CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125427Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Kadcyla (ado-trastuzumab emtansine)

Date: February 1, 2013
To: File for BLA 125427
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 of Dr. McGuin and labeling and secondary memorandum provided by Dr. Palmby. I concur with Dr. Palmby's analysis regarding risks associated with potential manufacturing (b) (4) and his conclusion that Kadcyla may be approved. No additional nonclinical studies are needed to support the proposed indication.

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/s/

JOHN K LEIGHTON
02/01/2013

MEMORANDUM

Date: January 31, 2013
From: Todd R. Palmby, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
To: File for BLA 125427 Kadcyla (ado-trastuzumab emtansine)
Re: Approvability for Pharmacology and Toxicology
Indication: as a single agent, for the treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination. Patients should have either:

- Received prior therapy for metastatic disease, or
- Recurred during or within six months of completing adjuvant therapy.

Non-clinical pharmacology and toxicology studies to support Kadcyla (ado-trastuzumab emtansine) BLA 125427 for the treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination, were reviewed by W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T. Studies conducted with intravenously administered ado-trastuzumab emtansine included pharmacology, pharmacokinetic/toxicokinetic, safety pharmacology, single- and repeat-dose toxicology (rat and monkey), and genetic toxicology (*in vivo* and *in vitro*). The studies cited in the review consist primarily of original research studies conducted by the Applicant with supporting information obtained from published literature.

Ado-trastuzumab emtansine is a humanized anti-HER2 monoclonal antibody, trastuzumab, covalently bound to a microtubule inhibitor, DM1, via a linker, succinimidyl trans-4-[maleimidylmethyl] cyclohexane-1-carboxylate (SMCC), which is commonly known as an antibody-drug conjugate (ADC). Trastuzumab was approved by the FDA in 1998 under the trade name Herceptin and is currently indicated for the treatment of HER2 overexpressing breast cancer and the treatment of HER2 overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma.

Pharmacology studies submitted to the BLA support that ado-trastuzumab emtansine retained similar binding affinity for the extracellular domain of HER2 as trastuzumab. When cancer cells were treated with ado-trastuzumab emtansine *in vitro*, internalization of the ADC and release of DM1 lead to decreased cellular proliferation, activated effector caspase-3 and -7 and apoptosis following internalization of the ADC. In contrast, trastuzumab inhibited cell proliferation, but was not directly cytotoxic. The addition of both trastuzumab and ado-trastuzumab emtansine to a cell line that is sensitive to trastuzumab and expresses a high level of HER2 caused a decrease in AKT phosphorylation. In

in vitro assays, the activity of ado-trastuzumab emtansine and trastuzumab were similar in mediating antibody-dependent cell-mediated cytotoxicity (ADCC) and inhibiting shedding of the HER2 extracellular domain from the cell surface. Therefore, the addition of emtansine to trastuzumab did not significantly alter the *in vitro* activity of trastuzumab. Ado-trastuzumab emtansine may retain the clinical activity of trastuzumab in addition to delivering the microtubule inhibitor, DM1, to tumor cells expressing HER2. These assays did not determine the relative contributions of these *in vitro* activities to the clinical efficacy of trastuzumab or ado-trastuzumab emtansine in the indicated population. Based on the data provided in the BLA, ado-trastuzumab emtansine was assigned a new Established Pharmacologic Class (EPC). The FDA text for the EPC that was determined to be both scientifically accurate and clinically meaningful is "HER2-targeted antibody and microtubule inhibitor conjugate." Including "microtubule inhibitor" is considered an important aspect of the EPC to distinguish toxicities associated with this type of compound compared to those associated with other types of cytotoxic compounds.

Nonclinical single- and repeat-dose toxicology studies submitted to this BLA were conducted in rats and Cynomolgus monkeys. Single-dose and short-term repeat-dose studies in rats and monkeys with ado-trastuzumab emtansine resulted in similar toxicity profiles across species. Likewise, studies in rats with ado-trastuzumab emtansine or DM1 alone resulted in similar toxicities, suggesting toxicities were mediated primarily by DM1. In addition, it was previously demonstrated that trastuzumab binds to the Cynomolgus monkey receptor with similar affinity as to human HER2, but does not bind with high avidity to the rat receptor indicating Cynomolgus monkey is the more relevant pharmacologic species. Therefore, long-term (e.g., 3- and 6-month) repeat-dose toxicology studies with ado-trastuzumab emtansine were conducted in monkeys.

