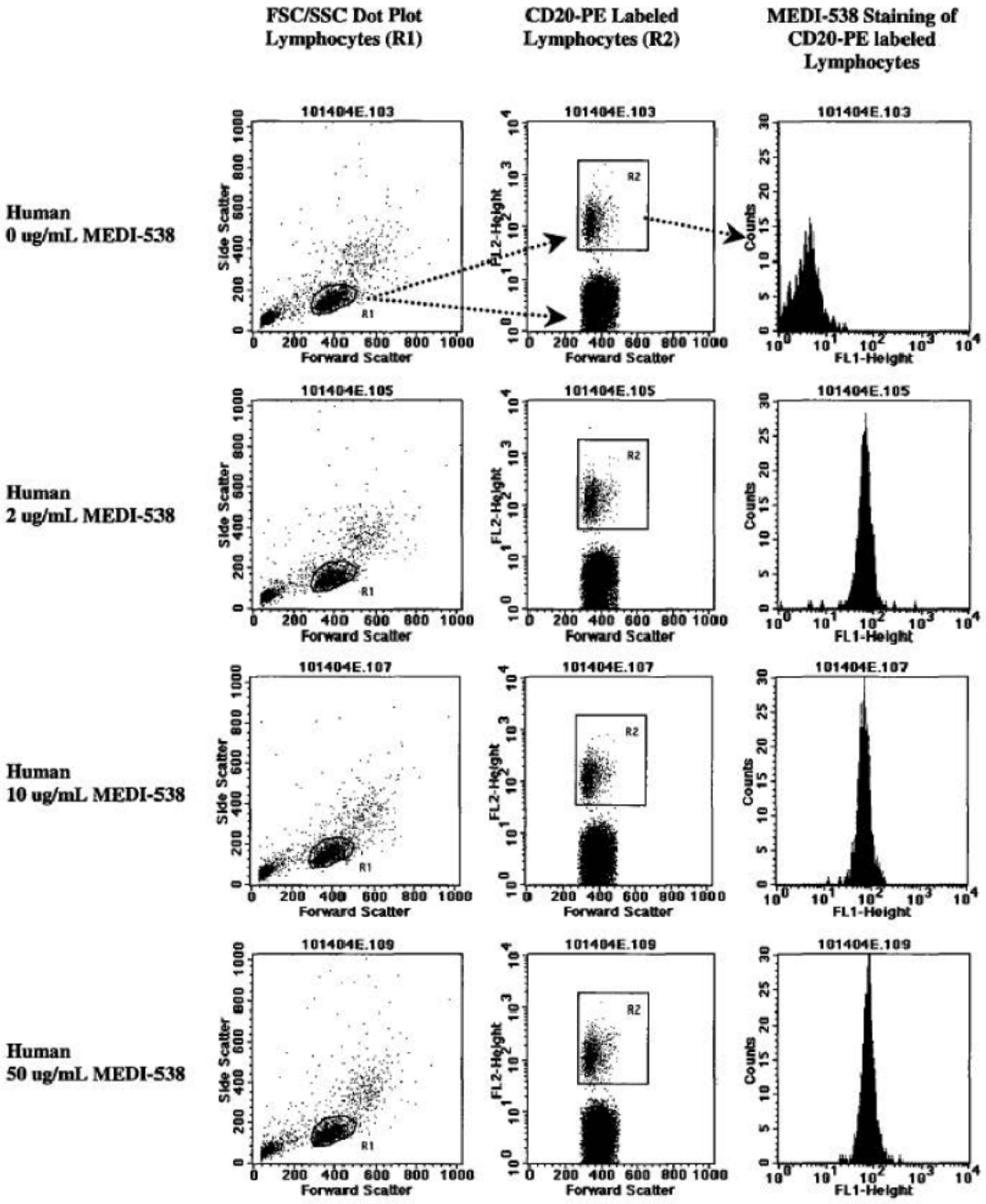
Results and Conclusions

Blinatumomab bound to human CD20$^+$ B lymphocytes and CD4$^+$ T lymphocytes but it did not bind to PBMCs from the African Green monkey, squirrel monkey, rat, and mouse.

Figure 4: Binding of blinatumomab to human CD4$^+$ T lymphocytes and CD20$^+$ B lymphocytes

(excerpted from Applicant's submission)




FSC/SSC Dot Plot Lymphocytes (R1)
CD20-PE Labeled Lymphocytes (R2)
MEDI-538 Staining of CD20-PE labeled Lymphocytes
Human 0 ug/mL MEDI-538
Human 2 ug/mL MEDI-538
Human 10 ug/mL MEDI-538
Human 50 ug/mL MEDI-538
101404E.103
101404E.105
101404E.107
101404E.109
Side Scatter
Forward Scatter
FL2-Height
FL1-Height
Counts
R1
R2

Study title: MT103-mediated redirected lysis of B cell lymphoma cell lines

Study No.:	103-PCD-0061
Report Date:	December 11, 2009
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Micromet AS, Staffelseestr. 2, 81477 Munich
GLP:	No

The objective of this study was to evaluate the blinatumomab-mediated redirected lysis of $CD19^+$ human chronic lymphocytic leukemia lymphoma (EHEB and MEC-1), mantle cell lymphoma (GRANTA-519, HBL-2, and NCEB-1), follicular lymphoma (Karpas-422), and B cell precursor leukemia NALM-6 cell lines by human cytotoxic T cells, in vitro. Briefly, freshly isolated human PBMCs and target cells were co-cultured at an effector-to-target (E:T) cell ratio of 10:1 for up to 20 hours and cytotoxicity was measured by flow cytometry.

Results and Conclusions

Blinatumomab treatment resulted in cell death of all seven cell lines with half-maximal effective concentration (EC_{50}) values ranging from 20 to 414 pg/mL. No correlation was observed between CD19 expression level and EC_{50} values.

Study title: MT103 mediated dose and time dependent expression of granzyme B

Study No.:	103-PCD-0063
Report Date:	January 29, 2010
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Micromet AG, Munich, Germany
GLP:	No

The objective of this study was to determine the expression of granzyme B in human T cells following treatment with blinatumomab. Briefly, human NALM-6 (pre B cell leukemia) cell line and human PBMC were co-cultured, at an E:T ratio of 10:1, for up to 48 hours. Granzyme B positive $CD4^+$ and $CD8^+$ T cells were quantified by flow cytometric analysis.

Results and Conclusions

Blinatumomab treatment in the presence of $CD19^+$ target cells resulted in time- and concentration-dependent induction of granzyme B expression in human $CD4^+$ and $CD8^+$ T cells. In vitro, granzyme B expression was detectable as early as 16 hours after treatment with blinatumomab and was still detectable at 48 hours (last measured timepoint) post-treatment. Based on EC_{50} values, $CD8^+$ cells were more sensitive to blinatumomab-induced granzyme B expression.

Reference ID: 3655615

Figure 5: Blinatumomab-mediated concentration- and time-dependent increases in granzyme B expression in CD8$^+$ and CD4$^+$ T cells
(excerpted from Applicant's submission)

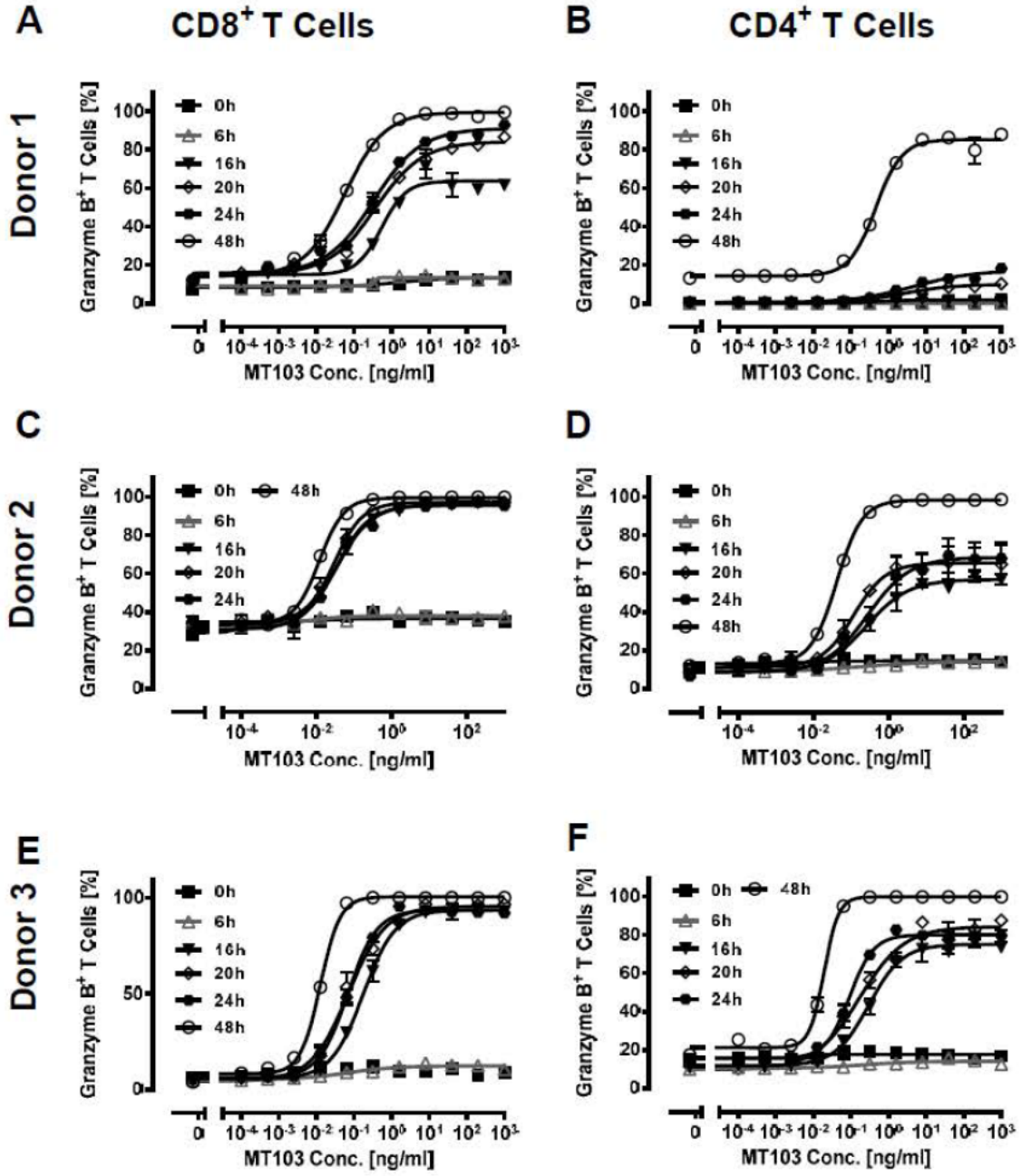


Table 3: EC_{50} values for granzyme B induction in human $CD8^+$ and $CD4^+$ cells
(excerpted from Applicant's submission)

EC50 values [ng/mL]	CD8+ T Cells			CD4+ T Cells		
Incubation Time [Hrs]	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16	0.54	0.04	0.18	n.a.	0.26	0.30
20	0.38	0.03	0.07	n.a.	0.12	0.17
24	0.33	0.03	0.07	n.a.	n.a.	0.09
48	0.06	0.01	0.01	0.46	0.04	0.02

n.a. = not applicable; dose-response curves were not considered valid

Study title: In vitro characterization of binding, cytotoxicity and mode of action

Study No.: 103-PCD-0065
Report Date: February 23, 2010
Study report location: eCTD 4.2.1.1.
Conducting Laboratory: Micromet AG
GLP: No

The objective of the studies in this study report was to evaluate pharmacologic properties of blinatumomab, in vitro.

Results and Conclusions

- Binding
  - Blinatumomab bound to $CD19^+$ Raji and NALM-6 B-cell lymphoma cell lines, human primary $CD20^+$ B and $CD4^+$ T cells, and immortalized human T cell clones derived from $CD8^+$ and $CD4^+$ T cells, in a concentration-dependent manner. Pre-incubation of T cells and NALM-6 cell line with anti-CD3 and anti-CD19 monoclonal antibodies, respectively, inhibited binding of blinatumomab to these cells.
  - Blinatumomab did not bind to the Chinese hamster ovary (CHO) cell line which does not express CD3 or CD19.
  - Dimerization of blinatumomab had no effect on CD19 target binding but increased blinatumomab binding to CD3.

- Cytotoxicity
  - Blinatumomab induced cytotoxicity towards $CD19^+$ NALM-6 cells in the presence of human T cells at E:T ratios ranging from 1.25:1 to 5:1, the lowest and highest ratios tested, respectively, using either a ^{51}chromium release or flow cytometry-based assay.
  - More potent blinatumomab-mediated cytotoxicity was noted when human $CD8^+$ cytotoxic T cells were used as the effector cells (EC_{50} < 10 pg/mL) when compared to human $CD4^+$ helper T cells. Within the cytotoxic T cell population, blinatumomab induced cell death of NALM-6 cells when they

Reference ID: 3655615

were incubated with primed $CD45RO^+CD8^+$ T cells (EC_{50} ~ 0.1 ng/mL) but not with naïve $CD45RA^+CD8^+$ T cells.
  - Both clone 3C (derived from human $CD8^+$ T cells) and clone CB15 (derived from human $CD4^+$ T cells) can mediate blinatumomab-dependent cytotoxicity of NALM-6 cells (EC_{50} = 11 pg/mL and 28 pg/mL, respectively), indicating that $CD4^+$ T cells can also function as effectors of blinatumomab-mediated cytotoxicity.
  - Similar blinatumomab-mediated cytotoxicity was noted at E:T cell ratios ranging from 20:1 to 2.5:1, with EC_{50} values ranging from 31 to 48 pg/mL.
  - Blinatumomab-mediated cytotoxicity was noted in $CD19^+$ NALM-6 cells but not in HT29 colon carcinoma cell line which does not express CD19.

- Mechanism of action
  - EGTA, a calcium chelator, inhibited blinatumomab-mediated cytotoxicity of NALM-6 cells in a concentration-dependent manner, in vitro. Since T-cell-mediated cytotoxicity by perforin is dependent on extracellular calcium, these data suggest blinatumomab-mediated cytotoxicity may be mediated by perforin.
  - In vitro, blinatumomab treatment of PBMC resulted in proliferation of T cells. No T cell proliferation was noted when PBMC depleted of B cells were treated with blinatumomab.
  - In vitro, treatment of cytotoxic T cells with blinatumomab in the presence of NALM-6, Raji, or primary B cells, resulted in detectable TNF-α, IFN-γ, IL-2, and soluble CD25 as soon as 2 hours following initiation of treatment. Maximum cytotoxicity was noted approximately 4 hours after initiation of treatment.
  - TNF-α neutralizing antibody abrogated increases in TNF-α levels but had no effect on blinatumomab-mediated cytotoxicity.
  - Dexamethasone inhibited blinatumomab-mediated increases in cytokine levels but had no effect on blinatumomab-mediated cytotoxicity.

Figure 6: Binding affinity analysis of blinatumomab to CD19 on NALM-6 cells
(excerpted from Applicant's submission)

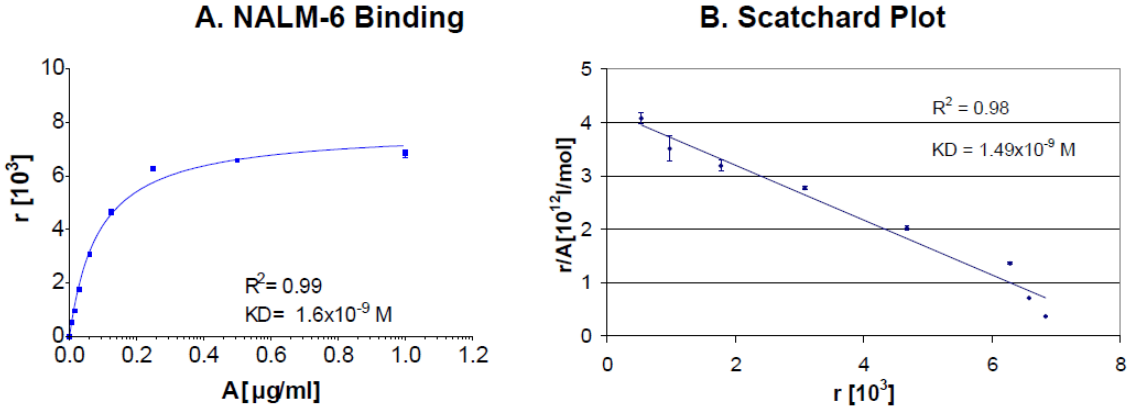


Figure 7: Binding affinity analysis of blinatumomab to (b) (4) on primary human T cells

(excerpted from Applicant's submission)

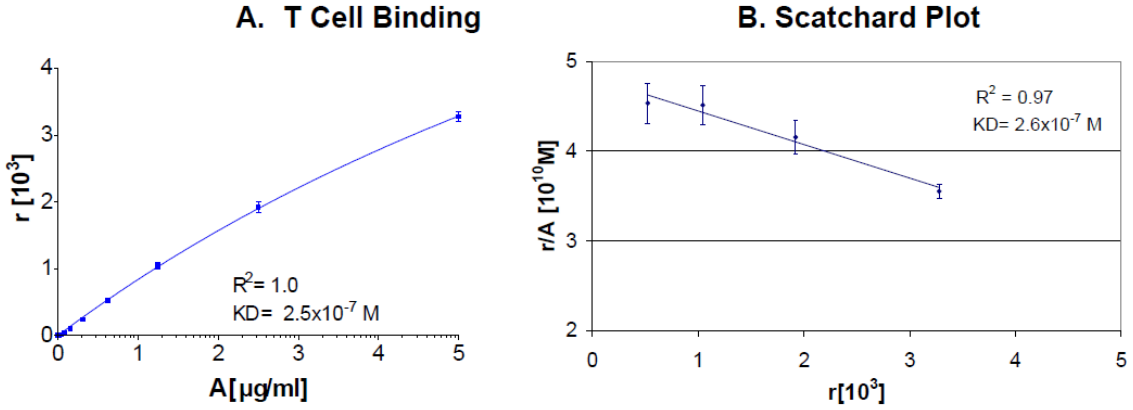


Figure 8: Blinatumomab-induced cytotoxicity measured by ^{51}chromium release assay or flow cytometry-based assay

(excerpted from Applicant's submission)

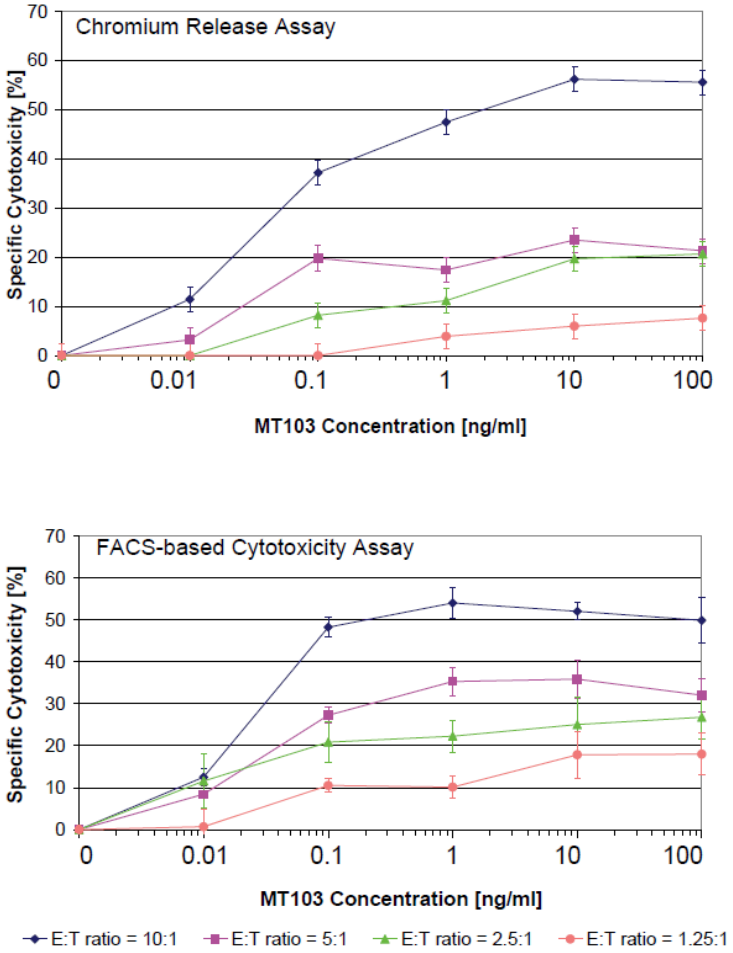


Figure 9: Blinatumomab-mediated cytotoxicity by human CD3$^+$, CD4$^+$, or CD8$^+$ T cells