Monkeys were administered repeat doses of ado-trastuzumab emtansine as high as 30 mg/kg every three weeks, or approximately 7 times the clinical exposure based on AUC. Increased transaminases and centrilobular vacuolization in monkeys were correlated with hepatic toxicity observed in patients. There was no significant evidence of cardiac toxicity in monkeys. Mononuclear infiltrates in monkeys correlated with pneumonitis observed in patients. Thrombocytopenia and anemia were observed both in monkeys and in patients during clinical trials, but were less severe in monkeys. The incidence of peripheral neuropathy in patients was about 21%, which correlated with the axonal degeneration in the sciatic nerve with Schwann cell hyperplasia and hypertrophy and axonal degeneration of the dorsal funiculus in the spinal chord in monkeys that received 30 mg/kg every three weeks (approximately 7 times the clinical exposure based on AUC). Whether ado-trastuzumab emtansine caused direct toxicity to Schwann cells or whether these effects were secondary to axonal degeneration of the associated neuronal cells is unclear, although axonal degeneration in the sciatic nerve was observed in animals that received a lower dose of 10 mg/kg every 3 weeks with no reported effects on Schwann cells.

No dedicated nonclinical toxicology studies were conducted with ado-trastuzumab emtansine to assess effects on fertility. However, results from nonclinical repeat-dose toxicology studies suggest ado-trastuzumab emtansine may impair male and female fertility in humans. Rats that received a single dose of ado-trastuzumab emtansine resulting in approximately 4 times the clinical exposure, based on AUC, had degeneration of seminiferous tubules with hemorrhage in the tested associated with increased testes and epididymides weights in males and signs of hemorrhage and necrosis of the corpus luteum in ovaries in females. Monkeys that received ado-trastuzumab emtansine every three weeks for 12 weeks resulting in 7 times the clinical exposure were observed with decreases in epididymides, prostate, testes, seminal vesicles and uterus organ weights. The interpretation of these organ weight changes was unclear as there were no microscopic correlates and the percent change of these organ weights compared to control were less when adjusted for body or brain weight. The Applicant attributed these changes as possible related to the varied sexual maturity of the animals used in this study.

Genetic toxicology studies submitted to the BLA were conducted with ado-trastuzumab emtansine or DM1. DM1 was positive in an *in vivo* rat bone marrow micronucleus assay at exposures similar to the mean maximum concentrations of DM1 in patients in clinical trials, which is consistent with its mechanism of action. A bone marrow micronucleus assay was incorporated into the 3-month repeat-dose toxicology study in monkeys administered ado-trastuzumab emtansine. Ado-trastuzumab emtansine did not induce micronuclei formation in bone marrow from monkeys, although the results of this study were deemed inconclusive due to minimal observed bone marrow toxicity. The delay in harvesting of the bone marrow following the final administration of ado-trastuzumab emtansine (i.e., 7 days), a lack of binding of trastuzumab or ado-trastuzumab emtansine to bone marrow from humans or monkeys in a tissue cross-reactivity assay and a low plasma exposure of free DM1 in monkeys may have contributed to a negative result. The validity of the monkey bone marrow micronucleus assay results were in question due to prior knowledge of the mechanism of action of DM1 and results from the rat micronucleus study with DM1 alone, (b) (4)

No reproductive and developmental toxicity studies were conducted with ado-trastuzumab emtansine or DM1. Toxicology studies in rats and monkeys with DM1 or ado-trastuzumab emtansine indicated toxic effects to rapidly dividing cells. In addition, DM1 was demonstrated to be genotoxic. Based on these findings and consistent with ICH S9, embryo-fetal developmental toxicity studies were not required for the indicated patient population. Data from trastuzumab in the post-marketing setting show that treatment during pregnancy has resulted in oligohydramnios, some associated with fatal pulmonary hypoplasia, skeletal abnormalities and neonatal death. Due to the expectation that Kadcyla can cause fetal harm when administered to a pregnant woman, it was assigned a

pregnancy category D in the package insert. The half-life of trastuzumab in humans is approximately 28 days. Since trastuzumab was detected in patients administered ado-trastuzumab emtansine, patients should be advised to use contraception during treatment with Kadcyla and for 6 months following the last dose of Kadcyla.