(excerpted from Applicant's submission)

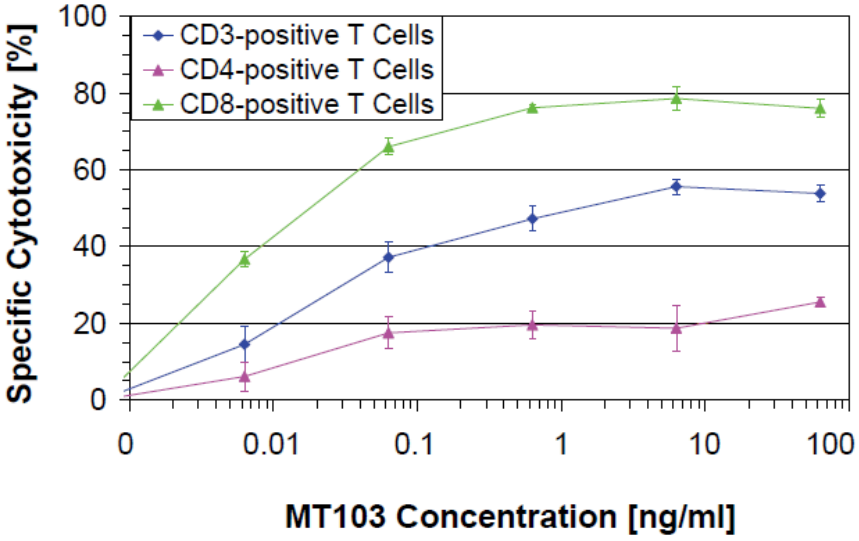


Figure 10: Blinatumomab-induced T cell proliferation in the presence or absence of CD19$^+$ cells

(excerpted from Applicant's submission)

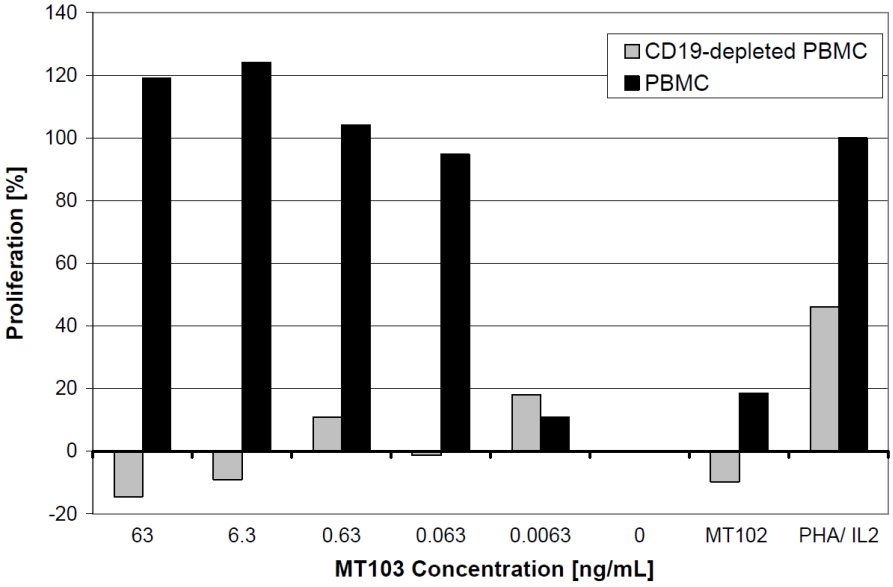
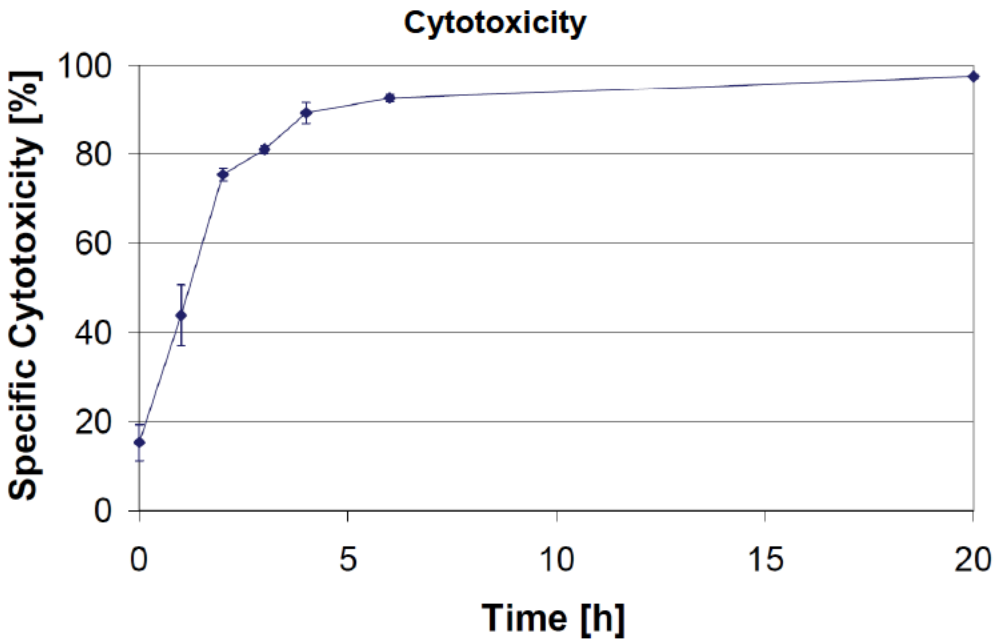
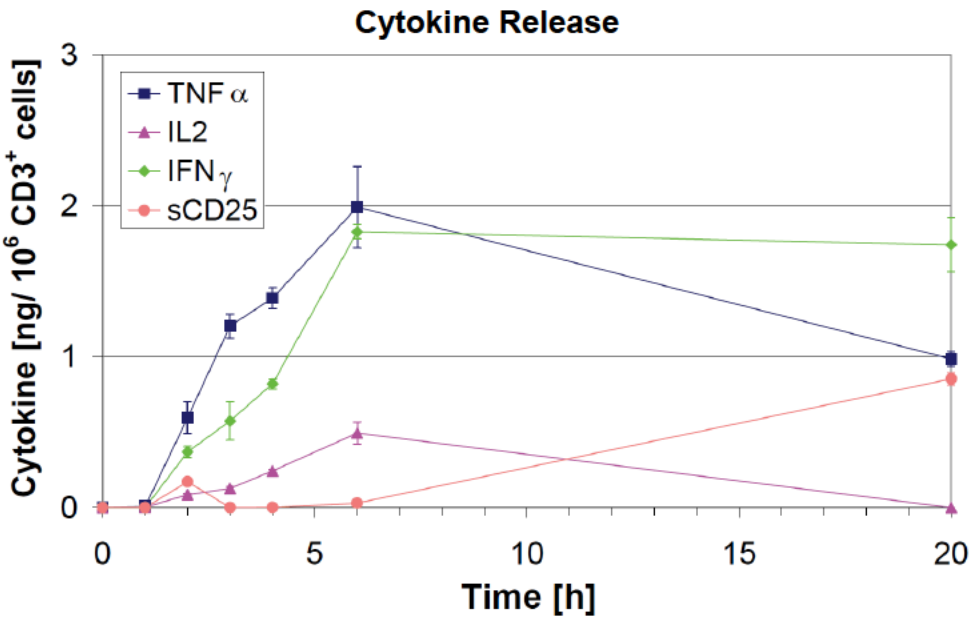


Figure 11: Time-dependent blinatumomab-mediated cytotoxicity in NALM-6 cells and cytokine production by T cells
(excerpted from Applicant's submission)





Study title: In vitro characterization of MT103 in human and chimpanzee test system

Study No.:	103-PCD-0066
Report Date:	June 18, 2012
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Amgen Research (Munich) GmbH
GLP:	No

The objective of the study was to compare binding and pharmacodynamic properties of blinatumomab between human and chimpanzee cells.

Results and Conclusions

- When blinatumomab was tested in a range of 0.08 to 50 μg/mL, no saturation of binding to $CD4^+$ lymphocytes was noted in either species. Saturation of blinatumomab binding to human $CD20^+$ lymphocytes was noted at 10 μg/mL while binding to chimpanzee $CD20^+$ lymphocytes plateaued at concentrations higher than 2 μg/mL.
- In autologous B cell depletion assays and cytotoxicity assays with NALM-6 cells, similar EC_{50} values were noted in human and chimpanzee.
- Similar IL-2, IFN-γ, and TNF-α profiles were noted between human and chimpanzee effector cells.

Study title: Impact of effector-to-target cell ratio on MT103-mediated redirected lysis and T cell activation

Study No.:	103-PCD-0067
Report Date:	November 22, 2011
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Micromet GmbH
GLP:	No

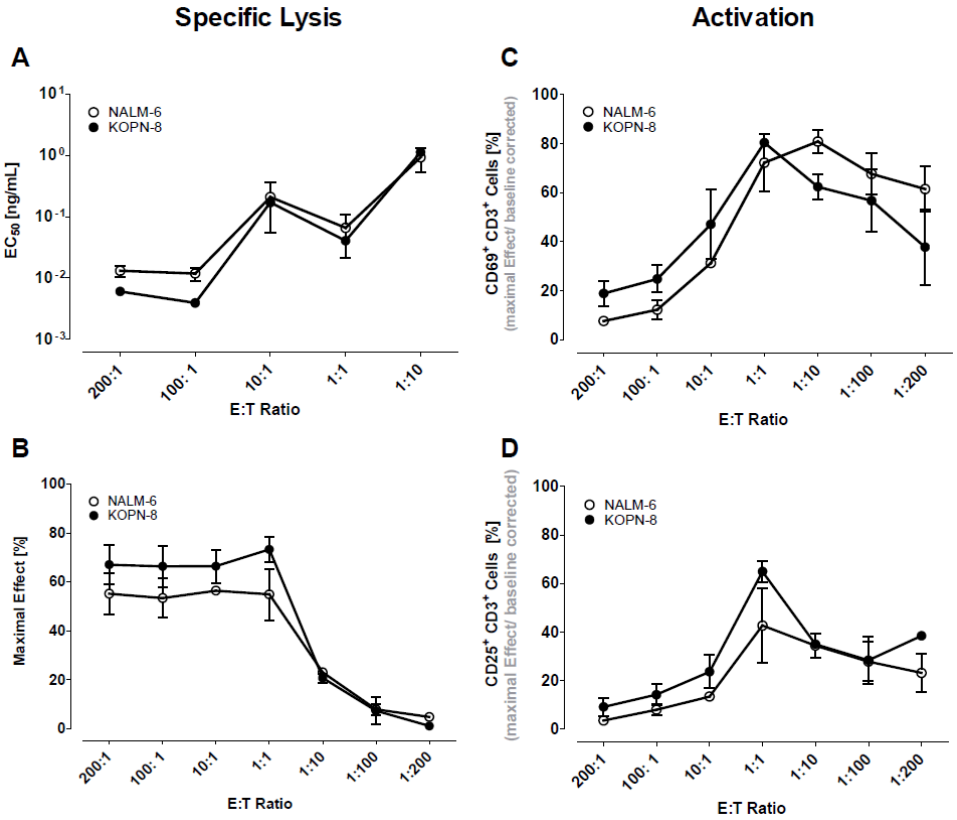
The objective of this study was to evaluate the impact of the effector-to-target cell ratio on blinatumomab-mediated redirected lysis of NALM-6 and KOPN-8 tumor cell lines and $CD3^+$ T cell activation, using flow cytometry. KOPN-8 is a human pre B cell leukemia cell line which was established from a 3-month-old female subject.

Results and Conclusions

- In vitro, blinatumomab-mediated cytotoxicity of NALM-6 and KOPN-8 cells was noted at E:T cell ratios greater than 1:10. EC_{50} values increased with decreasing E:T cell ratios in a concentration-dependent manner.
- In vitro, maximal activation of $CD3^+$ T cells, as measured by increased expression of activation markers CD25 and CD69, was noted at an E:T cell ratio of 1:1.

Reference ID: 3655615

Figure 12: Effect of E:T cell ratios on blinatumomab-mediated redirected lysis and T cell activation, in vitro
(excerpted from Applicant's submission)



Study title: MT103-mediated redirected lysis of human pediatric B cell acute lymphoblastic leukemia cell lines

Study No.:	103-PCD-0076
Report Date:	May 4, 2011
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Micromet AG, Staffelseestr. 2, 81477 Munich
GLP:	No

The objective of the study was to evaluate blinatumomab-mediated redirected cytotoxicity of human B cell acute lymphoblastic leukemia cell lines derived from pediatric subjects.

Results and Conclusions

- Blinatumomab-mediated redirected lysis was observed in all cell lines tested (i.e. KOPN-8, SEM_c, MHH-CALL-3, 380, REH, NALM-6).
- EC_{50} values ranged from 14 to 101 pg/mL following 48-hour incubation. Lower EC_{50} values were noted for $CD8^+$ T cells when compared to $CD4^+$ T cells.

- Consistent with previous studies, blinatumomab-mediated redirected lysis was directly correlated with CD19 expression on target cells, resulting in increased expression of activation markers CD25 and CD69 and cytokine release, including IL-2, IL-4, IL-6, IL-10, TNF, and IFN-γ.

Study title: Influence of dexamethasone and indomethacin on MT103-mediated cell lysis and cytokine release

Study No.:	103-PCD-0071
Report Date:	July 28, 2011
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Micromet AG
GLP:	No

The objective of the study was to evaluate the effect of dexamethasone and indomethacin pre-treatment on blinatumomab-mediated cell lysis and cytokine release. Cytokine release in the cell culture supernatant and cell viability were analyzed by flow cytometric methods.

Results and Conclusions

- Pre-treatment of PBMC with dexamethasone for 1 or 14 hours resulted in inhibition of cytokine release. However, dexamethasone had no effect on blinatumomab-mediated cytotoxicity of NALM-6 cells.
- Indomethacin had no effect on blinatumomab-mediated cytokine release or cytotoxicity.

Reference ID: 3655615

Figure 13: Effect of dexamethasone on cytokine release
(excerpted from Applicant's submission)

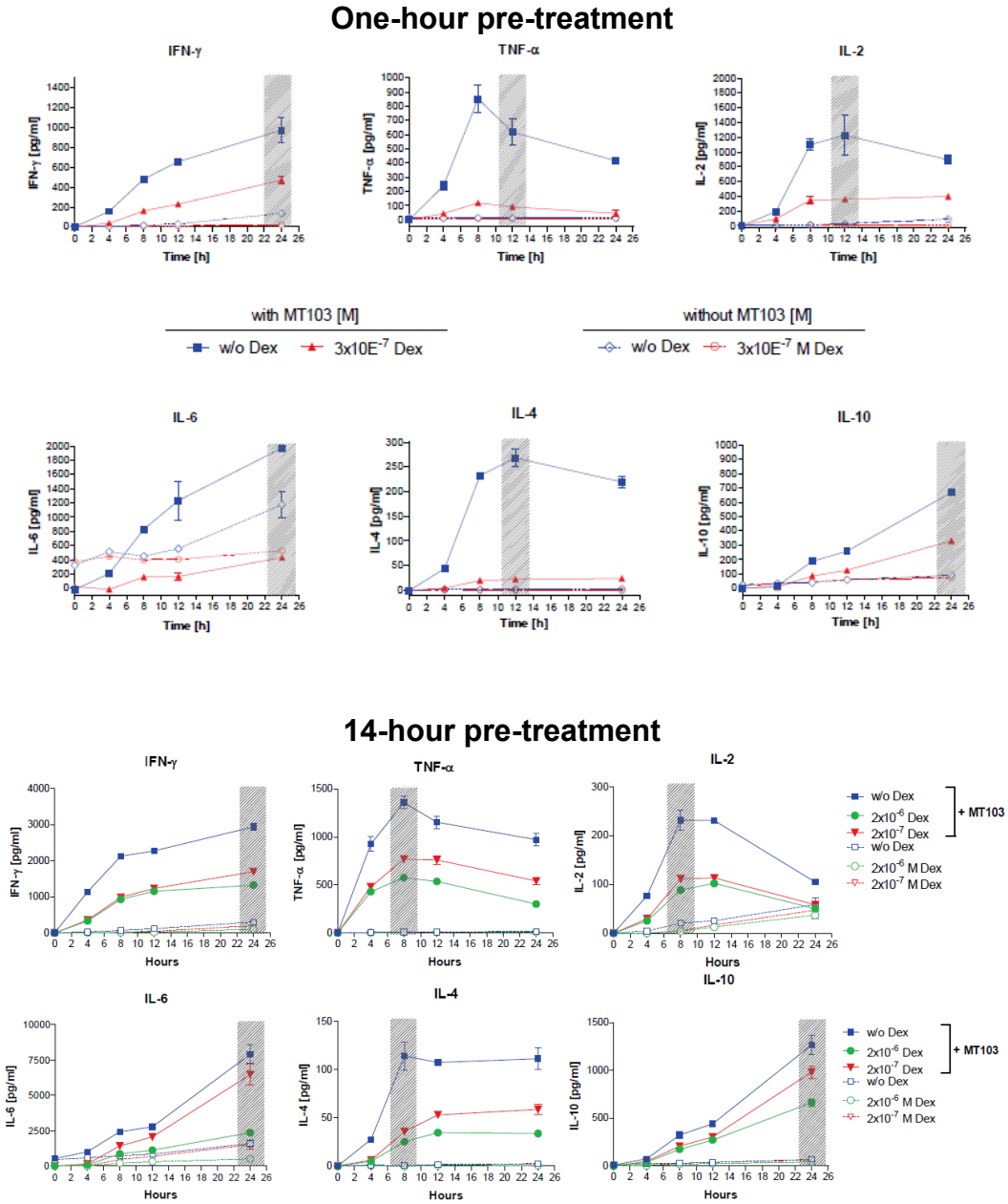
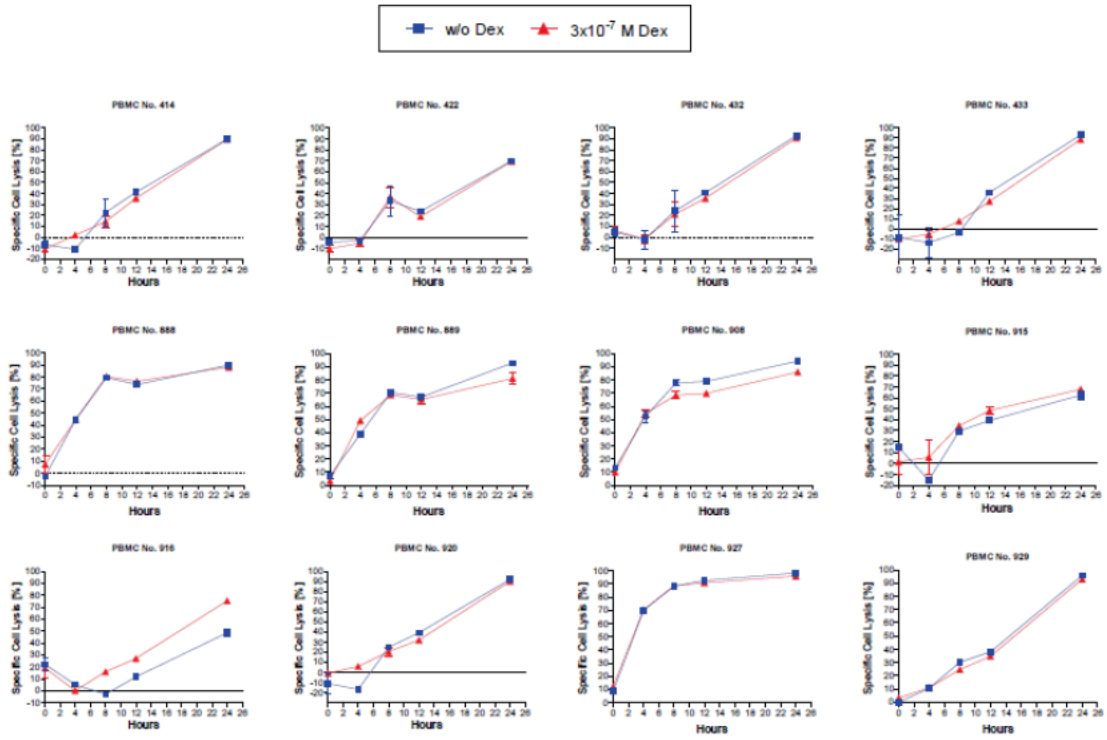


Figure 14: Effect of dexamethasone on blinatumomab-mediated cytotoxicity
(excerpted from Applicant's submission)



Study Title: MuS103new BiTE antibody generation of a murine-like MT103 surrogate molecule with paired affinities

Study No.:	DR-RE-103-001
Report Date:	June 5, 2011
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Micromet AG Staffelseestr. 2 81477 Munich, Germany
GLP:	No

Because blinatumomab binds only to human and chimpanzee CD19 and CD3, the Applicant generated a murine surrogate for toxicology studies in mice. The original surrogate molecule, muS103, caused a significant immune response in mice and had poor production yields, according to the Applicant. Additionally, the binding characteristics differed from blinatumomab, in particular the ratio of CD19 to CD3 binding. Blinatumomab binds human CD19-expressing cells with higher affinity (K_D = (b) (4) nM) than CD3-expressing cells (K_D = (b) (4) nM). The surrogate molecule displayed the opposite binding characteristics (muCD19 K_D = (b) (4) nM vs muCD3 K_D = (b) (4) nM).