Potential (b) (4) of Kadcyla During Manufacturing

During review of the BLA submission, members of the review team in the Division of Good Manufacturing Practice Assessment (DGMPA) within the Office of Compliance determined that the manufacturing contractor for the Kadcyla drug product (b) (4) The reviewer in DGMPA, Reyes Candauchacon, requested a consult from the Pharmacology/Toxicology review team to determine if the proposed limits (b) (4) in the Kadcyla drug product were acceptable.

(b) (4)

(b) (4)

(b) (4)

Kadcyla is administered once every three weeks. The indicated patient population has HER2-positive metastatic breast cancer. The median duration of survival of patients treated with Kadcyla in the randomized clinical trial was 30.9 months and the median age of patients was 53 years (ranged from 24-85 years). The median duration of treatment with Kadcyla was 5.7 months. In teleconferences between the Applicant and the review team held during the review cycle for this BLA, the Applicant stated that cleaning verification/validation data will be collected (b) (4), and that Kadcyla would be produced (b) (4)

Therefore, from a Pharmacology/Toxicology perspective, the proposed limit (b) (4) in the Kadcyla drug product is acceptable.

(b) (4)

In response to another information request from the review team, the Applicant submitted cleaning verification/validation data (b) (4)

(b) (4)

(b) (4)

Therefore, from a Pharmacology/Toxicology perspective, the amount (b) (4) in batch A6540 of ado-trastuzumab emtansine drug product is acceptable.

Recommendation: I concur with Dr. McGuinn's conclusion that pharmacology and toxicology data support the approval of BLA 125427 for Kadcyla. There are no outstanding nonclinical issues that would preclude the approval of Kadcyla for the proposed indication.

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/s/

TODD R PALMBY
01/31/2013

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

PHARMACOLOGY and TOXICOLOGY BLA REVIEW and EVALUATION

Application number	BLA 125427
Supporting document	0 (Electronic Submission)
Applicant's letter date	June 12, 2012 (rolling submission; Module 4)
CDER stamp date	August 27, 2012
Product	Kadcyla (trastuzumab emtansine; T-DM1)
Indication	Patients with human epidermal growth factor 2 (HER2)-positive (b) (4) metastatic breast cancer who have received prior treatment with trastuzumab and a taxane
Applicant	Genentech
Review Division	Division of Hematology Oncology Toxicology (Division of Oncology Products 1)
Reviewers	W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T. Whitney Helms, Ph.D. Anne Pilaro, Ph.D.
Acting Supervisor	Todd Palmby, Ph.D.
Division Directors	John Leighton, Ph.D., D.A.B.T. (DHOT) Robert L. Justice, M.S., M.D. (DOP1)
Medical Officer	Gideon Blumenthal, M.D.
Project Manager	Lisa Skarupa

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125427 are owned by Genentech or are data for which Genentech has obtained a written right of reference. Any information or data necessary for approval of BLA 125427 that Genentech 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 125427.

Table of Contents

1 Executive Summary

1.1 Introduction

Trastuzumab emtansine (TDM1, or trastuzumab-MCC-DM1) is an antibody covalently bound to a cytotoxic drug This type of molecule is commonly known as an antibody-drug conjugate (ADC). Trastuzumab is a humanized anti-HER2 IgG1 (isotype 1) that the FDA approved as a cancer drug therapy for breast cancer under the trade name Herceptin. In trastuzumab emtansine, the antibody is linked to a hetero-bifunctional reagent, succinimidyl trans-4-[maleimidylmethyl] cyclohexane-1-carboxylate (SMCC). The other end of the SMCC linker molecule is covalently bound to DM1, a microtubule-inhibitory maytansinoid, by a thioether bond. The antibody binds to the linker predominantly at lysine residues with a net stoichiometry of approximately 3.5. This ratio varies somewhat with lot and time.

The trastuzumab antibody is directed against the extracellular domain of HER2 (Human Epidermal Growth Factor Receptor 2) also known as Neu, ErbB-2, CD340 or p185. HER2 is a member of the epidermal growth factor receptor (EGFR/ErbB) family encoded by the ERBB2 gene. An acquired somatic alteration of this gene resulting in amplification and overexpression occurs in approximately 20 to 25% of human breast cancers.[1,2,3] Amplification and overexpression result in an increase in the proliferative stimuli associated with HER2. This increase in proliferative stimuli results in increased tumor growth. When trastuzumab binds to HER2 it inhibits the growth stimulating signal of this tyrosine kinase by preventing the necessary dimerization of HER2 or heterodimerization with other members of the HER family (EGFR, HER3 and HER4), slowing the growth of HER2 overexpressing ($HER2^+$) breast cancers.