Briefly, the Applicant conducted studies to improve the pharmacological properties of muS103. To reduce immunogenicity in mice, the Applicant used (b) (4)

(b) (4). In addition, the CD19 binding region was affinity optimized to match the binding affinities displayed by blinatumomab.

Results and Conclusions

- The Applicant selected muS103new (b) (4) from (b) (4) candidate antibody constructs based on its monomer yield, cytotoxic activity (Figure 15), and binding affinities to exogenous murine CD19 expressed on CHO cells and native CD3 expressed on mouse splenocytes (Table 4).
- Collectively, the species-specific binding characteristics and pharmacological activity of muS103new (b) (4)) were comparable to blinatumomab in vitro (Table 5).

Figure 15: Cytotoxic activity of muS103new candidates
(excerpted from Applicant's submission)

(b) (4)

Table 4: Binding affinities of muS103new candidates
(modified from Applicant's submission)

MuS103new	CHO/Mu CD19 KD [nM]	Mouse spleen cells KD [nM]
(b) (4)		

Table 5: Binding affinities of blinatumomab and muS103new (b) (4)
(excerpted from Applicant's submission)

	CHO/muCD19 KD [nM]	Mouse splenocytes KD [nM]
(b) (4)		

Study Title: Comparison of the pharmacodynamic effects of MT103 and its murine surrogate muS103new in vitro

Study No.: 103-PCD-0094
Report Date: July 31, 2012
Study report location: eCTD 4.2.1.1.
Conducting Laboratory: Amgen Research (Munich) GmbH
GLP: No

The objective of these studies was to compare the in vitro activity of blinatumomab and the murine surrogate, muS103new using flow cytometric methods. Briefly, blinatumomab was tested on co-cultures of isolated $CD3^+$ T cells from PBMC and either isolated B cells from PBMC or $CD19^+$ tumor cell lines, NALM and KOPN-8. muS103new was tested on co-cultures of $CD3^+$ T cells isolated from mouse splenocytes and CD19-expressing cell lines, BLC-1, A20 and CHO-mCD19. Cell lines lacking CD19 expression, PC-3-huPSA and CHO-DHFR[-], were used as negative controls.

Results and Conclusions

- Both blinatumomab and muS103new induced a concentration-dependent increase in T cell activation, cytokine release, and target cell lysis of CD19-positive cells but not CD19-negative cells (Figure 16 to Figure 20).
- EC_{50} values for species-specific T cell activation were comparable for blinatumomab (EC_{50} = 111-200 pg/ml) and muS103new (126-170 pg/ml; Appendix 1).
- EC_{50} values for cytotoxicity were higher for muS103new (EC_{50} = 37.9-1324 pg/ml) compared to blinatumomab (0.3-56.6 pg/ml) for the target cells tested (Appendix 1).
- While the cytokine profiles were similar between the two bispecific constructs, muS103new induced greater cytokine release compared to blinatumomab in vitro.

- Collectively, the pharmacological activity of muS103new was comparable to blinatumomab in vitro (Appendix 1).

Figure 16: Blinatumomab induces T cell-mediated cytotoxicity of CD19$^+$ target cells

(excerpted from Applicant's submission)

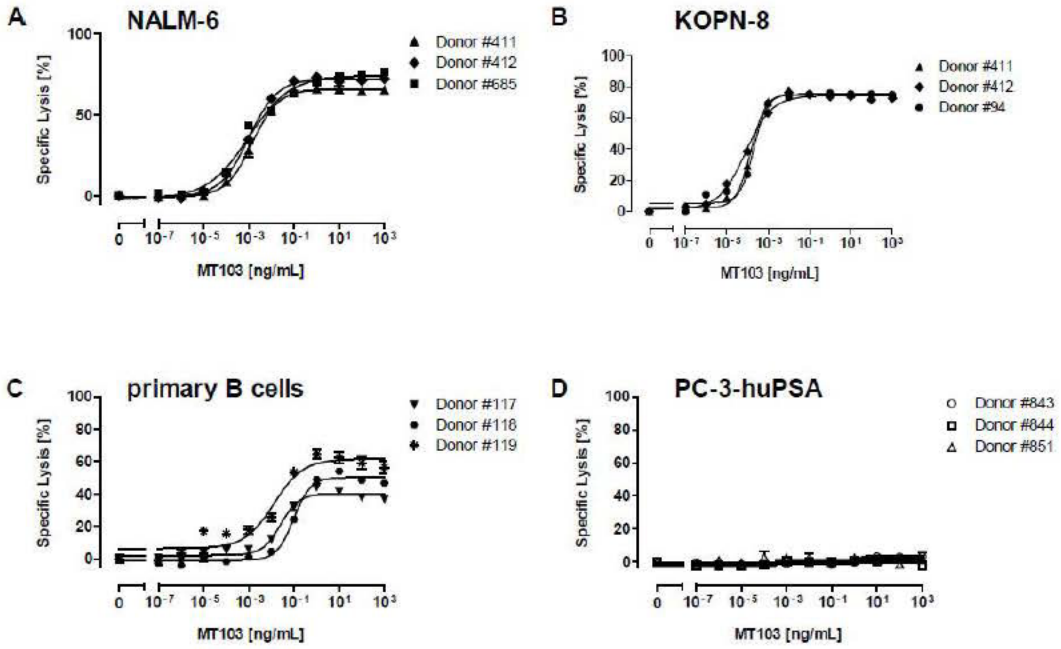


Figure 17: muS103new induces T cell-mediated cytotoxicity of CD19$^+$ target cells
(excerpted from Applicant's submission)

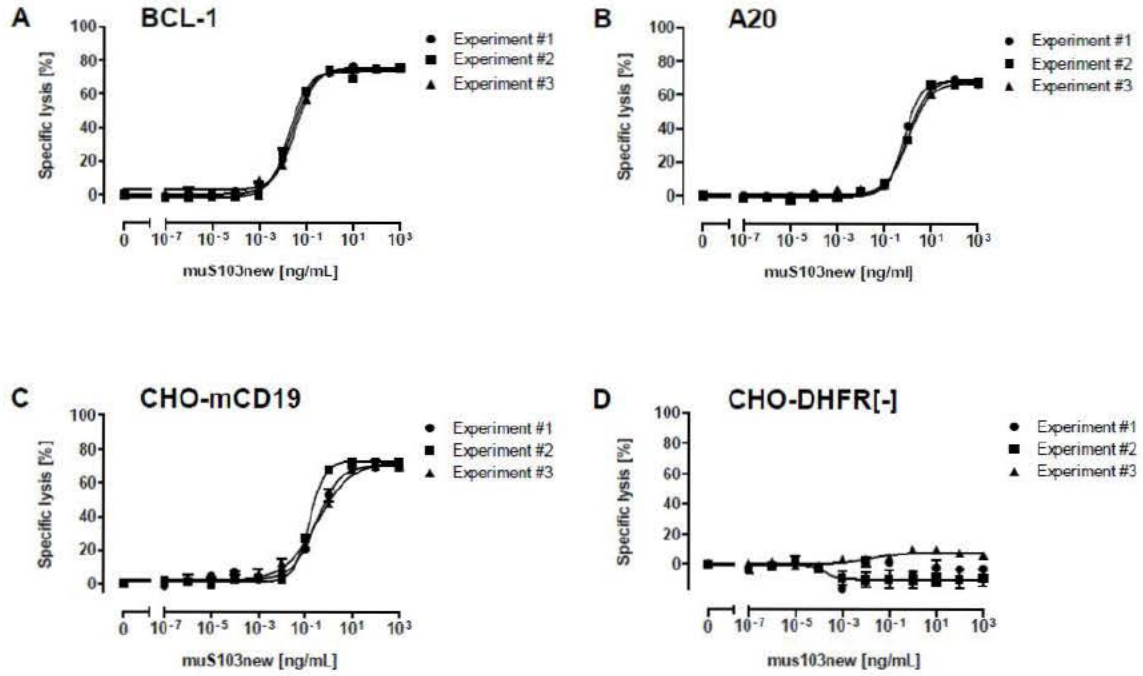


Figure 18: Blinatumomab-mediated T cell activation
(excerpted from Applicant's submission)

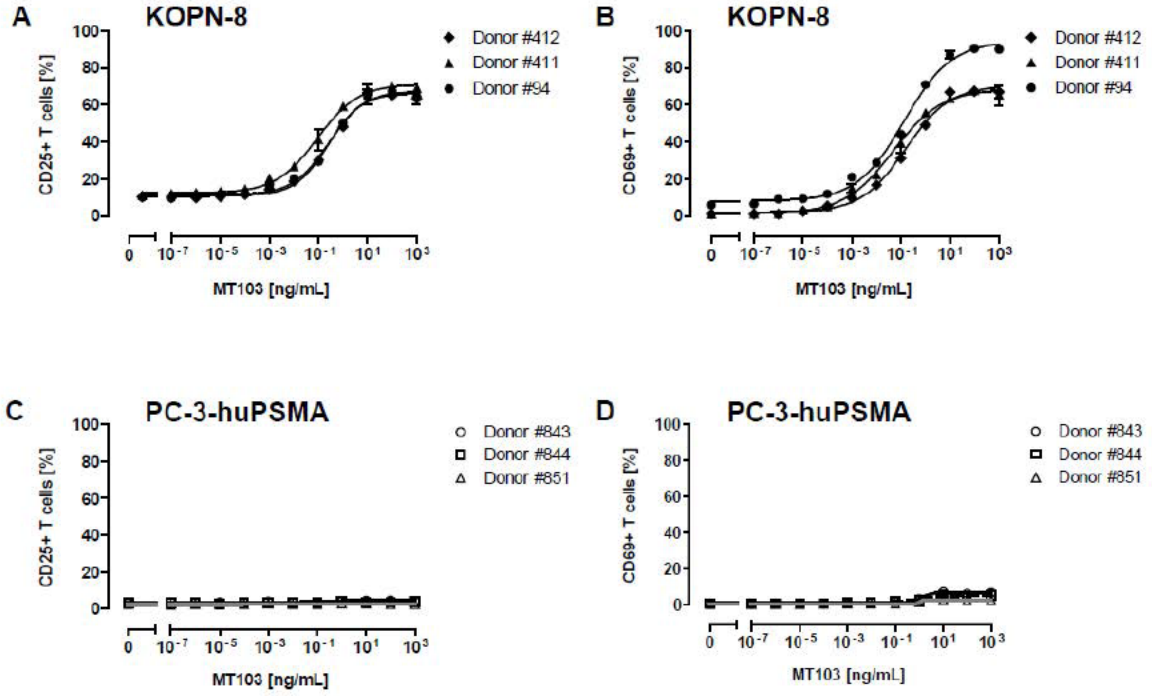


Figure 19: muS103new-mediated T cell activation
(excerpted from Applicant's submission)

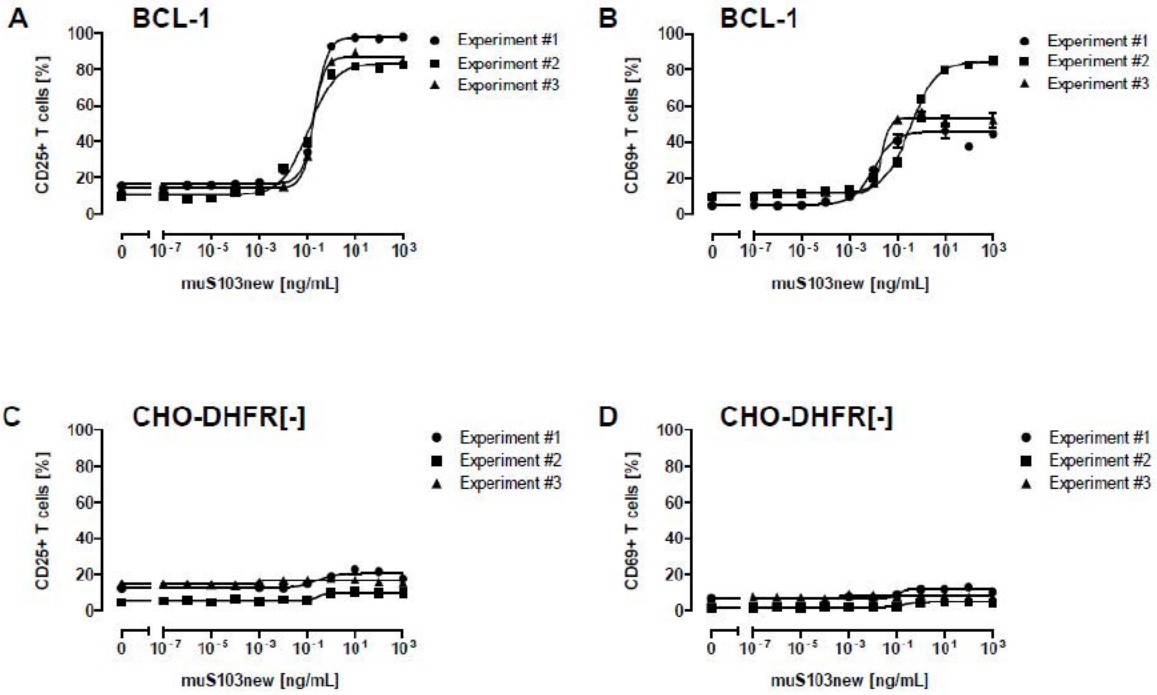
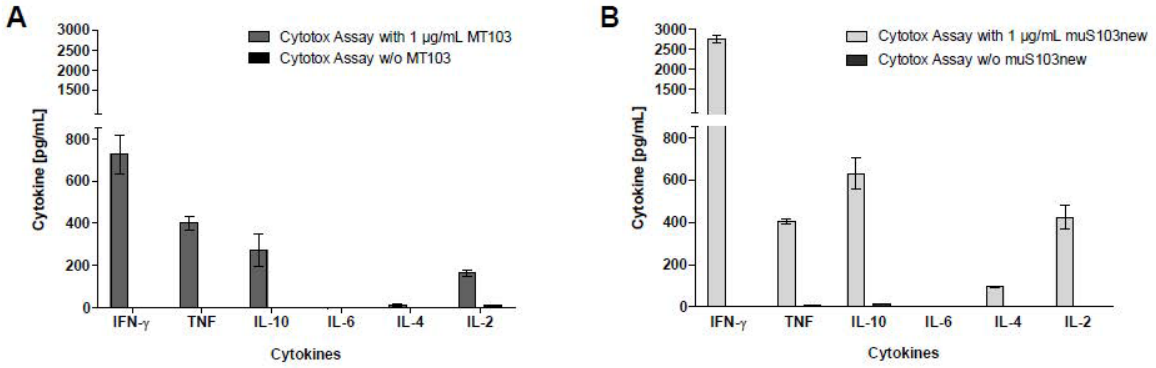


Figure 20: Blinatumomab (MT103)- and muS103new-induced cytokine release in the presence of CD3$^+$ T cells and CD19$^+$ target cells
(excerpted from Applicant's submission)



4.2 Secondary Pharmacology

Study title: AMG 103-induced T cell-adhesion to endothelial cells and its mitigation by anti-adhesive agents

Study No.:	R20140011
Report Date:	June 19, 2014
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Amgen Research (Munich) GmbH
GLP:	No

The objective of the study was to evaluate blinatumomab-induced activation of T-cell interaction with endothelial cells, in the absence of CD19$^+$ target cells, using an in vitro flow chamber system.

Results and Conclusions

- Pre-treatment of human T cells with blinatumomab resulted in reduced mean rolling velocity of T cells on human umbilical vein endothelial cells (HUVEC) and human brain microvascular endothelial cells (HBMEC), and increased adherence of T cells to HUVEC, when compared to vehicle control, in vitro. These effects were correlated with activation of HUVEC as measured by increased surface expression of adhesion molecules, including ICAM-1, P-selectin, and VCAM-1 (on HBMEC).
- Pre-treatment of T cells with pharmacology agents known to block the interactions between T cells and endothelial cells (i.e. pentosan polysulfate, natalizumab, minocycline, anti-ICAM-1 antibody, anti-P-selectin antibody, anti-CD11a antibody, anti-CD162 antibody, recombinant P-selectin, and PP2) inhibited blinatumomab-mediated reduction in T-cell rolling velocity.
- Overall, the Applicant concluded that these findings correlated with pharmacodynamic effects of blinatumomab in clinical trials in which T cell redistribution was noted as early as 45 minutes following the start of

blinatumomab infusion. Furthermore, the Applicant concluded that, unlike blinatumomab-mediated cytotoxicity and cytokine release, these effects can occur in the absence of $CD19^+$ target cells.

Study title: T cell activation by AMG 103 alters expression of adhesion molecules on endothelial cells

Study No.:	R20140012
Report Date:	May 2, 2014
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Amgen Research (Munich) GmbH
GLP:	No

The objective of this study was to evaluate the effect of blinatumomab-induced TNF-α on expression of adhesion molecules on T cells and endothelial cells, in vitro.

Results and Conclusions:

- In an in vitro co-culture of PBMC, HUVEC, and NALM-6 cells, blinatumomab-mediated increased expression of adhesion molecules on HUVEC (ICAM-1 and VCAM-1) and release of chemokine (MCP-1) and cytokine (IL-8) were induced by TNF-α. These blinatumomab-mediated effects could be blocked by treatment with entanercept.
- Blinatumomab treatment of T cells induced TNF-independent ICAM-1 expression but not VCAM-1 expression on HUVEC in the absence of $CD19^+$ target cells. However, in the presence of NALM-6 cells, blinatumomab treatment up-regulated TNF-dependent expression of both ICAM-1 and VCAM-1. Up-regulation of ICAM-1 in the presence of NALM-6 was up to 30-fold higher when compared to co-culture studies in the absence of $CD19^+$ cells.

4.3 Safety Pharmacology

Two CNS safety pharmacology studies were conducted with the murine surrogate muS103new in mice, an Irwin test following intravenous bolus administration of muS103new daily for 5 days and an exploratory study with 7-day continuous intracerebroventricular infusion of muS103new.

Study title: Evaluation of the effects of muS103new on behavior using the primary observation (Irwin) test in the mouse following intravenous (bolus) administration

Study no.:	103-PCD-0078
Study report location:	eCTD 4.2.1.3.
Conducting laboratory and location:	(b) (4)

Reference ID: 3655615

(b) (4)

Date of study initiation:	May 30, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	muS103new (surrogate bispecific antibody construct), lot # 100910MMa07, Purity: 98.8% monomer

Key Study Findings

- Intravenous administration of muS103new at doses of 0.2 and 5 mg/kg/day did not induce any CNS changes.
- At 1 mg/kg/day muS103new, a variety of CNS effects including decreased activity and reactivity, piloerection, and motor signs were observed in one animal starting on the third day of treatment.

Methods

Doses:	0 (saline and vehicle), 0.2, 1, or 5 mg/kg/day muS103new
Frequency of dosing:	Once daily for 5 days
Route of administration:	Intravenous bolus injection
Dose volume:	5 mL/kg (saline, vehicle, and muS103new) 10 mL/kg (midazolam)
Formulation/Vehicle:	Aqueous solution of 10 mM citric acid monohydrate, 75 mM L-lysine hydrochloride, 4% (w/v) Trehalose and 0.03% (w/v) Tween 80
Positive control:	Midazolam, 30 mg/kg (free-base)
Species/Strain:	BALB/c mice
Number/Sex/Group:	6 males/group, 6 groups total
Age:	Not provided
Weight:	17-25 g

The effects of muS103new on behavior and physiological function were evaluated following intravenous administration using the Irwin test in mice. muS103new (0, 0.2, 1, or 5 mg/kg/day) was administered intravenously immediately before the test on Days 1 and 5 and 15 minutes before the test on Days 2 to 4. The positive control, midazolam (30 mg/kg), was administered intravenously immediately before the test on Day 1. Observations were measured prior to dosing and 15, 60, and 180 minutes after administration on Days 1 and 5 and prior to dosing and 15 minutes after dosing on Days 2 to 4.

Results

- The vehicle and low and high doses (0.2 and 5 mg/kg/day) of muS103new did not induce any observable changes or affect body weight on Days 1 to 5.

Reference ID: 3655615

- At the mid dose (1 mg/kg/day) of muS103new, various changes were observed in one of the six animals.
    - On Day 3, the mouse displayed a slight decrease in activity, decreased abdominal muscle tone, piloerection, and ptosis before administration and at 15 minutes after administration.
    - On Day 4, the mouse had abnormal gait (rolling), loss of traction, decreased respiration and decreased reactivity to touch in addition to the same signs observed on Day 3; the decrease in activity was moderate.
    - On Day 5, the mouse exhibited the same signs observed on Days 3 and 4 and a decreased fear/startle response.
    - Body temperature was reduced on Days 4 and 5 to as low as 32.2 °C at 15 minutes after dosing on Day 5 compared to a vehicle group mean ranging from 36.3-37.5 °C at various time points on Day 5.
    - A decrease in body weight was observed from Days 3 to 5 with a loss of 5 g at Day 5 from a weight of 21 g on Day 1.
- The positive control midazolam (30 mg/kg) induced several CNS depressant effects consistent with its pharmacological profile including hypothermia, decreased activity, abnormal gait (rolling), motor incoordination, decreased abdominal muscle tone, decreased respiration, decreased or increased fear/startle response, akinesia, catalepsy, and analgesia.

Study title: Exploratory 7-day continuous intracerebroventricular infusion of muS103new to BALB/c mice

Study no.:	103-PCD-0103
Study report location:	eCTD 4.2.1.3.
Conducting laboratory and location:	Amgen Research (Munich) GmbH Staffelseestr. 2 81477 Munich Germany
Date of study initiation:	September 15, 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	muS103new (surrogate bispecific antibody construct), lot # 101004DAe01, Purity: 99.4% monomer

Key Study Findings

- Intracerebroventricular administration of muS103new did not produce any behavioral modifications or monitorable evidence of physiological change or neurotoxicity in mice.

Reference ID: 3655615

Methods

Doses:	0, 0.042, or 0.978 mg/kg/day
Frequency of dosing:	7-day continuous infusion
Route of administration:	Intracerebroventricular infusion into the left ventricle via a catheter from a subcutaneously-implanted osmotic pump
Dose volume:	1.06 µL/hour
Formulation/Vehicle:	Aqueous solution of 10 mM citric acid monohydrate, 75 mM L-lysine hydrochloride, 4% (w/v) Trehalose and 0.03% (w/v) Tween 80
Species/Strain:	BALB/c mice
Number/Sex/Group:	10 males/group
Age:	Not provided
Weight:	Means of 24.5-30.8 g on Day -1

To evaluate the effects of muS103new on behavior and physiological function, mice were observed during and following the continuous 7-day intracerebroventricular administration of muS103new (0, 0.042, or 0.978 mg/kg/day) on Days 1-10 (except on Day 7). Observations were conducted at least twice daily to monitor for the incidence and onset of death, convulsion, tremor, piloerection, jumping, motor incoordination and changes in activity. The start of exposure was estimated based on the technical specifications provided by the manufacturer and was considered to be on the morning of Day 2.

Results

Between Days 2 and 4, piloerection was observed in all animals including controls, and slightly decreased activity was observed in three animals administered the low dose (0.042 mg/kg/day) of muS103new. These findings were considered by the Applicant to be related to the surgical intervention to replace the pre-implanted artificial CSF-filled osmotic pumps that occurred on Day 1. No other findings were observed. Based on these results, intracerebroventricular administration of muS103new did not produce any behavioral modifications or monitorable evidence of physiological change or neurotoxicity.

5 Pharmacokinetics/ADME/Toxicokinetics

No pharmacokinetics studies were reviewed.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicology studies were submitted.

Reference ID: 3655615

6.2 Repeat-Dose Toxicity

Study title: muS103new: Toxicity study by intravenous (bolus) administration to BALB/c mice for 4 weeks followed by a 4-week recovery period

Study no.:	103-PCD-0075
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	Primary location: (b) (4) [redacted] Histology: (b) (4) [redacted]
Date of study initiation:	June 2, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	muS103new (surrogate bispecific antibody construct), lot # 100910MMa07, Purity: 98.8% monomer

Methods

Doses:	0 (saline and vehicle controls), 0.2, 1, or 5 mg/kg/day
Frequency of dosing:	Once daily for 4 weeks, 4-week recovery period
Route of administration:	Intravenous bolus injection
Dose volume:	5 mL/kg/dose
Formulation/Vehicle:	Aqueous solution of 10 mM citric acid monohydrate, 75 mM L-lysine hydrochloride, 4% (w/v) Trehalose and 0.03% (w/v) Tween 80
Species/Strain:	BALB/c mice
Number/Sex/Group:	Main study: 12/sex/group Recovery: 6/sex/group, vehicle control and muS103new groups
Age:	63 to 69 days at start of treatment
Weight:	Males: 15.9-28.0 g at start of treatment Females: 17.9-22.3 g at start of treatment
Satellite groups:	Immunogenicity: 6/sex/group, vehicle control and muS103new groups Pharmacodynamics: 9/sex/group

Reference ID: 3655615

Toxicokinetics: 6/sex for vehicle control group
and 18/sex/group for muS103new groups

This 4-week mouse toxicology study was conducted with intravenous administration of murine surrogate molecule, muS103new. Due to feasibility issues, muS103new was administered subcutaneously instead of intravenously in the 13-week study in mice. Since the 13-week study is the toxicology study of the longest duration with muS103new, it has been fully reviewed. The results of the 4-week study are summarized below.

Summary of results

Toxicities observed following intravenous administration of muS103new for 4 weeks are similar to those observed in the 13-week study with subcutaneous administration. muS103new-related hematology changes included decreases in WBC, lymphocytes, and large unstained cell (LUC) counts at all doses compared to the vehicle control group. Immunophenotyping showed that treatment with muS103new depleted lymphocyte populations including B and T cells (including $CD4^+$ and $CD8^+$ T cell subsets), NK cells and NKT cells in the blood and lymphoid tissues (spleen and lymph node). Decreases in lymphocytes showed some recovery following the 4-week recovery period; however, overall, lymphocyte cell numbers were still lower in muS103new-treated animals than controls. Spleen weights were lower at all doses of muS103new in males and at 1 mg/kg/day in females. Histopathology findings included decreased cellularity of the white pulp of the spleen, and decreased cellularity or germinal center development in the mandibular, mesenteric, axillary, and inguinal lymph nodes and Peyer's patches at all doses of muS103new.

Reference ID: 3655615

Study title: muS103new: Toxicity study by twice-daily subcutaneous administration to BALB/c mice for 13 weeks followed by a 4-week recovery period

Study no.:	103-PCD-0082
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	Primary location: (b) (4) Histology: (b) (4)
Date of study initiation:	February 16, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	muS103new (surrogate bispecific antibody construct), lot # 111201SEi04, Purity: 99.8% monomer

Key Study Findings

- Surrogate bispecific antibody construct muS103new decreased lymphocytes including total B cells and T cells and the $CD4^+$ and $CD8^+$ T lymphocyte subsets in the blood, spleen, and lymph node.
- Toxicities of muS103new included decreased cellularity or decreased germinal center development in lymphoid tissues (mandibular, mesenteric, axillary, and inguinal lymph nodes, Peyer's patches, and spleen) and increased dermal or subcutis/panniculus inflammation at treated sites and skin.

Reference ID: 3655615

Methods

Doses:	0, 1, or 5 mg/kg/dose (0, 2, or 10 mg/kg/day)
Frequency of dosing:	Twice daily (8-hour interval between doses) for 13 weeks, 4-week recovery period
Route of administration:	Subcutaneous injection
Dose volume:	5 mL/kg/dose
Formulation/Vehicle:	Aqueous solution of 10 mM citric acid monohydrate, 75 mM L-lysine hydrochloride, 4% (w/v) Trehalose and 0.03% (w/v) Tween 80
Species/Strain:	BALB/c mice
Number/Sex/Group:	Main study: 12/sex/group Recovery: 8/sex/group
Age:	63-73 days at start of treatment
Weight:	Males: 22.0-29.6 g at start of treatment Females: 18.4-22.4 g at start of treatment
Satellite groups:	Immunogenicity/pharmacodynamics: 18/sex/group Immunogenicity/toxicokinetics: 6/sex/group for muS103new groups only Toxicokinetics: 6/sex for control group and 18/sex/group for muS103new groups

This 13-week mouse toxicology study was conducted with a murine surrogate molecule, muS103new. According to the Applicant, the maximum feasible dose in mice for subcutaneous administration was 5 mg/kg based on a test substance concentration of 1.02 mg/mL and a dose volume of 5 mL/kg/injection. The drug was administered twice daily to achieve increased exposure (10 mg/kg/day).

Observations and times:

Mortality:	Twice daily
Clinical signs:	Cage observations: Twice daily during dosing, once daily during acclimatization and recovery periods Detailed observations: Weekly
Body weights:	Weekly
Food consumption:	Weekly, mean weekly consumption per animal (g/animal/week) was calculated for each cage
Ophthalmoscopy:	Before treatment (all animals), Week 13 (control and 10 mg/kg/day groups only)
Hematology:	At necropsy
Clinical chemistry:	At necropsy
Coagulation:	Not conducted
Immunophenotyping in blood:	Days 43, 71, 92, and 120
Immunophenotyping in spleen and lymph node:	Days 72, 93, and Day 121
Urinalysis:	Not conducted

Reference ID: 3655615

Gross pathology:	At necropsy
Organ weights:	At necropsy
Histopathology:	At necropsy
Immunogenicity	Days 43, 71, 92, and 121
Toxicokinetics:	From toxicokinetics animals: • Day 1: At 30 minutes and 1 hour after first daily dose for control group and at 30 minutes, and 1, 2, 4, 8, and 10 hours after first daily dose for muS103new groups • Day 92: At 30 minutes and 1 hour after second daily dose for control group and at 30 minutes, and 1, 2, 4, 8, and 12 hours after second daily dose for muS103new groups From immunogenicity/toxicokinetics animals: • Days 43 and 71 at 2 hours after first daily dose

Results

Mortality

There were five mortalities during the study. Two were in the main study/recovery animals and the other three were in satellite animals. Two of the mortalities were clearly accidental and another in a control animal was not drug-related.

The mortalities in the two animals treated with 10 mg/kg/day muS103new were not considered muS103new-related by the Applicant; however, since both animals were in poor clinical condition and had toxicities, a possible relationship to muS103new treatment cannot be ruled out.

- The 10 mg/kg/day male (Animal #117) euthanized on Day 12 due to poor clinical condition was cold to touch with reduced activity, piloerection, fast breathing, and a dark ventral body surface. Findings included a trauma in the right horn/seminal vesicles with loss of seminal fluid that correlated to necrosis and hemorrhage in one of the seminal vesicles and inflammatory and degenerative lesions in the urinary bladder with hemorrhage.
- The 10 mg/kg/day male in the immunogenicity/pharmacodynamics group (Animal # 139) euthanized on Day 111 during the recovery phase had clinical signs of hunched posture, reduced activity, and thin appearance. Renal toxicity consisted of chronic nephropathy with papillary necrosis that correlated to pale and granular kidneys observed macroscopically. Renal changes were not observed in other animals in this study.

Reference ID: 3655615

Table 6: Mortality in 13-week mouse study

Dose/group	Animal #	Sex	Found dead or euthanized	Day of death/ euthanasia	Cause of death or reason for euthanasia
Control	13	Male	Euthanized	Day 79	Accidental; trapped left hind limb in cage and had limited use of limb
Control (IMM/PD)	36	Male	Found dead	Day 47	Cause of death not identified; had cannibalized left hind paw and broken tail
2 mg/kg/day (TK)	86	Male	Died	Day 90	Accidental; died following traumatic injury in cage (animal jumped from the cage when lid was being closed and head was hit by cage lid)
10 mg/kg/day	117	Male	Euthanized	Day 12	Poor clinical condition; had urinary/genital lesions
10 mg/kg/day (IMM/PD)	139	Male	Euthanized	Day 111	Poor clinical condition; chronic nephropathy with papillary necrosis observed microscopically

IMM/PD=Immunogenicity/pharmacodynamics animal
TK= Toxicokinetics animal

Clinical Signs

Clinical signs for animals euthanized in poor clinical condition are listed in the mortality section above. No muS103new-related clinical signs were observed in surviving animals.

Body Weights

Unremarkable

Food Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Not conducted

Reference ID: 3655615

Hematology

Table 7: Hematology findings for 13-week mouse study

Index	Mean		Percentage deviation from Control			
	Control 0 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	Males	Females	Males	Females	Males	Females
WBC ($x10^9$)						
Main study necropsy	7.72	3.80	↓52**	↓13	↓75**	↓66**
Recovery necropsy	4.50	4.77	-	↓8	-	↓25↓
Lymphocytes ($x10^9$)						
Main study necropsy	6.00	2.72	↓74**	↓40**	↓93**	↓90**
Recovery necropsy	2.57	3.62	↑60**	↓14	-	↓38
Neutrophils ($x10^9$)						
Main study necropsy	1.50	0.87	-	↑37*	↓31*	↓24
Recovery necropsy	1.66	0.91	-	-	-	-
LUC ($x10^9$)						
Main study necropsy	0.07	0.01	↓86**	-	↓100**	↓100**
Recovery necropsy	0.03	0.04	-	-	-	-

↓= decrease ↑= increase - = no test-article related changes
* P≤0.05 ** P≤0.01

Clinical Chemistry

Unremarkable

Urinalysis

Not conducted

Gross Pathology

Table 8: Macroscopic findings for 13-week mouse study

Treatment-Related Macroscopic Findings		No. of animals affected					
		Males			Females		
Dose (mg/kg/day)		0	2	10	0	2	10
Number of animals examined		1*/12/**7**	0/12/**8**	1*/11/**8**	0/12/**8**	0/12/**8**	0/12/**8**
Organ	Finding						
Left axillary lymph node	Small	-	0/4/**0**	0/7/**4**	-	0/3/**3**	0/7/**6**
Left inguinal lymph node	Small	-	0/11/**0**	0/8/**6**	-	0/2/**2**	0/10/**5**
Right inguinal lymph node	Small	-	0/10/**0**	0/7/**2**	-	0/3/**3**	0/10/**3**

Number of animals examined and affected: Early deaths*/Main necropsy / **Recovery necropsy**
- = no test-article related changes

Organ Weights

Table 9: Spleen weights for 13-week mouse study

Group and Dose		Mean		Percentage deviation from Control			
		Control 0 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
Sex		Males	Females	Males	Females	Males	Females
Number of animals examined (Main study/**recovery**)		11/**7**	12/**8**	12/**8**	12/**8**	11/**8**	12/**8**
Spleen (Unadjusted; g)	Main study	0.082	0.087	↓45	↓33	↓37	↓29
	Recovery	0.123	0.094	↓22	-	↓20	-
Spleen (Adjusted‡; g)	Main study	0.081	0.089	↓44**	↓35**	↓37**	↓33**
	Recovery	0.104	0.093	↓10	-	↓9	-

‡ Means adjusted for body weight by conducting an analysis of covariance using terminal bodyweight as covariate
↓=decrease - = no test-article related changes
** p<0.01

Histopathology

Microscopic examination was conducted on all tissues in all animals euthanized early and all main study animals in the control and 10 mg/kg/day muS103new groups. The spleen, gut-associated lymphoid tissue (GALT), mesenteric and mandibular lymph nodes, left axillary lymph node, left and right inguinal lymph nodes, injection sites, and skin were also examined for all main study animals in the 2 mg/kg/day muS103new group and all recovery animals.

One control male (Animal # 3) that had enlarged kidneys, spleen, and lymph nodes at necropsy had widespread malignant lymphoma at histopathological examination. Data (including hematology, clinical chemistry, and organ weights) for this control animal were excluded from the calculation of the group means throughout the report based on the finding. Lymphoma was not observed in any of the other animals in the study.

The muS103new-related findings only are presented in the table below. The number of animals examined listed in the table is the total of number of animals for which histopathology was conducted for each group; however, the number of animals examined varied with each tissue.

Adequate Battery: Yes

Peer Review: No

Reference ID: 3655615

Histological Findings

Table 10: Microscopic findings for 13-week mouse study

Treatment-Related Microscopic Findings			No. of animals affected					
			Males			Females		
Dose (mg/kg/day)			0	2	10	0	2	10
Number of animals examined			1*/12/7	0/12/8	1*/11/8	0/12/8	0/12/8	0/12/8
Organ	Finding							
Left axillary lymph node	Decreased cellularity, cortex/paracortex	Total	-	0/4/0	0/8/1	-	0/8/0	0/10/0
		Minimal	-	0/3/0	0/7/0	-	0/5/0	0/8/0
		Slight	-	0/1/0	0/1/1	-	0/3/0	0/2/0
	Decreased germinal center development	Total	-	0/10/1	0/10/5	-	0/10/0	0/11/0
		Minimal	-	0/0/1	-	-	-	-
		Slight	-	0/3/0	0/0/4	-	-	-
		Moderate	-	0/2/0	-	-	0/2/0	-
		Marked	-	0/5/0	0/10/1	-	0/8/0	0/11/0
Left inguinal lymph node	Decreased cellularity, cortex/paracortex	Total	-	0/8/1	1*/10/5	-	0/4/1	0/11/1
		Minimal	-	0/8/1	0/10/5	-	0/4/1	0/10/1
		Slight	-	-	1*/0/0	-	-	0/1/0
	Decreased germinal center development	Total	-	0/8/1	1*/10/4	-	0/11/0	0/9/2
		Minimal	-	0/0/1	0/0/4	-		0/0/1
		Moderate	-	-	-	-	0/2/0	0/0/1
		Marked	-	0/8/0	1*/10/0	-	0/9/0	0/9/0
Right inguinal lymph node	Decreased cellularity, cortex/paracortex	Total	-	0/8/1	1*/9/0	-	0/5/0	0/10/1
		Minimal	-	0/8/1	1*/9/0	-	0/5/0	0/7/1
		Slight	-	-	-	-	-	0/3/0
	Decreased germinal center development	Total	-	0/9/0	1*/9/2	-	0/9/0	0/12/2
		Minimal	-	-	0/0/2	-	-	0/0/1
		Slight	-	-	-	-	-	0/0/1
		Moderate	-	-	-	-	0/2/0	-
		Marked	-	0/9/0	1*/9/0	-	0/7/0	0/12/0
Mandibular lymph node	Decreased cellularity, cortex/paracortex	Total	-	0/8/1	1*/10/3	-	0/8/0	0/12/4
		Minimal	-	0/5/1	0/0/3	-	0/2/0	0/0/4
		Slight	-	0/3/0	1*/10/0	-	0/6/0	0/12/0
	Decreased germinal center development	Total	-	0/9/2	1*/10/4	-	0/11/0	0/12/3
		Slight	-	0/0/1	0/0/3	-	-	0/0/1
		Moderate	-	-	0/0/1	-	0/1/0	0/0/2
		Marked	-	0/9/1	1*/10/0	-	0/10/0	0/12/0
Mesenteric lymph node	Decreased cellularity, cortex/paracortex	Total	-	0/9/0	1*/10/3	-	0/9/1	0/12/4
		Minimal	-	0/7/0	0/0/3	-	0/6/1	0/1/4
		Slight	-	0/2/0	1*/10/0	-	0/3/0	0/11/0
	Decreased germinal center development	Total	-	0/12/3	1*/10/6	-	0/12/0	0/12/1
		Minimal	-	0/0/3	0/0/2	-	-	0/0/1
		Slight	-	-	0/0/2	-	-	-
		Moderate	-	0/6/0	0/0/2	-	0/2/0	-
		Marked	-	0/6/0	1*/10/0	-	0/10/0	0/12/0
Peyer's patches (GALT)	Decreased cellularity	Total	-	0/5/1	1*/8/0	-	0/6/0	0/9/0
		Minimal	-	0/4/1	1*/8/0	-	0/4/0	0/7/0
		Slight	-	0/1/0	-	-	0/2/0	0/2/0
	Decreased germinal center development	Total	-	0/6/0	1*/6/0	-	0/6/0	0/8/0
		Minimal	-	0/1/0	-	-	-	-
		Slight	-	0/1/0	-	-	-	-
		Moderate	-	-	-	-	0/2/0	-
		Marked	-	0/4/0	1*/6/0	-	0/4/0	0/8/0
Skin	Subcutis/panniculus inflammation	Total	1*/3/0	0/6/0	1*/8/1	0/3/0	0/7/0	0/5/1
		Minimal	1*/2/0	0/6/0	0/7/1	0/3/0	0/6/0	0/5/1
		Slight	0/1/0	-	1*/1/0	-	0/1/0	-
Spleen	Decreased cellularity,	Total	-	0/10/0	1*/11/0	-	0/7/0	0/12/0