The DM1 component of trastuzumab emtansine is an inhibitor of tubulin polymerization; it binds to the beta subunit of tubulin at the same binding site as the vinca alkaloids. Nevertheless, DM1 derivatives are as much as 100-fold more cytotoxic than vinca alkaloids and taxanes.[4] Trastuzumab emtansine binds to HER2 with an affinity similar to that of trastuzumab. When trastuzumab emtansine binds to HER2, the damaged receptor protein is invaginated into the cell. Following internalization, the intracellular lysosomes degrade the TDM1 bound antibody and release DM1-containing cytotoxic catabolites into the cell.[5, 6] The applicant hypothesizes that the released DM1 then binds to tubulin and disrupts the microtubule network, resulting in inhibition of cell division and cell growth and eventually cell death. They further postulate that the combined action of trastuzumab diminishing the cancer cell mitogenic signals and DM1 cytotoxicity will affect a greater degree of clinical benefit that trastuzumab alone.

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[1] Yarden Y, Sliwkowski MX. Untangling the ErbB signaling network. *Nat Rev Mol Cell Biol* 2001;**2**:127-37.

[2] Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177-82.

[3] Slamon D., et al. Adjuvant Trastuzumab in HER2-Positive Breast Cancer, october 6, 2011 vol. 365 no. 14, 1273-1284.

[4] Kovtun YV, Audette CA, Mayo MF, et al. Antibody-maytansinoid conjugates designed to bypass multidrug resistance. Cancer Res 2010;70(6):2528−37.

[5] Erickson HK, Park PU, Widdison WC, et al. Antibody-maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing. Cancer Res 2006;66(8):4426−33.

[6] Erickson HK, Widdison WC, Mayo MF, et al. Tumor delivery and in vivo processing of disulfide-linked and thioether-linked antibody-maytansinoid conjugates. Bioconjug Chem 2010;21(1):84−92.

1.2 Brief Discussion of Nonclinical Findings

The binding of trastuzumab emtansine to the extracellular domain of HER2 and the high-affinity Fc gamma (Fcγ) cell surface receptor RIa were comparable with that of trastuzumab. Likewise binding to the complement component C1q was comparable, but of relatively low affinity. Binding of trastuzumab emtansine to low-affinity Fcγ receptors was somewhat more avid than that of trastuzumab.

The activities of trastuzumab emtansine and trastuzumab were investigated *in vitro.* Data suggests that following internalization of trastuzumab emtansine, DM-1 causes sufficient cell damage to initiate apoptosis in cancer cells. Trastuzumab emtansine caused cytotoxicity, decreased cellular proliferation, and activated effector caspase-3 and -7 in cancer cell lines. These changes were not observed with trastuzumab, which inhibits cellular proliferation, but is not directly cytotoxic. These experiments demonstrated that cell cycle arrest induced by trastuzumab emtansine was predominantly at G_2/M, attributed to inhibition of microtubules by DM1. Addition of both trastuzumab emtansine and trastuzumab to a cell line that expresses HER2 and is sensitive to trastuzumab caused a decrease in phosphorylation of AKT. Trastuzumab emtansine had similar activity as trastuzumab in mediating antibody-dependent cell-mediated cytotoxicity (ADDC) when human breast cancer cells were used as target cells and purified peripheral blood mononuclear cells from healthy donors were used at effector cells. In other studies, binding of trastuzumab or trastuzumab emtansine diminished the extent of shedding of the extracellular domain of HER2 from the cell surface *in vitro*, although the contribution of this activity to the clinical efficacy of trastuzumab emtansine is unclear.

When injected intravenously, trastuzumab emtansine distributes relatively slowly, $t_{1/2}\alpha$ ~ 1 day in monkeys. DM1 is slowly cleaved from the molecule to form free trastuzumab; little DM1 is detectable in the blood as the drug is cleared rapidly in the liver. At the clinical dose, the elimination half-life for trastuzumab emtansine is about 4 days in humans reflecting the cleavage of DM1. The half-life for free trastuzumab is about 7 days. In monkeys, clearance for both trastuzumab and trastuzumab emtansine decreases with increasing dose reflecting some non-linear process, possibly the *de novo* synthesis of new HER2 receptors. Clearance is much lower than hepatic flow. A dose of 3 mg/kg trastuzumab emtansine in monkeys gave approximately the same pharmacokinetic characteristics as the clinical dose, 3.6 mg/kg. The antibodies are both confined to the plasma space. Tissue distribution parallels blood flow. Excretion of a radiolabeled dose is predominantly fecal but there is substantial retention of the label.