Treatment-Related Microscopic Findings			No. of animals affected					
			Males			Females		
Dose (mg/kg/day)			0	2	10	0	2	10
Number of animals examined			1*/12/**7**	0/12/**8**	1*/11/**8**	0/12/**8**	0/12/**8**	0/12/**8**
Organ	Finding							
	white pulp	Minimal	-	0/6/**0**	-	-	0/5/**0**	-
		Slight	-	0/4/**0**	0/10/**0**	-	0/2/**0**	0/10/**0**
		Moderate	-	-	1*/1/	-	-	0/2/
	Extramedullary hemopoiesis	Total	0/0/**1**	0/3/**1**	0/5/**3**	-	0/8/**1**	0/6/**0**
Treated site 1	Dermal inflammation	Total	-	0/3/**0**	0/4/**0**	0/3/**0**	0/6/**0**	0/6/**0**
		Minimal	-	0/3/**0**	0/2/**0**	0/2/**0**	0/4/**0**	0/3/**0**
		Slight	-	-	0/2/**0**	0/1/**0**	0/2/**0**	0/3/**0**
	Subcutis/panniculus inflammation	Total	0/2/**0**	0/8/**1**	1*/8/**0**	0/8/**0**	0/11/**0**	0/11/**0**
		Minimal	0/2/**0**	0/6/**1**	1*/6/**0**	0/8/**0**	0/5/**0**	0/5/**0**
		Slight	-	0/2/**0**	0/2/**0**	-	0/6/**0**	0/6/**0**
Treated site 2	Dermal inflammation	Total	0/3/**0**	0/3/**0**	1*/4/**0**	0/1/**0**	0/3/**0**	0/3/**0**
		Minimal	0/3/**0**	0/3/**0**	1*/3/**0**	0/1/**0**	0/2/**0**	-
		Slight	-	-	0/1/**0**	-	0/1/**0**	0/3/**0**
	Subcutis/panniculus inflammation	Total	1*/4/**0**	0/9/**0**	1*/9/**0**	0/6/**0**	0/6/**0**	0/6/**0**
		Minimal	1*/4/**0**	0/9/**0**	1*/7/**0**	0/5/**0**	0/4/**0**	0/5/**0**
		Slight	-	-	0/2/**0**	0/1/**0**	0/2/**0**	0/1/**0**

Number of animals examined and affected: Early deaths*/Main necropsy / **Recovery necropsy**
- = no test-article related changes
GALT= Gut-associated lymphoid tissue

Special Evaluation

Immunogenicity

- Anti-muS103new antibodies were observed on Day 121 in one serum sample from one animal treated with 10 mg/kg/day (Animal # 140) with a titer value of 729 in the confirmation assay.
- No anti-muS103new antibodies were observed in serum samples from animals in the control or 2 mg/kg/day muS103new groups or other animals in the 10 mg/kg/day muS103new group on Days 43, 71, 92, and 121.

Immunophenotyping

The muS103new-related changes in lymphocyte populations were measured in the blood, spleen, and lymph node.

- muS103new produced large decreases in both B and T lymphocytes and the $CD4^+$ and $CD8^+$ T lymphocyte subsets with similar patterns in the blood, spleen, and lymph node. Decreases in other lymphocytes (NK and NKT cells) were also observed.
- The decreases in B cells were marked at both dose levels (2 and 10 mg/kg/day) and the decreases in the T cell populations were generally greater at the 10 mg/kg/day dose.

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Figure 21: Total B and T lymphocytes in peripheral blood in 13-week mouse study

Index	Mean		Percentage deviation from Control			
	Control 0 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	Males	Females	Males	Females	Males	Females
Total T lymphocytes (cells/µL)						
Day 43	1247	1559	↓67	↓58	↓97	↓96
Day 71	1794	2508	↓44	↓45	↓97	↓95
Day 92	1686	1516	↓51	↓17	↓94	↓94
Day 120 (recovery)	1665	2015	↓60	↓28	↓70	↓65
Total B lymphocytes (cells/µL)						
Day 43	1778	1829	↓99	↓99	↓99	↓99
Day 71	2733	2643	↓99	↓99	↓99	↓99
Day 92	2927	2187	↓99	↓99	↓99	↓99
Day 120 (recovery)	3042	2841	↓65	↓70	↓58	↓71

↓= decrease

Table 11: $CD4^+$ and $CD8^+$ T lymphocytes in peripheral blood, spleen, and lymph node in 13-week mouse study

Index	Mean		Percentage deviation from Control			
	Control 0 mg/kg/day		2 mg/kg/day		10 mg/kg/day	
	Males	Females	Males	Females	Males	Females
Blood						
$CD4^+$ (cells/µL)						
Day 43	919	1368	↓56	↓61	↓95	↓95
Day 71	1343	1660	↓18	↓40	↓95	↓94
Day 92	1563	1653	↓45	↓7	↓94	↓95
Day 120 (recovery)	1532	1960	↓44	↓11	↓67	↓63
$CD8^+$ (cells/µL)						
Day 43	316	461	↓83	↓84	↓98	↓98
Day 71	488	503	↓73	↓84	↓99	↓98
Day 92	579	533	↓88	↓75	↓98	↓98
Day 120 (recovery)	553	645	↓75	↓69	↓80	↓80
Spleen						
$CD4^+$ (cells/spleen x 10^6)						
Day 72	4.36	8.71	↓55	-	↓92	↓78
Day 93	5.89	9.38	-	↓55	↓87	↓95
Day 121 (recovery)	3.22	8.31	-	-	↓33	↓30
$CD8^+$ (cells/spleen x 10^6)						
Day 72	1.63	3.31	↓75	↓51	↓95	↓89
Day 93	2.30	3.64	↓63	↓82	↓93	↓98
Day 121 (recovery)	1.16	2.82	↓44	↓29	↓51	↓46
Lymph node						
$CD4^+$ (cells/g x 10^6)						
Day 72	41.31	57.14	↓37	-	↓98	↓87
Day 93	42.62	49.77	↓70	-	↓98	↓99
Day 121 (recovery)	29.01	44.62	-	-	↓30	↓13
$CD8^+$ (cells/g x 10^6)						
Day 72	13.03	16.9	↓61	-	↓88	↓80
Day 93	15.46	17.86	↓87	↓54	↓96	↓98
Day 121 (recovery)	9.92	15.04	↓54	↓42	↓54	↓38

↓= decrease - = no test-article related changes

Toxicokinetics

Table 12: Toxicokinetics of muS103new for 13-week mouse study

Dose level	Sex	C_{max} (ng/mL)	T_{max} (hours)	AUC_{0-8} (ng·h/mL)	AUC_{0-12} (ng·h/mL)	$t_{1/2}$ (hour)
Day 1						
1 mg/kg/dose (2 mg/kg/day)	Male	388	2	1340	-	1.3
	Female	378	2	1180	-	1.5
	Combined	383	2	1260	-	-
5 mg/kg/dose (10 mg/kg/day)	Male	2760	2	10900	-	1.4
	Female	2400	1	10400	-	-
	Combined	2580	-	10700	-	-
Day 92						
1 mg/kg/dose (2 mg/kg/day)	Male	304	2	1600	1760	-
	Female	398	2	1700	1990	-
	Combined	351	2	1650	1875	-
5 mg/kg/dose (10 mg/kg/day)	Male	2770	2	12600	13800	2.5
	Female	3010	2	13200	14100	2.1
	Combined	2890	2	12900	13950	-

- = Data not available

Dose level	Sex	C_{max} ratio	AUC ratio	Accumulation ratio
Day 1				
1 mg/kg/dose (2 mg/kg/day)	Male	1	1	NA
	Female	1	1	NA
5 mg/kg/dose (10 mg/kg/day)	Male	7.1	8.1	NA
	Female	6.3	8.8	NA
Day 92				
1 mg/kg/dose (2 mg/kg/day)	Male	1	1	1.2
	Female	1	1	1.4
5 mg/kg/dose (10 mg/kg/day)	Male	9.1	7.8	1.2
	Female	7.6	7.1	1.3

NA=Not applicable
AUC ratio calculated for AUC_{0-8} for Day 1 and AUC_{0-12} for Day 92
Accumulation ratios based on AUC_{0-8}

Dosing Solution Analysis

The relative bioactivity of the formulated muS103new material for the 1 and 5 mg/kg/dose muS103new doses was within specifications; therefore, the muS103new formulations administered to the animals were bioactive and had the expected concentrations.

Histopathology inventory

Study	Mouse
Species	103-PCD-0082
Adrenals	X*
Aorta	X
Bone Marrow smear	X
Bone	X
Brain	X*

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Study	Mouse
Species	103-PCD-0082
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymides	X*
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	X
Heart	X*
Ileum	X
Injection site	X
Jejunum	X
Kidneys	X*
Lacrimal gland	X
Larynx	X
Liver	X*
Lungs	X*
Lymph nodes, axillary	X
Lymph nodes, inguinal	X
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	X
Ovaries	X*
Pancreas	X
Parathyroid	X
Peripheral nerve	
Pharynx	
Pituitary	X
Prostate	X*
Rectum	X
Salivary gland	X*
Sciatic nerve	X
Seminal vesicles	X*

Reference ID: 3655615

Study	Mouse
Species	103-PCD-0082
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Sternum	X
Stomach	X
Teeth	
Testes	X*
Thymus	X*
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X*
Vagina	X
Zymbal gland	

X, histopathology performed
*, organ weight obtained

Study title: Multiple dose study for the bispecific single chain monoclonal antibody MT103 (CD19XCD3) in chimpanzees

Study no.: 103-PCD-0015 (MT103-PE-006)
Study report location: eCTD 4.2.3.2.
Conducting laboratory and location: (b) (4)
Date of study initiation: May 23, 2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MT103, lot # ChB103010130-2, Purity: Contained 92.5% monomeric and 7.5% dimeric product

Key Study Findings

- Blinatumomab-related effects included increases in body temperature and heart rate and decreases in blood pressure following infusion of blinatumomab.
- Infusion with blinatumomab decreased lymphocyte levels including B cells (CD19 and CD20) and T cells ($CD3^+/CD4^+$ and $CD3^+/CD8^+$).
- Infusion with blinatumomab increased the expression/levels of T cell activation markers sCD25, CD69, and HLA-DR and cytokines IL-2, IL-6, and INFγ.

Methods

Doses:	0 or 0.1 µg/kg
Frequency of dosing:	Once weekly for 5 weeks on Days 0, 7, 14, 21, and 28
Route of administration:	Intravenous infusion (2-hour, 25 mL per hour; under anesthesia)
Dose volume:	50 mL total volume, followed by an extra 10 mL of 5% HSA to flush the extension set
Formulation/Vehicle:	Isotonic phosphate buffer solution containing: 0.1% human serum albumin (HSA), sodium chloride, disodium monohydrogen phosphate, potassium dihydrogen phosphate, potassium chloride, and water for injection; diluent was 5% HSA
Species/Strain:	Chimpanzee (*Pan troglodytes*)
Number/Sex/Group:	1 female control (Animal 4X0330); 1 male (Animal 4X0352) and 1 female (Animal 4X0360) at 0.1 µg/kg
Age:	10-13 years old
Weight:	45.6 to 63.2 kg at time of baseline sampling
Satellite groups:	None

Observations and times:

Mortality:	Daily
Clinical signs:	Twice daily
Body weights:	Days -5, -3 (-6 and -4 for Animal 4x0360), 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 42, 50, and 56
Physical examinations:	Days -5, -3 (-6 and -4 for Animal 4x0360), 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 42, 50, and 56
Food consumption:	Twice daily; “semi-quantitative” (0%, 50%, or 100%)
Vital signs (Body temperature, blood pressure, ECG and heart rate):	Days -5 and -3, dosing Days 0, 7, 14, 21, and 28 (pre-dose, end of infusion, and 1, 2, 4, and 8 hours after infusion), and Days 35, 42, 50, and 56 Body temperature, mean blood pressure and heart rate measured every minute and reported at 5 minute intervals during 2-hour drug infusion and for 2 hours after infusion
Ophthalmoscopy:	Days -5 (-6 for Animal 4x0360), 28, 35, and 56
Hematology:	Days -5, -3, 0, 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 42, 50, and 56 At pre-dose and 8 hours after infusion on dosing Days 0, 7, 14, 21, and 28
Clinical chemistry:	Days -5, -3, 0, 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 42, 50, and 56

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	At pre-dose and 8 hours after infusion on dosing Days 0, 7, 14, 21, and 28
Immunophenotyping:	Days -5, -3, 0, 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 42, 50, and 56 At pre-dose and 8 hours after infusion on dosing Days 0, 7, 14, 21, and 28
Coagulation:	Days -5, -3, 0, 1, 7, 8, 14, 15, 21, 22, 28, 29, 35, 42, 50, and 56
Urinalysis:	Days -5, -3, 0, 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, 35, 42, 50, and 56
Injection site observations:	Days -3 (-4 for Animal 4x0360), 0, 1, 3, 7, 8, 10, 14, 15, 17, 21, 22, 24, 28, 29, 31, and 35
Lymph node biopsy samples:	Days -5 (-6 for Animal 4x0360) and 28 (at end of the last infusion)
Gross pathology:	Not conducted
Organ weights:	Not conducted
Histopathology:	Not conducted
Toxicokinetics:	Days 0, 1, 7, 14, 21, 28, 29, 31, 35, and 42; MT103-treated animals only Pre-dose, end of infusion, and 1, 2, 4, and 8 hours after infusion on Day 0 Pre-dose and end of infusion on Days 7, 14, 21, and 28
Cytokine analysis:	Days 0, 1, 7, 8,14, 15, 21, 22, 28, and 29 Pre-dose, end of infusion, and 1, 2, 4, and 8 hours after infusion on dosing Days 0, 7, 14, 21, and 28

Results

Mortality

None

Clinical Signs and physical examination

- A swollen right ear was observed in the male treated with MT103 (Animal 4X0352) on Day 6 and he was examined on Day 7. The ear canal was very inflamed with pus in the canal and the diagnosis was an infection in the right ear canal. The ear was flushed with warm saline and the chimpanzee was placed on parenteral antibiotics; the infection was resolving within 7 days.

Body Weights

Unremarkable

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Food Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

Vital signs (Body temperature, blood pressure, ECG and heart rate):

No MT103-related effects were observed for ECG measurements and no MT103-related changes in vital signs were observed on non-infusion days. MT103-related effects on body temperature, blood pressure, and heart rate were observed on infusion days.

- **Body temperature:** Body temperature was slightly increased in MT103-treated animals.
  - In the male treated with MT103 (Animal 4X0352), body temperature gradually increased by 0.5-2°F over the 4 hour monitoring period for the first, second, third, and fifth infusions with the largest change following the first infusion.
  - In the female treated with MT103 (Animal 4X0360), body temperature increased 0.9-2.8°F over the 4 hour monitoring period for the first, third, fourth, and fifth infusions with the largest change following the first infusion.
- **Blood pressure:** MT103 induced a decreasing trend in blood pressure during and immediately after MT103 infusion.
  - In the male treated with MT103 (Animal 4X0352), blood pressure dropped from 91-51 mm Hg during the fifth infusion, which contrasts with a range of 80-90 mm Hg observed during the first four infusions.
  - In the female treated with MT103 (Animal 4X0360), there was a general decreasing trend for mean blood pressure that was the most pronounced during the fourth infusion. For all infusions mean blood pressure values increased to approximately 100 mm Hg during the first hour of the infusion and then decreased to approximately 60 mm Hg during the monitoring period. At the end of the fourth infusion mean blood pressure dropped to 40 mm Hg for more than 5 minutes.
- **Heart rate:** Increases in heart rate were more pronounced in MT103-treated animals than in the control animal.
  - In the male treated with MT103 (Animal 4X0352), heart rate increased about 8-33 bpm for all infusions.
  - In the female treated with MT103 (Animal 4X0360), heart rate increased 3-42 bpm over the four hour monitoring period for all infusions.

Hematology and coagulation

- Overall, hematology was unremarkable.
- Lymphocytes were decreased compared to baseline in both the control animal and the MT103-treated animals throughout the study.

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- In the control animal, a large decrease in lymphocytes was observed 8 hours after the infusion compared to the pre-dose measurement for the first infusion. During the study there were sustained decreases in lymphocytes compared to baseline with values lower than the normal range.
- In MT103-treated animals, large decreases in lymphocytes were observed 8 hours after the infusion compared to the pre-dose measurements for all 5 infusions; the levels of lymphocytes during these decreases were lower than those of the control animal at the same time point. In general, lymphocyte levels did increase to levels similar to baseline between infusions in the MT103-treated animals.

Table 13: Lymphocyte counts in 5-dose chimpanzee study

Day and time		Lymphocytes (1000/mm^3)		
			MT103-treated animals	
		Control animal	Male	Female
Day -5 (baseline)		4.3	4.2	3.7
Day -3 (baseline)		5.4	5.3	5.1
Day 0 (infusion 1)	Pre-dose	3.5	3.9	4.7
	8-hours post-dose	0.9	0.4	0.2
Day 7 (infusion 2)	Pre-dose	1.5	2.6	5.5
	8-hours post-dose	2.1	0.4	0.2
Day 14 (infusion 3)	Pre-dose	3.2	2.5	4.5
	8-hours post-dose	3.5	0.3	2.8
Day 21 (infusion 4)	Pre-dose	2.5	3.0	5.2
	8-hours post-dose	2.2	0.3	0.4
Day 28 (infusion 5)	Pre-dose	2.3	3.4	5.9
	8-hours post-dose	2.1	0.3	0.4

Clinical Chemistry

- Total bilirubin values were increased approximately 1.4-fold at 8 hours after infusion compared to the pre-dose measurement in the control animal and the male treated with MT103 (Animal 4X0352). In the female treated with MT103 (Animal 4X0360), the increase in total bilirubin at 8 hours after infusion was 2-3 fold for infusions 2, 3, and 4.
- The levels of the acute phase protein C-reactive protein (CRP) were elevated 24 hours after each infusion with values higher in the MT103-treated animals (2.2-15.1 mg/dL) compared to the control animal (3-8.5 mg/dL).

Urinalysis

Unremarkable

Gross Pathology

Not conducted

Organ Weights

Not conducted

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Histopathology

Not conducted

Special Evaluation

Injection Site Observations

Unremarkable

Lymph Node Biopsy Samples

Half of each of the lymph node biopsy sample was evaluated by histopathology and the other half was evaluated by immunohistopathology.

- There were no histopathology findings.
- There were no alterations in $CD3^+$, $CD4^+$, $CD10^+$, $CD19^+$, $CD20^+$, or $CD69^+$ cell populations between pre- and post-dose lymph node biopsies for the control or MT103-treated animals. Normal $CD19^+$ cells do not appear to be affected by MT103 in the lymph nodes.
- CD8 and CD25 antigens were not detected in the lymph nodes of the control or MT103-treated animals.

Immunophenotyping

T cell activation markers

- **CD69:** Expression of the early T cell activation marker CD69 was increased in the MT103-treated animals following each infusion of MT103. The CD69 expression peaked 8 hours after the infusion with increases 3 to 19-fold of the pre-dose values.
- **HLA-DR:** HLA-DR was another activation marker that was increased with MT103 treatment. Expression of HLA-DR was evaluated separately for $CD4^+$ and $CD8^+$ lymphocyte subpopulations.
    - For the $CD4^+$ lymphocyte population, HLA-DR expression was increased in the female treated with MT103 (Animal 4X0360) following the first, second, third, and fifth infusions. HLA-DR levels peaked at 24 hours after the first and second infusions (~2-fold of pre-dose values) and peaked at 8 hours after the third and fifth infusions (9 to 26-fold of pre-dose values). Only slight increases in HLA-DR expression on $CD4^+$ cells were observed in the male treated with MT103 (Animal 4X0352).
    - For the $CD8^+$ lymphocyte population, HLA-DR expression was increased in the female treated with MT103 (Animal 4X0360) following the first through fourth infusions with levels peaked at 8 hours after the infusion (3 to 7-fold of pre-dose values). In the male treated with MT103 (Animal 4X0352), HLA-DR expression was increased on $CD8^+$ cells following the first infusion with peak levels at 8 and 24 hours after the infusion (5-fold of pre-dose).