Trastuzumab emtansine binds to the HER2 receptor site with an equilibrium binding constant of about 1.0×10^{-9} M. The antibody binds to similar tissues in monkeys and humans but with somewhat greater avidity in humans. Trastuzumab emtansine causes apoptosis *in vitro* whereas trastuzumab does not. *In vitro*, trastuzumab emtansine causes cell cycle arrest at the G_2/M interface while trastuzumab causes arrest at G_0/G_1, thus reflecting the cytotoxicity of DM1. The IC_{50} for inhibition of cell growth by trastuzumab emtansine is sub-nanomolar; that for trastuzumab is considerably greater.

Free DM1 does not inhibit the cardiac slow rectifier current at physiologically relevant concentrations. Trastuzumab emtansine does not cause changes in the monkey ECG but it does cause some elevation in blood pressures. Hypertension occurred in about 5% of treated patients in clinical trials. HER2 signaling is necessary for the maintenance of mitochondrial function in the heart. Inhibition of this signaling causes mitochondrial apoptosis and diminished cardiac function. This correlates with the incidence of diminished left ventricular ejection fraction seen clinically (about 1.8% incidence). This toxicity could limit long term trastuzumab emtansine treatment.

Monkeys tolerated repeat doses of trastuzumab emtansine as high as 30 mg/kg or about 8 times the clinical dose. Even at this high dose, trastuzumab emtansine was less toxic to

monkeys than the lower clinical dose was to humans. Thrombocytopenia was seen in both species but was much less severe in monkeys. Likewise, anemia was also less severe in monkeys. Mononuclear infiltrates in monkeys predicted the pneumonitis seen in humans. Increased transaminases and centrilobular vacuolization in monkeys predicted the more severe hepatic toxicity seen in cancer patients. There was some evidence of toxicity in the tongue in monkeys that anticipates the stomatitis seen clinically. Diarrhea was also seen in both monkeys and patients. The incidence of peripheral neuropathy in patients is about 21%, this correlated with the axonal degeneration in the sciatic nerve with Schwann cell hyperplasia and hypertrophy and axonal and axonal degeneration of the dorsal funiculus in the spinal chord in monkeys at approximately 7 times the clinical exposure, based on AUC.

In nonclinical toxicity studies, trastuzumab emtansine caused effects that suggest it may impair male and female fertility in humans. Effects in rats that received a single dose of trastuzumab emtansine included degeneration of seminiferous tubules with hemorrhage in the testes associated with increased weights of testes and epididymides in males and signs of hemorrhage and necrosis of the corpus luteum in ovaries in females at approximately 4 times the clinical exposure, based on AUC. In monkeys that received trastuzumab emtansine once every three weeks for 12 weeks at up to 7 times the clinical exposure, based on AUC, there were decreases in the weights of epididymides, prostate, testes, seminal vesicles and uterus. The percent change of these organ weights compared to control when adjusted for body or brain weight were less. The Applicant attributed some of these changes on the varied sexual maturity of the animals used in this study. Effects on reproductive organs were not unexpected due to the mechanism of action of the small molecule portion, DM1.

In studies with trastuzumab administration to pregnant monkeys at about 7 times the clinical dose, trastuzumab crossed the placental barrier, resulting in concentrations in fetal blood and amniotic fluid approximately 33% and 25%, respectively, of those present in the maternal serum. However, these concentrations of trastuzumab in monekys were not associated with adverse findings. Nevertheless, trastuzumab is a known embryo-fetal toxin in humans, causing oligohydramnios that is potentially fatal. DM1 was negative in an *in vitro* bacterial reverse mutation (Ames) assay. However, DM1 is a microtubule inhibitor, which caused micronuclei formation *in vivo* in rat bone marrow cells and is known to be toxic to rapidly dividing cells (e.g., GI tract and bone marrow). Thus, the Applicant did not conduct any new reproductive and developmental toxicity studies with trastuzumab emtansine, relying instead on the results for trastuzumab and the mechanism of action of DM1. Trastuzumab emtansine can likely cause embryo-fetal toxicity and has been labeled accordingly.

1.3 Recommendations

1.3.1 Approvability

There are no non-clinical findings of unacceptable toxicity that would prevent the approval of trastuzumab emtansine for this indication.