Depletion of B lymphocyte cells

B cells were depleted in the MT103-treated animals following infusion with MT103.

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- **CD19:**

Figure 22: CD19 counts in male chimpanzee treated with MT103
(excerpted from Applicant's submission)

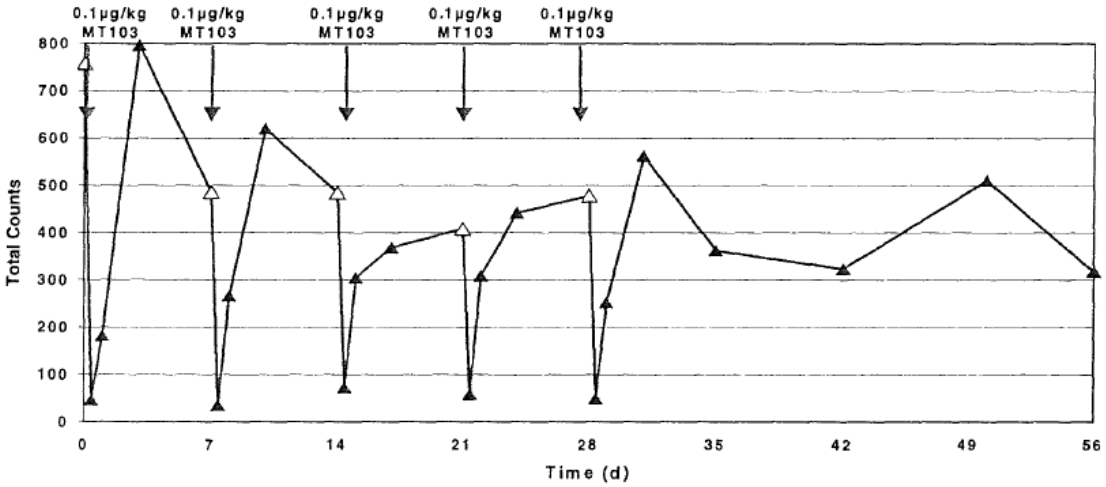
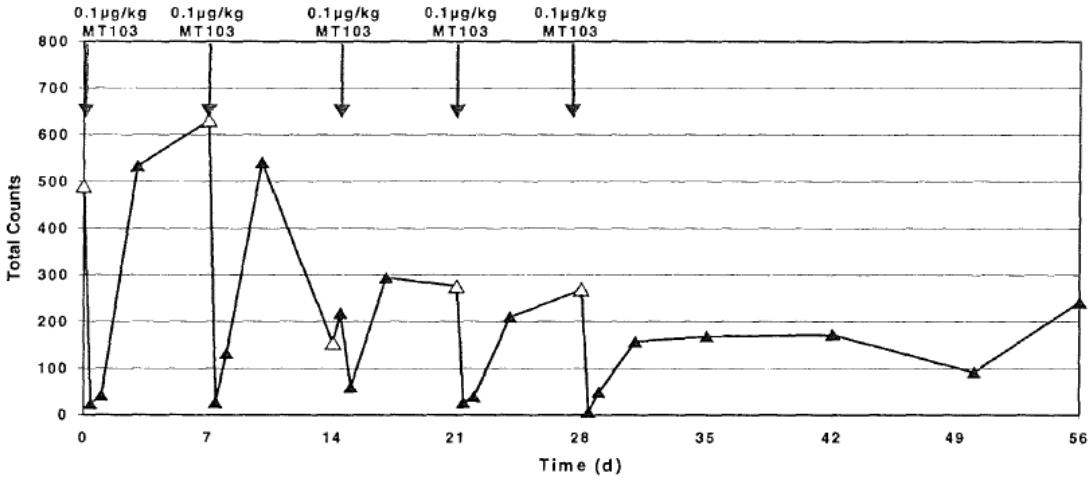


Figure 23: CD19 counts in female chimpanzee treated with MT103
(excerpted from Applicant's submission)



- **CD20:**

Figure 24: CD20 counts in male chimpanzee treated with MT103
(excerpted from Applicant's submission)

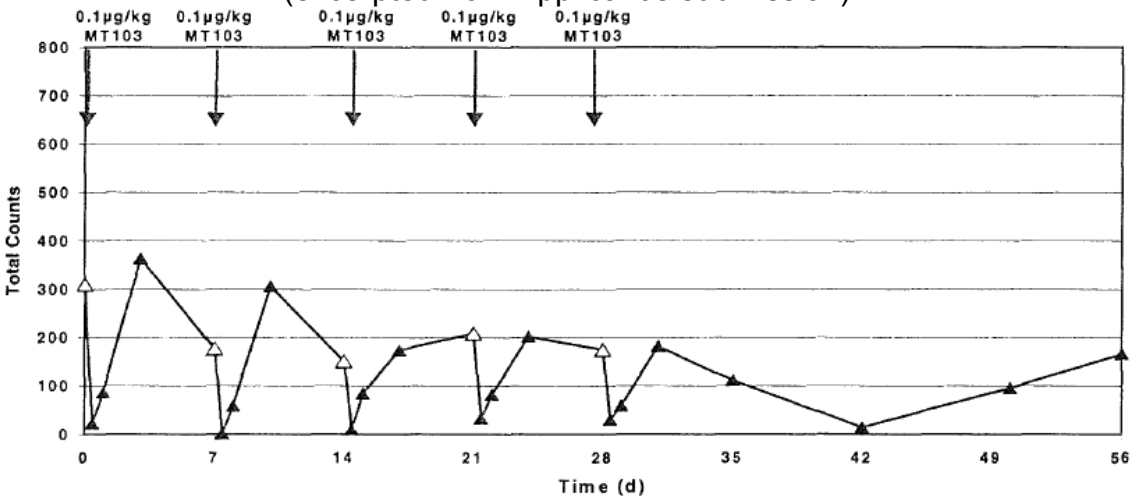
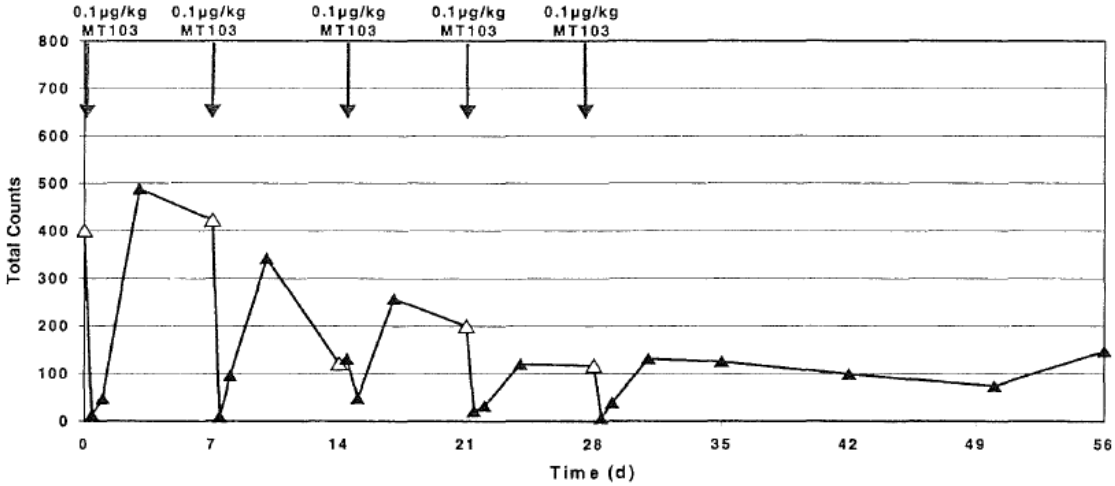


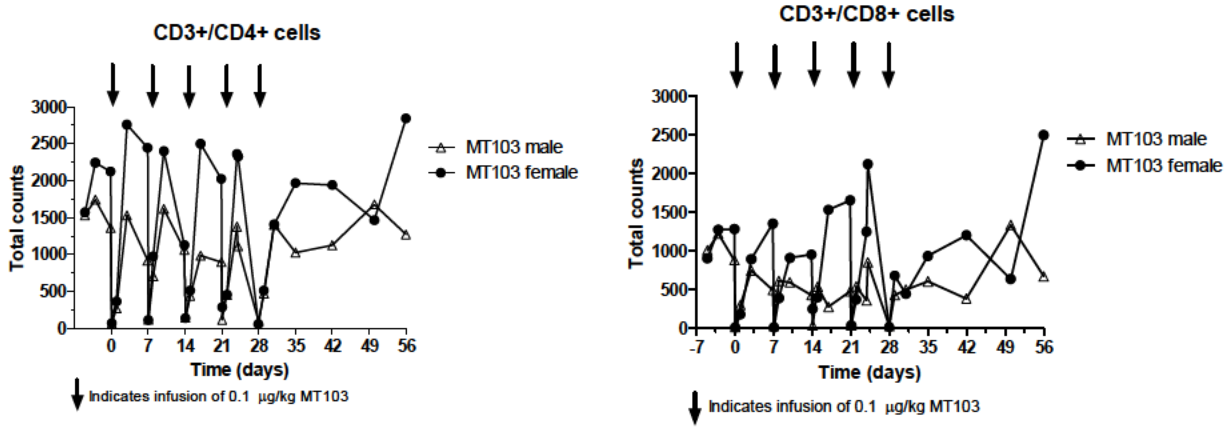
Figure 25: CD20 counts in female chimpanzee treated with MT103
(excerpted from Applicant's submission)



T lymphocyte cells

T cells including $CD3^+/CD4^+$ and $CD3^+/CD8^+$ were also depleted in the MT103-treated animals following infusion with MT103.

Figure 26: CD3$^+$/CD4$^+$ and CD3$^+$/CD8$^+$ cells in chimpanzees treated with MT103



Cytokine Analysis

- **IL-6:** IL-6 levels were increased in MT103-treated animals after each infusion. The highest levels for both animals (approximately 1400 pg/mL) were detected after the first infusion of MT103.

Figure 27: IL-6 levels in male chimpanzee treated with MT103
(excerpted from Applicant's submission)

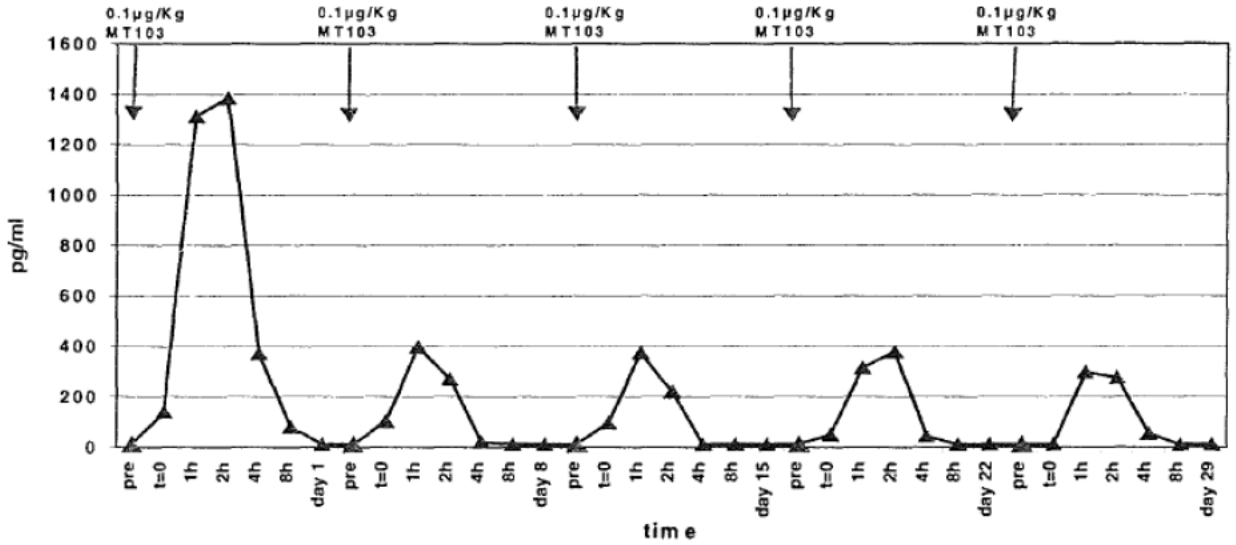
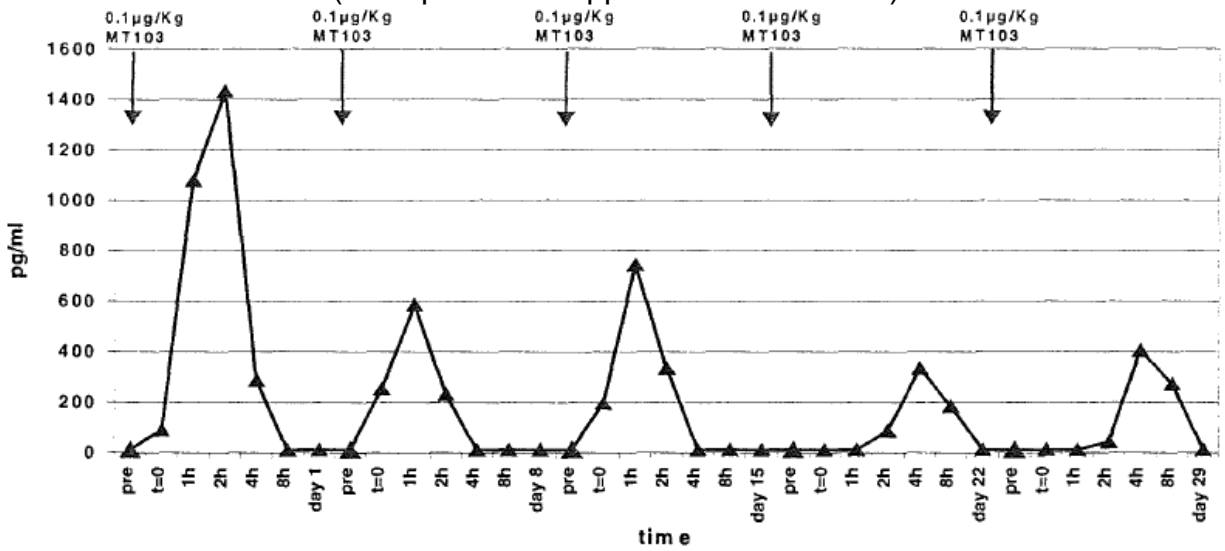


Figure 28: IL-6 levels in female chimpanzee treated with MT103
(excerpted from Applicant's submission)



- **IL-2:** IL-2 levels were increased in MT103-treated animals.
  - In the male treated with MT103 (Animal 4X0352), the highest IL-2 levels (220 pg/mL) were observed after the first infusion and lower IL-2 levels induced by MT103 (36-41 pg/mL) were observed following infusions 2, 3, and 4.
  - In the female treated with MT103 (Animal 4X0360), MT103 induced increases in IL-2 following each infusion. An IL-2 level of 443 pg/mL was observed at the pre-dose measurement for the third infusion and an IL-2 level of 346 pg/mL was observed during the fourth infusion.

Figure 29: IL-2 levels in male chimpanzee treated with MT103
(excerpted from Applicant's submission)

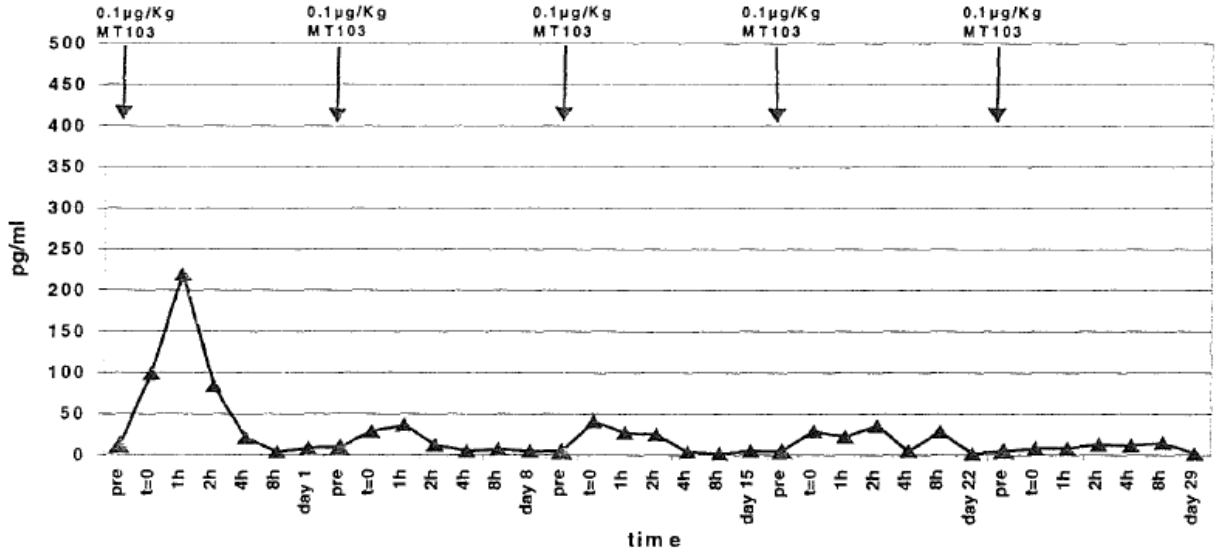
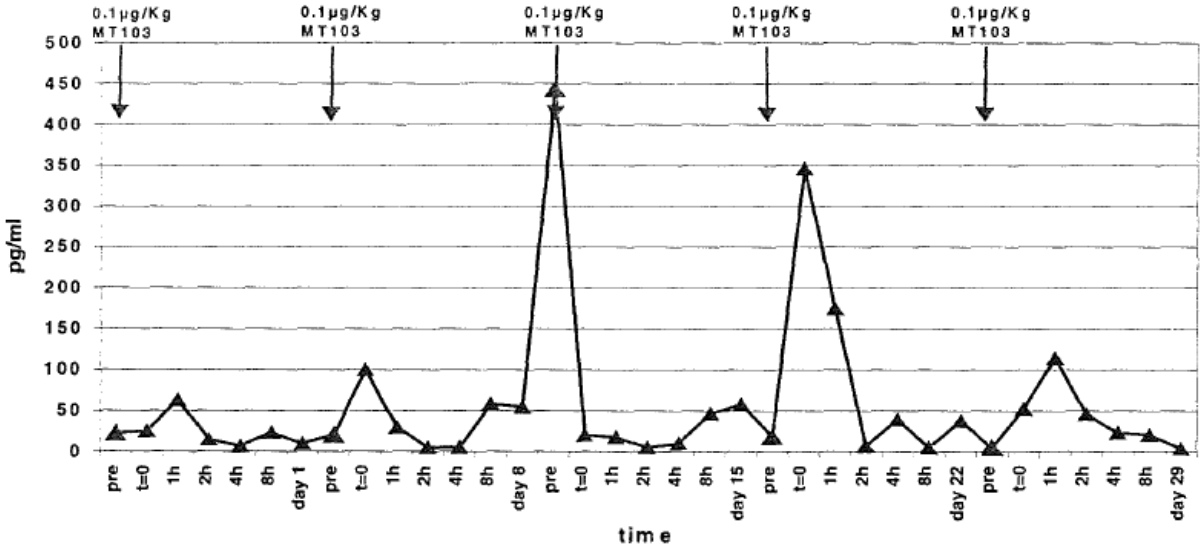


Figure 30: IL-2 levels in female chimpanzee treated with MT103
(excerpted from Applicant's submission)



- **INFγ:** INFγ levels were increased in MT103-treated animals with the highest levels for both animals (31 and 33 IU/mL) detected after the first infusion of MT103. In the female treated with MT103 (Animal 4X0360), MT103 induced increases in INFγ following each infusion.

Figure 31: INFγ levels in male chimpanzee treated with MT103
(excerpted from Applicant's submission)

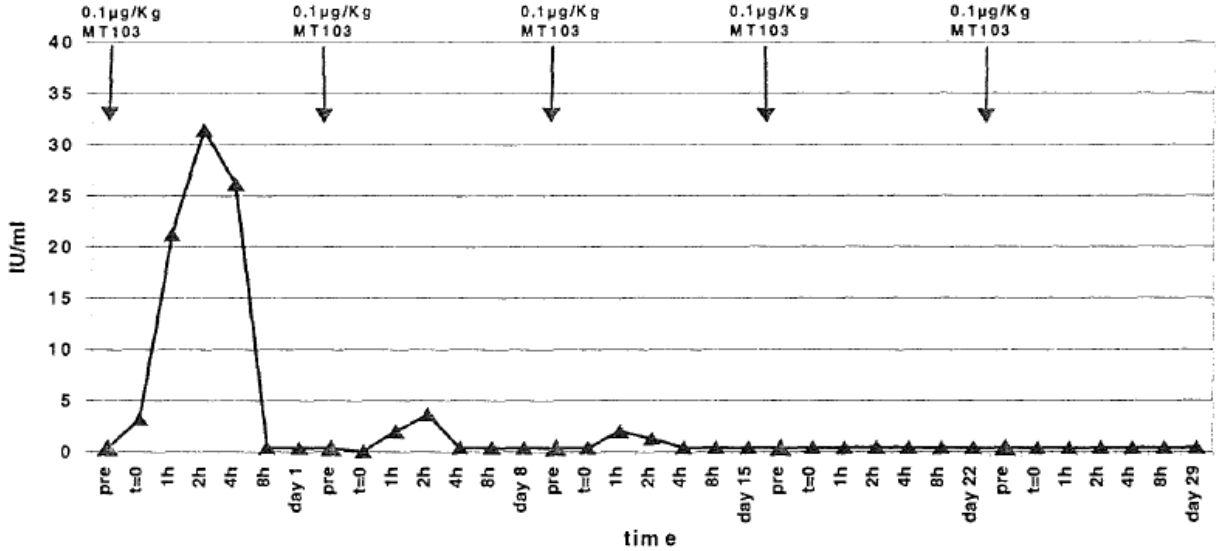
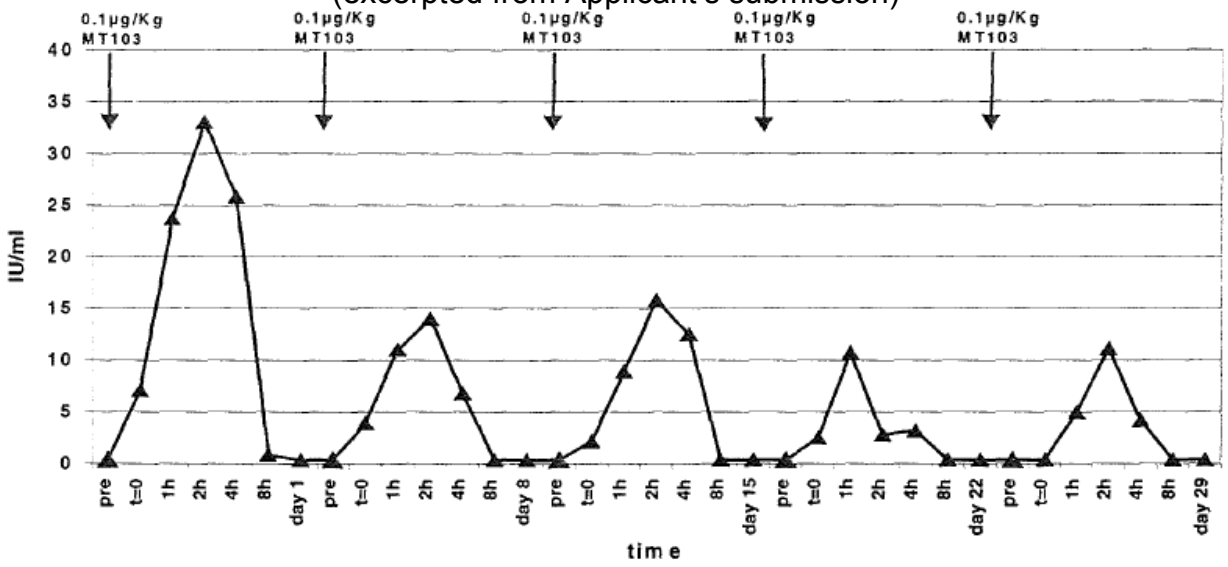


Figure 32: INFγ levels in female chimpanzee treated with MT103
(excerpted from Applicant's submission)



- **sCD25:** Levels of the late T cell activation marker sCD25 were also increased in MT103-treated animals.

Figure 33: sCD25 levels in male chimpanzee treated with MT103
(excerpted from Applicant's submission)

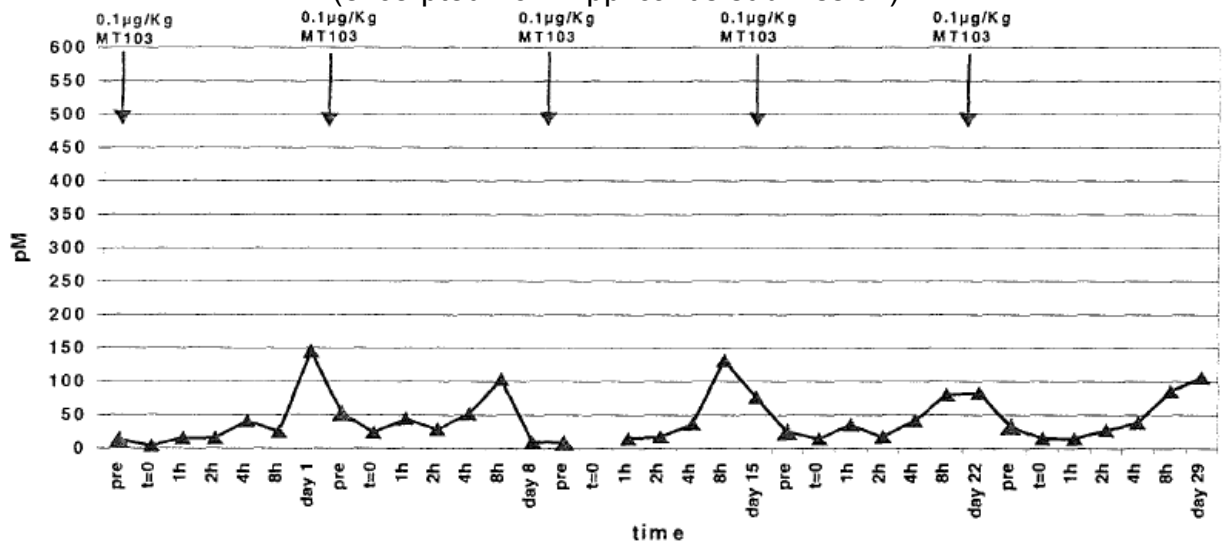
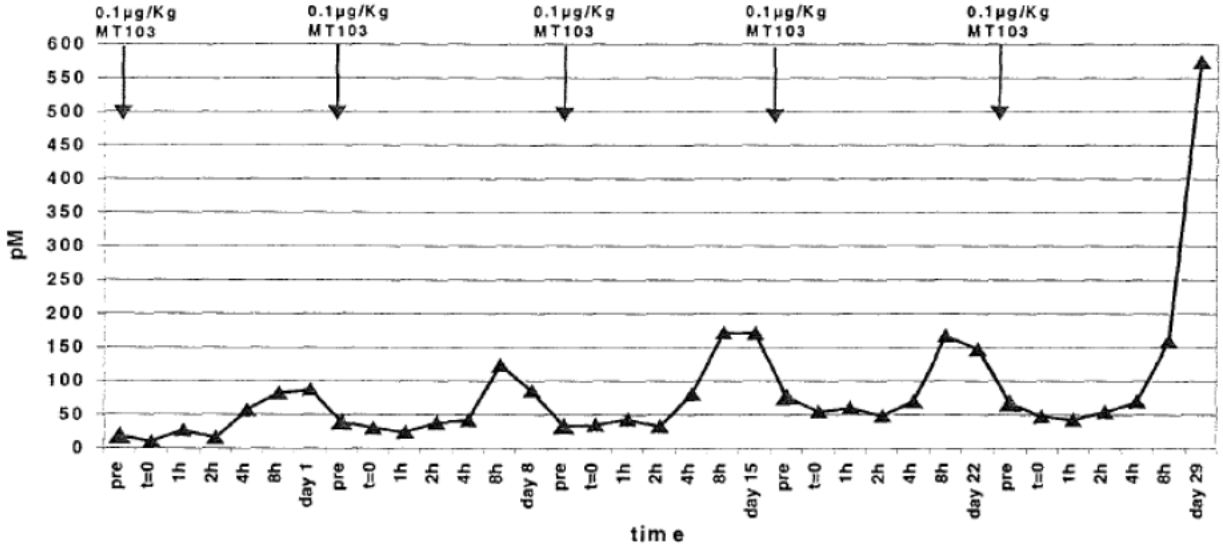


Figure 34: sCD25 levels in female chimpanzee treated with MT103
(excerpted from Applicant's submission)



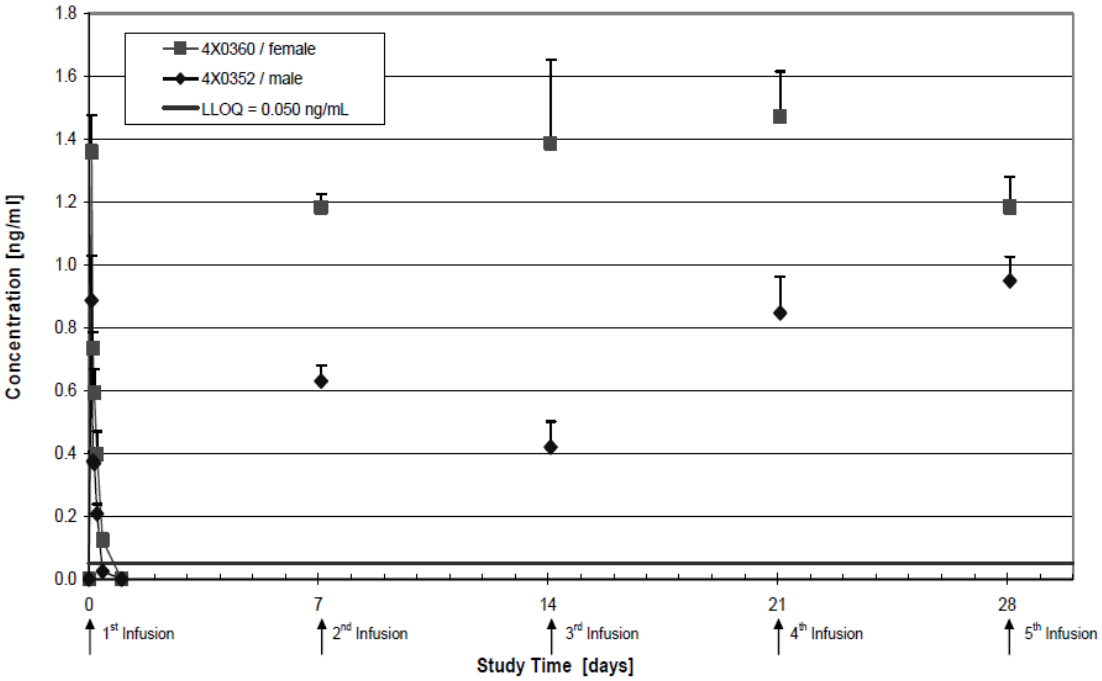
Toxicokinetics

These results were presented in a separate report (Study # 103-PCD-0024), which was submitted as a pharmacokinetic absorption study under Module 4.2.2.2.

Table 14: Toxicokinetics MT103 in chimpanzees following the first infusion of MT103 on Day 0

Animal # (sex)	Dose µg/kg	T_{max} hours	C_{max} ng/mL	$t_{1/2}$ hours	AUC_{last} hr*ng/mL	AUC_{INF} hr*ng/mL	AUC_{INF}/D hr*kg*ng/mL/µg	Vz mL/kg	Cl mL/hr/kg
4X0352 (male)	0.1	2.0	0.887	1.506	2.933	2.987	29.87	72.7	33.47
4X0360 (female)	0.1	2.0	1.360	2.628	5.109	5.582	55.82	67.9	17.91

Figure 35: Concentrations of MT103 in chimpanzees following each of five infusions of MT103



7 Genetic Toxicology

No genetic toxicology studies were submitted.

8 Carcinogenicity

No carcinogenicity studies were submitted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

No fertility and early embryonic development studies were submitted.

9.2 Embryonic Fetal Development

Study title: Preliminary study for effects on embryo-fetal development by intravenous route (bolus) in mice

Study no.:	103-PCD-0079
Study report location:	eCTD 4.2.3.5.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 22, 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	muS103new, lot # 100910MMa07, Purity: 98.8% monomer

Key Study Findings

- The mean fetal serum concentration of muS103new was 10.8 ng/ml (~20 nM).

Methods

Doses:	0 and 5 mg/kg (Groups 1 and 2)
Frequency of dosing:	Daily from Day 6 to Day 15 post-mating
Dose volume:	5 mL/kg
Route of administration:	Intravenous bolus injection
Formulation/Vehicle:	10 mM citric acid monohydrate, 75 mM L-lysine hydrochloride, 4% (w/v) Trehalose, 0.03% (w/v) Tween 80, pH 7.0
Species/Strain:	Swiss RjOrl
Number/Sex/Group:	10 females/group (main study) 12 females/group (immunophenotyping)
Satellite groups:	24 females in Group 2 (TK analysis)
Study design:	Pregnant mice received 0 or 5 mg/kg/day IV muS103new from Day 6 to Day 15 post-mating. Five TK satellite animals received muS103new from Day 6 until the end of gestation on Day 18 to evaluate muS103new in maternal and fetal serum. Necropsy occurred on Day 18 post-mating.
Deviation from study protocol:	Deviations from the study protocol did not impact the study design or the interpretation of the results.

Reference ID: 3655615

Observations and Schedule

Reproductive parameters: early and late resorptions, uterine scars, implantation sites, uterus weight, viable fetuses, intrauterine deaths, sex, fetal weight, macroscopic examination of placenta, and external fetal examination

Mortality	Twice daily
Clinical signs	At least twice daily
Body weights	Days 2, 4, 6, 9, 12, 16 and 18 post-mating
Food Consumption	Days 2-4, 4-6, 6-9, 9-12, 12-16 and 16-18 post-mating
Immunophenotyping[1]	Day 6 and Day 15 post-mating
Anti-drug antibody analysis	Day 15
Toxicokinetics	Day 6 and Day 15 post-mating at 0.083 (5 min), 0.5, 1, 2, 4, and 8 h post-dose Day 18 post-mating at 10 min post-dose only

[1]Lymphocyte subsets: T helper cells ($CD3^+/CD4^+$), T cytotoxic/suppressor cells ($CD3^+/CD8^+$), activated T helper cells ($CD3^+/CD4^+/CD25^+$), activated T cytotoxic/suppressor cells ($CD3^+/CD8^+/CD25^+$), total T cells ($CD3^+$), B cells (CD45R $B220^+$), natural killer cells ($CD3^-/NK1.1^+$), activated T cells ($CD3^+/CD69^+$)

Summary of results

A preliminary embryo-fetal development study tested doses of 0 or 5 mg/kg/day IV muS103new in pregnant mice (10/group) during the period of major organogenesis. Five satellite TK dams received muS103new through the end of gestation on Day 18 to measure maternal and fetal serum levels of muS103new. Dams treated with muS103new showed a decrease (> 80%) in lymphocyte subsets, consistent with the pharmacological activity. There were no anti-drug antibodies detected on Day 15. A dose of 5 mg/kg/day was not embryotoxic, and there were no test article-related findings in external fetal examinations. The mean fetal serum concentration was 10.8 ng/mL (~20 nM), approximately 2- and 8-fold greater than the K_D value for muS103new binding to murine CD3- and CD19-expressing cells, respectively (Study No. DR-RE-103-001). In addition, fetal serum concentrations were above the EC_{50} values for T cell activation and cytotoxicity in vitro (0.04-1.3 ng/mL). These results indicate a potential risk of fetal exposure at pharmacologically active concentrations. The study did not include an evaluation of fetal lymphocyte counts.

Study title: Study for effects on embryo-fetal development by intravenous route (bolus) in mice

Study no.:	103-PCD-0080
Study report location:	eCTD 4.2.3.5.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 25, 2012
GLP compliance:	Yes, OECD Principles of GLP
QA statement:	Yes
Drug, lot #, and % purity:	muS103new, lot # 100910MMa07, Purity: 98.8% monomer

Reference ID: 3655615

Key Study Findings

- Murine surrogate muS103new did not cause embryo-fetal toxicity or teratogenicity in mice.

Methods

Doses:	0, 1, 5 mg/kg (Groups 1, 2, 3)
Frequency of dosing:	Daily on Day 6 to Day 15 post-mating
Dose volume:	5 mL/kg
Route of administration:	Intravenous bolus injection
Formulation/Vehicle:	10 mM citric acid monohydrate, 75 mM L-Lysine monohydrochloride, 4% (w/v) Trehalose, 0.03% (w/v) Tween 80, pH 7.0
Species/Strain:	Swiss RjOrl mice
Number/Sex/Group:	25 females/group (main study)
Satellite groups:	12 females/group (immunophenotyping) 6 females for Control (TK analysis) 18 females/Groups 2-3 (TK analysis)
Study design:	Time-mated female mice were administered 0, 1, or 5 mg/kg/day IV muS103new on Days 6 to 15 post-mating during the period of major organogenesis. Scheduled necropsy occurred on Day 18 post-mating.
Deviation from study protocol:	Deviations from the study protocol did not affect the study design or interpretation of the results.

Observations and Schedule

Reproductive parameters: early and late resorptions, uterine scars, implantation sites, uterus weight, viable fetuses, intrauterine deaths, sex, fetal weight, macroscopic examination of placenta, and external, visceral, and skeletal malformations/variations

Mortality	Twice daily
Clinical signs	Twice daily
Body weights	Days 2, 4, 6, 9, 12, 16 and 18 post-mating
Food Consumption	Days 2-4, 4-6, 6-9, 9-12, 12-16 and 16-18 post-mating
Immunophenotyping[1]	Day 6 and Day 15 post-mating
Toxicokinetics	Day 6 and Day 15 post-mating at 0.083 (5 min), 0.5, 1, 2, 4, and 8 h post-dose

[1]Lymphocyte subsets: T helper cells ($CD3^+/CD4^+$), T cytotoxic/suppressor cells ($CD3^+/CD8^+$), activated T helper cells ($CD3^+/CD4^+/CD25^+$), activated T cytotoxic/suppressor cells ($CD3^+/CD8^+/CD25^+$), total T cells ($CD3^+$), B cells (CD45R $B220^+$), natural killer cells ($CD3^-/NK1.1^+$), activated T cells ($CD3^+/CD69^+$)

Dose Justification

The maximum feasible dose was 5 mg/kg/day muS103new given intravenously.

Mortality

All animals survived to scheduled necropsy on Day 18.

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Clinical Signs

Unremarkable

Body Weight

Unremarkable

Feed Consumption

Unremarkable

Hematology

- Hematology analysis only included data on total lymphocyte counts.
- Doses of 1 and 5 mg/kg/day muS103new reduced lymphocytes in dams by >80% compared to vehicle controls, consistent with the pharmacological activity in mice.

Immunophenotyping

- Following single and repeat dosing, muS103new caused a marked decrease in lymphocyte subtypes including B cells and helper T cells, consistent with the mechanism of action.
- After the first day of dosing, the percent of activated $CD4^+$ and $CD8^+$ T cells increased by approximately 2-fold in muS103new-treated cohorts compared to vehicle controls, reflecting an increase in activation of non-depleted T cell subsets.

Table 15: Results of immunophenotyping following daily doses of muS103new in pregnant mice (% change relative to controls)

	Day 6 post-mating		Day 15 post-mating	
	1 mg/kg/day	5 mg/kg/day	1 mg/kg/day	5 mg/kg/day
$CD4^+$ T cells	-99.7**	-99.8**	-97.9	-99.2**
$CD8^+$ T cells	-99.7**	-99.7**	-99.2	-99.7**
Activated $CD4^+$ T cells	-92.6*	-90.3*	-84.2	-91.4**
Activated $CD8^+$ T cells	-89.4**	-85.7**	-87.2	-91.9**
Total T cells	-99.6**	-99.7**	-98.0*	-98.8*
B cells	-95.5**	-93.2**	-97.9*	-97.5*
NK cells	-85.6**	-83.1**	23.7	34.7
Activated total T cells	-50.3	-57.0*	-89.4*	-90.3*

Significant finding, *$p < 0.05$, **$p < 0.01$

Toxicokinetics

- Following single and repeat dosing, C_{max} and AUC increased with increasing dose and were approximately dose proportional across the tested dose range.
- Systemic exposure was slightly higher on Day 15 compared to Day 6, indicating some accumulation following repeat dosing.
- The terminal half-life was approximately 1.4 to 2.3 hours.

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Table 16: Mean toxicokinetic parameters of muS103new in pregnant female mice following single and repeat dosing

	Dose (mg/kg)	$T_{1/2}$ (hours)	C_{max} (ng/ml)	C_{max}/D (ng/ml)/ (mg/kg)	$AUC_{(0-8)}$ (ng·h/ml)	$AUC_{(0-8)}$/D (ng·h/ml)/ (mg/kg)
Day 6 post-mating	1	1.4	6909	6909	4590	4590
	5	-	42002	8400	28000	5600
Day 15 post-mating	1	2.3	8696	8696	6610	6610
	5	1.7	42874	8575	38500	7700

(-) Could not be calculated

Dosing Solution Analysis

The sample formulations were within the accepted bioactivity range. The pH was within the acceptance criteria of (b) (4). The osmolality of the control and low dose formulation was 344 and 326 mOsm/kg, which was outside the acceptance range of (b) (4) mOsm/kg. The Applicant considered the formulations to be suitable for IV administration in mice.

Necropsy

There were no macroscopic findings reported.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Cesarean section results were unremarkable.

Table 17: Cesarean section observations in pregnant mice administered IV muS103new during organogenesis

Parameters	0 mg/kg/day	1 mg/kg/day	5 mg/kg/day
Number pregnant and evaluated	20	22	18
Mean implantation sites/litter	14.6	15.4	14.2
Mean fetuses/litter	14.0	15.0	13.9
Number of live fetuses/litter	13.9 (94.8%)	14.9 (96.7%)	13.9 (98.1%)
Mean post-implantation loss	5.2%	3.3%	1.9%
Number of early resorptions/litter	0.4	0.4	0.1
Number of late resorptions/litter	0.3	0.0	0.1
Mean gravid uterus weight (g)	24.73	26.56	25.28
Mean fetal weight/litter (g)	1.39	1.39	1.44
Fetal sex ratio/animal (% male)	48.0%	51.6%	48.5%

Offspring (Malformations, Variations, etc.)

- There were no effects on external or visceral variations or malformations.
- Skeletal variations in the sternebrae (7% fetal incidence, 24% litter incidence) and talus (20% fetal incidence, 65% litter incidence) were slightly higher than the reported historical control values but were not biologically relevant.

  Reported historical control data
  - 1st to 4th incomplete ossification of sternebrae: 0.7 to 4.0% fetal incidence, 4.0 to 16.7% litter incidence
  - Incomplete ossification of talus: 0.0 to 13.9% fetal incidence, 0.0 to 40.7% litter incidence

Table 18: Summary of mouse fetal examinations following daily doses of IV muS103new to pregnant mice during organogenesis

	Group 1 0 mg/kg/day	Group 2 1 mg/kg/day	Group 3 5 mg/kg/day
Skeletal Variations Number of fetuses (number of litters)	144 (20)	168 (22)	122 (17)
Skullcap • Extra ossification bone……	14 (9)	30* (11)	17 (9)
Sternebrae • 1st to 4th incomplete ossification…	2 (2)	3 (3)	8* (4)
Thoracic vertebrae • Ossification point on 14th thoracic vertebra……..……..	2 (2)	5 (5)	7 (4)
Ribs • Supernumerary 14th…….	30 (11)	40 (19*)	26 (11)
Talus • Incomplete ossification……	15 (6)	34* (14*)	25* (11)

Significant finding, *p < 0.05

9.3 Prenatal and Postnatal Development

No prenatal and postnatal development studies were submitted.

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10 Special Toxicology Studies

Study title: Immunopathology Report: Cross-reactivity of MEDI-538 with normal tissues

Study no.:	103-PCD-0041
Study report location:	eCTD 4.2.3.7.7.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 8, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MEDI-538 (MT103), lot # 2254-030, Purity: 100% monomer

A tissue cross-reactivity study was conducted to determine the potential cross-reactivity of MEDI-538 (MT103) with cryosections of normal human tissues and to identify potential target organs for toxicity. As mentioned in the drug information section, a (b) (4) MEDI-538 (10 and 50 µg/mL) was applied to cryosections of human tissues (3 donors per tissue). Cryosections of human tonsil and thymus were used as positive control tissue and normal human cerebellum served as a negative control tissue. The assay control was created by eliminating the MEDI-538 from the procedure and following the remainder of the immunoperoxidase staining procedure. These slides were stained with the anti-His tag detection antibody as well as an additional isotype control antibody for the anti-His tag detection antibody (mouse IgG1). No comparable, relevant negative control antibody or antibody fragment could be located; therefore, tissues that stained positively with MEDI-538 and the secondary (anti-HIS) antibody were also stained with the following mouse parental antibodies: HD37 anti-human CD19 mouse IgG1, L2K-07 (L2Kpm0611) anti-human CD3 mouse IgG1, and 1A7 anti-E. coli fimbrial protein (isotype control monoclonal antibody for HD37 and L2K-07). Binding of these antibodies to the test or control tissues was detected with the Dako EnVision horseradish peroxidase conjugated goat anti-mouse IgG polymer. As a tissue control, horseradish peroxidase-labeled rabbit anti-β_2 microglobulin was reacted with cryosections of human tissues. An indirect immunoperoxidase procedure was used for the study. The overall reaction sequence for immunoperoxidase staining was the following:

Primary antibody	Anti-HIS tag	Mouse IgG	Peroxidase labeled polymer	DAB
MEDI-538 (10 or 50 µg/mL)	X		X	X
None	X		X	X
None		X	X	X

Tissues examined (3 human donors per tissue):

Species	Human
Adrenal	X
Bladder	X
Blood cells	X
Bone Marrow	X
Breast	X
Brain-cerebellum	X
Brain-cerebral cortex	X
Colon	X
Endothelium-blood vessels	X
Eye	X
Fallopian tube	X
GI tract-stomach	X
GI tract-small intestine	X
GI tract-esophagus	X
Heart	X
Kidney	X
Liver	X
Lung	X
Lymph node	X
Ovary	X
Pancreas	X
Parathyroid	X
Peripheral nerve	X
Pituitary	X
Placenta	X
Prostate	X
Salivary gland	X
Skin	X
Spinal cord	X
Spleen	X
Striated muscle	X
Testes	X
Thymus	X
Thyroid	X
Tonsil	X
Ureter	X
Uterus-cervix	X
Uterus-	X

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Species	Human
endometrium	

Results

In the normal human tissues, MEDI-538 stained lymphocytes (determined to be T cells and B cells based on their morphology and location) that were abundant in lymphoid tissues such as the tonsil, spleen, thymus, and lymph node. In other tissues, very rare or occasional intravascular, migrating, or interstitial lymphocytes were stained by MEDI-538. There was no staining of the negative control cryosections of human cerebellum attributable to MEDI-538. The results of this study indicate that MEDI-538 stained only lymphocytes in the normal human tissue panel and that there was no unexpected tissue binding or cross-reactivity attributable to MEDI-538.

11 Integrated Summary and Safety Evaluation

Pharmacology

The Applicant conducted pharmacology studies to evaluate the species specificity and binding affinity of blinatumomab to CD3 and CD19 and the mechanism of action of blinatumomab-mediated redirected cytotoxicity. Blinatumomab bound to B and T lymphocytes from human and chimpanzee PBMC but it did not bind to PBMC from the mouse, rat, beagle dog, squirrel monkey, African Green monkey, cynomolgus monkey, rhesus monkey, and baboon. In vitro binding affinity (K_D) of blinatumomab to human (b) (4) and CD19 were 260 nM and 1.49 nM, respectively. Binding of blinatumomab to $CD3^+$ T cells and $CD19^+$ target cells (isolated B cells or tumor cell lines), resulted in cytokine release (i.e. IL-2, TNF-α, and IFN-γ), T cell proliferation, increased expression of granzyme B, and redirected cytotoxicity of $CD19^+$ target cells. More potent blinatumomab-mediated redirected cytotoxicity was noted when human $CD8^+$ cytotoxic T cells were used as the effector cells when compared to $CD4^+$ helper T cells. Furthermore, within the cytotoxic T cell population, blinatumomab-mediated redirected cytotoxicity was only noted when $CD19^+$ target cells were incubated with primed $CD45RO^+CD8^+$ T cells but not with naïve $CD45RA^+CD8^+$ T cells. In vitro, blinatumomab-mediated redirected cytotoxicity was found to be dependent on extracellular calcium and correlated with cytokine release and increased expression of granzyme B expression within 2 and 16 hours post-treatment, respectively. However, blinatumomab-mediated redirected cytotoxicity was not dependent on cytokine release since either treatment with TNF-α neutralizing antibody or dexamethasone resulted in decreased cytokine levels but had no effect on cytotoxicity.

Because blinatumomab binds only to human and chimpanzee CD3 and CD19, the Applicant generated the murine surrogate muS103new for toxicology studies in mice and compared its activity to blinatumomab. Flow cytometry analysis demonstrated that blinatumomab and muS103new had similar binding affinities and cytokine release profiles in vitro (Appendix 1). EC_{50} values for species-specific T cell activation were comparable for blinatumomab (111-200 pg/ml) and muS103new (126-170 pg/ml). EC_{50} values for cytotoxicity were higher in studies conducted with muS103new (37.9-1324

pg/ml) compared to blinatumomab (0.3-56.5 pg/ml) for the target cells tested. Collectively, the pharmacological activity of muS103new was comparable to blinatumomab in vitro.

Safety Pharmacology

To evaluate the potential effects on the central nervous system (CNS), two CNS safety pharmacology studies were conducted with the murine surrogate muS103new in mice. In the first, an Irwin test was conducted following intravenous bolus administration of muS103new (0, 0.2, 1, or 5 mg/kg/day) daily for 5 days. Intravenous administration of muS103new at doses of 0.2 and 5 mg/kg/day did not induce any CNS changes. At 1 mg/kg/day muS103new, a variety of CNS effects including decreased activity and reactivity, piloerection, and motor signs were observed in one animal starting on the third day of treatment. While there were no effects at the highest dose tested, these findings suggest that there may be inter-individual differences in the sensitivity to muS103new in mice. The second study was an exploratory study to evaluate the effects of 7-day continuous intracerebroventricular infusion of muS103new on behavior and physiological function. Intracerebroventricular administration of muS103new did not produce any behavioral modifications or monitorable evidence of physiological change or neurotoxicity in mice.

General Toxicology

General toxicology studies conducted to support the development of blinatumomab include repeat-dose studies of subcutaneous and intravenous administration of the murine surrogate muS103new in mice and repeat-dose studies of intravenous administration of blinatumomab in chimpanzees. For this review, the 4-week (summarized) and 13-week studies of muS103new in mice and the 5-weekly dose study of blinatumomab in chimpanzees were reviewed.

In the 4-week repeat-dose toxicology study of muS103new in mice, BALB/c mice were administered muS103new (0, 0.2, 1, or 5 mg/kg/day) by intravenous bolus injection once daily for 4 weeks with a 4-week recovery period. Due to feasibility issues, muS103new was administered subcutaneously instead of intravenously in the 13-week study in mice, and the drug was administered twice daily to achieve increased exposure. Therefore, in the 13-week repeat-dose toxicology study in mice, BALB/c mice were administered muS103new (0, 1, or 5 mg/kg/day; 0, 2, or 10 mg/kg/day) by subcutaneous injection twice daily with an 8-hour interval between doses for 13 weeks with a 4-week recovery period. The toxicities observed with muS103new were similar in the two studies and are consistent with the pharmacology of the drug. muS103new decreased lymphocytes including total B cells and T cells and the $CD4^+$ and $CD8^+$ T lymphocyte subsets in the blood, spleen, and lymph node. In the lymph nodes (mandibular, mesenteric, left axillary, and left and right inguinal) and Peyer's patches, the microscopic findings were decreased cellularity and decreased germinal center development. Decreased cellularity was also observed in the white pulp of the spleen and corresponded to decreased spleen weight. In the 13-week study, increased

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subcutis/panniculus inflammation was observed at treated sites and the skin and increased dermal inflammation was observed at the treated sites.

The longest duration repeat-dose toxicology study of blinatumomab in chimpanzees was a 5-dose, non-terminal study in which only in-life measurements were conducted. One female control was administered the vehicle and two chimpanzees (one male and one female) were administered 0.1 µg/kg blinatumomab by a 2-hour intravenous infusion once weekly for 5 weeks. Blinatumomab-related effects included increases in body temperature and heart rate and decreases in blood pressure following infusion of blinatumomab. Infusion with blinatumomab decreased lymphocyte levels including B cells ($CD19^+$ and $CD20^+$) and T cells ($CD3^+/CD4^+$ and $CD3^+/CD8^+$) and increased the expression/levels of T cell activation markers sCD25, CD69, and HLA-DR. Increases in cytokines IL-2, IL-6, and INFγ were also observed following infusion with blinatumomab in chimpanzees, a finding consistent with the cytokine release syndrome observed in the clinical trial in patients with ALL. An ear infection was observed in the male chimpanzee starting around Day 6; while it is unclear if the infection was related to treatment with blinatumomab, infection was one of the major adverse events observed with blinatumomab in patients with ALL.

Reproductive and Developmental Toxicology

The Applicant conducted an embryo-fetal development study in mice using muS103new. Pregnant mice received 0, 1, or 5 mg/kg/day muS103new intravenously from gestation Day 6 to Day 15 and were euthanized on Day 18. muS103new reduced maternal B cells, T cells, and NK cells by > 80%, consistent with the mechanism of action. muS103new did not cause embryo-fetal toxicity or teratogenicity in mice at the doses tested. Skeletal variations (delayed ossification) in the sternebrae and talus were slightly higher than reported historical control values but were not biologically relevant.

In a preliminary embryo-fetal development study, a subset of dams received 5 mg/kg/day muS103new from Day 6 through the end of gestation on Day 18 to measure maternal and fetal serum levels of muS103new. The mean fetal serum concentration was 10.8 ng/ml (~20 nM). Fetal serum concentrations were above the EC_{50} values for T cell activation and cytotoxicity in vitro (0.04-1.3 ng/ml) and approximately 2- and 8-fold higher than the K_D value for CD3 and CD19 binding, respectively. Collectively, these findings suggest a potential risk of fetal exposure at pharmacologically active concentrations. The study did not evaluate effects of muS103new on fetal lymphocyte counts.

(b) (4)

However, there are no reproductive and developmental toxicology studies with blinatumomab, and it is not known whether blinatumomab can cause fetal harm. Based on its mechanism of action and placental transfer observed with muS103new in mice, there is a potential risk of fetal lymphocyte depletion. Other approved products that target B cells include rituximab, obinutuzumab and ofatumumab. In product labels, these agents were not

teratogenic in cynomolgus monkeys but did cause B cell depletion and immunosuppression in offspring. In pre- and post-natal studies with rituximab and obinutuzumab, B cell counts and immunologic function recovered within 6 months after birth. Postmarketing data showed that infants exposed to rituximab in utero experienced B cell depletion for less than 6 months. Consequently, rituximab, obinutuzumab, and ofatumumab are labeled as Pregnancy category C in approved package inserts. Based on its mechanism of action, the Pharmacology/ Toxicology team recommends Pregnancy category C for blinatumomab. Blinatumomab should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Special Toxicology Studies

A tissue cross-reactivity study was conducted to determine the potential cross-reactivity of blinatumomab in normal human tissues. Blinatumomab (10 and 50 µg/mL) was applied to cryosections of human tissues (3 donors per tissue). Binding of blinatumomab was limited to lymphocytes primarily in lymphoid tissues. There was no unexpected tissue binding or cross-reactivity attributable to blinatumomab.

Reference ID: 3655615

12 Appendix/Attachments

Appendix 1: Comparison of blinatumomab and murine surrogate muS103new
(excerpted from Applicant's submission)

Category	Parameter	Blinatumomab	muS103new
Molecule	Parental Antibody source	(b) (4)	
	MW, #aa	54.(b)(4)kDa, (b) (4)	(b) (4)
Binding affinity[c]	CD19 K_d (nM)	CHO-huCD19: 1.8	CHO-muCD19: 2.4
	CD3 K_d (nM)	Human PBMC: 55.4	Mouse splenocytes: 10.6
Cytotoxicity[c]	EC_{50} (pg/mL) Mean ± SEM	NALM-6: 1.6 ± 0.5 KOPN-8: 0.3 ± 0.2 Primary Hu B cells: 56.5 ± 21.6	BCL-1: 37.9 ± 10.2 A20:1324 ± 373 CHO-mCD19: 361± 165
T cell activation markers[c]	CD25: EC_{50} (pg/mL) Mean ± SEM	KOPN-8: 200 ± 56	BCL-1: 170 ± 29
	CD69: EC_{50} (pg/mL) Mean ± SEM	KOPN-8: 111 ± 33	BCL-1: 126 ± 110
Cytokine release in vitro[c]	Cytokines at 24 hr (human) and 72 hr (mouse)	KOPN-8: IL-2, IL-4, IL-10, IFN-γ, TNF-α	A20: IL-2, IL-4, IL-10, IFN-γ, TNF-α
Maximal B cell depletion in vivo[d]		100%[e]	100%[f]
T cell redistribution in vivo[d]		Transient[e,g]	Persistent[f]
Cytokine release in vivo[d]	Cytokines elevated during 1st 24 hr	IL-2, IL-6, IL-10, IFN-γ,TNF-α[e]	IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ,TNF-α[h]

aa = amino acid; A20 and BCL-1 = murine B cell lymphoma lines; CHO = Chinese hamster ovary; cIV = continuous IV infusion; EC_{50} = 50% effective concentration; hu = human; KOPN-8 and NALM-6 = human pre-B cell ALL cell lines ; MW = molecular weight; mu = murine; PBMC = peripheral blood mononuclear cells; SEM = standard error of the mean.
[a] Section 2 of Module 3.2.S.1.2 Structure
[b] Report DR-RE-103-001
[c] Report 103-PCD-0094
[d] B cell depletion, T cell redistribution, and cytokine release comparisons are in peripheral blood in humans (blinatumomab) and mice (muS103new).
[e] Klinger et al, 2012
[f] Reports 103-PCD-0074, 103-PCD-0075, 103-PCD-0082, 103-PCD-0079, 103-PCD-0080; 2.6.7 Toxicology Tabulated Summary.
[g] T cell redistribution recovers approximately 8-9 days after the start of the cIV infusion (Klinger et al, 2012).
[h] Reports 103-PCD-0091, 103-PCD-0092

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/s/

BRENDA J GEHRKE
11/07/2014

HAW-JYH CHIU
11/07/2014

TIFFANY RICKS
11/07/2014

CHRISTOPHER M SHETH
11/07/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR BLA

BLA Number: 125557 **Applicant: Amgen Inc.** **Stamp Date: 9/19/2014**

Drug Name: Blinatumomab **BLA Type: BLA (351 a)**

On **initial** overview of the BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	✓		BLA is submitted in the eCTD format
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	✓		Electronic submission
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	✓		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	✓		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	✓		Mouse studies were conducted with a murine surrogate antibody (muS103new), formulation for muS103new appears to be similar to that of blinatumomab. Chimpanzee studies were conducted with a different formulation of blinatumomab.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	✓		The intended clinical route is intravenous infusion. Most animal studies were conducted with the intravenous route. The 13-week mouse study was conducted with subcutaneous administration; intravenous bolus injection was not considered feasible by the applicant for this study.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	✓		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	✓		Table with side-by-side comparisons for blinatumomab vs. murine surrogate antibody (muS103new) requested at pre-

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR BLA

	Content Parameter	Yes	No	Comment
				BLA meeting was submitted.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	✓		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			This is a review issue. No impurity issues have been identified at this time.
11	Has the applicant addressed any abuse potential issues in the submission?			Not Applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not Applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes_____

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Brenda J. Gehrke, Ph.D. 9/30/2014
Reviewing Pharmacologist Date

Haw-Jyh Chiu, Ph.D. 9/30/2014
Reviewing Pharmacologist Date

Tiffany K. Ricks, Ph.D. 9/30/2014
Reviewing Pharmacologist Date

Haleh Saber, Ph.D. 9/30/2014
Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

BRENDA J GEHRKE
09/30/2014

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