1.3.2 Additional Non-Clinical Recommendations

None

1.3.3 Labeling

Calculations of AUC comparisons in animals from nonclinical study data and in patients from clinical trial data were made to add to relevant nonclinical sections of the package insert. The

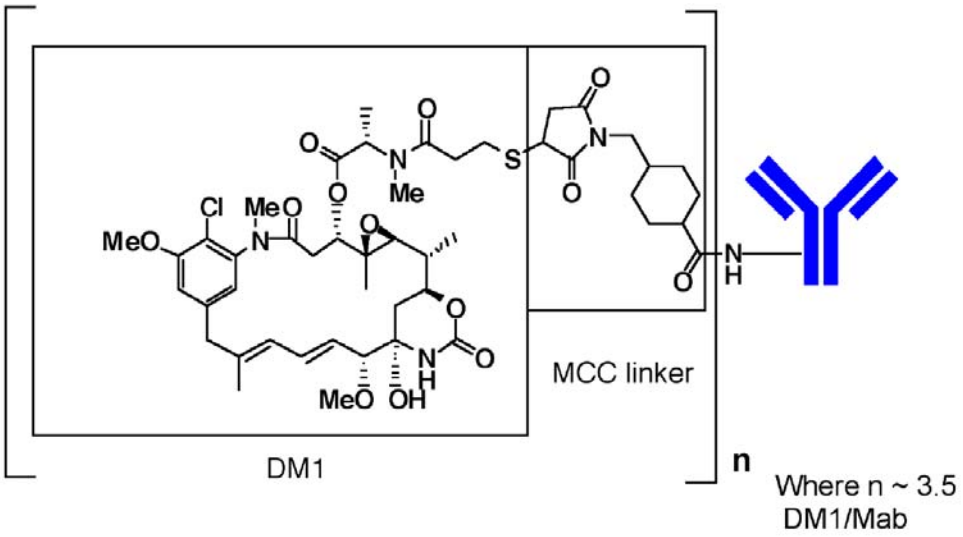
clinical AUC that was used for these calculations was (475 μg*day/mL), which was reported in patients in Study # Trial TDM4370g/BO21977 cycle 4.

2 Drug Information

2.1 Drug

Generic Name	Trastuzumab emtansine
Code Name	T-DM1, Trastuzumab-MCC-DM1, xHER2-DM1, Tmab-MCC-DM1, Herceptin-DM1, Herception-SMCC-DM1, Herceptin-MCC-DM1, Hu Tmab-MCC-DM1, Trastuzumab-SMCC-DM1PR0132365, RO530420
Chemical Name	humanized mouse monoclonal rhuMab HER2 γ1 heavy chain (223-214')-disulfide with humanized mouse monoclonal rhuMab HER2 light chain, dimer (229-229":232-232")-bisdisulfide in which the 6-amino groups of an average of 3.5 lysine residues are amidified by 4-({3-[(3-{[(1*S*)-2-{[(1*S*,2*R*,3*S*,5*S*,6*S*,16*E*,18*E*,20*R*,21*S*)-11-chloro-21-hydroxy-12,20-dimethoxy-2,5,9,16-tetramethyl-8,23-dioxo-4,24-dioxa-9,22-diazatetracyclo[19.3.1.110,14.03,5]hexacosa-10,12,14(26),16,18-pentaen-6-yl]oxy}-1-methyl-2-oxoethyl]methylamino}-3-oxopropyl)sulfanyl]-2,5-dioxopyrrolidin-1-yl}methyl)cyclohexylcarbonyl groups
Molecular Formula	$C_{6448}H_{9948}N_{1720}O_{2012}S_{44}\cdot(C_{47}H_{62}ClN_4O_{13}S)n$ Where n ~ 3.5
Molecular Weight	148.781 kDa
CAS Number	1018448-65-1

Structure



Note: The bracketed structure is DM1 plus MCC. The n is on average 3.5 per trastuzumab molecule.

Pharmacological Class HER2-targeted antibody and microtubule inhibitor conjugate

2.2 Relevant IND

IND 71072 originally submitted by Genentech

2.3 Clinical Formulation

Upon reconstitution with 8.0 mL of sterile water, the lyophilized T-DM1 yields a solution of 20 mg/mL T-DM1, 10 mM sodium succinate, 6% w/v sucrose, and 0.02% w/v polysorbate 20, pH 5.0.

The following table from the submission shows the formulation

Ingredients	Strength		Function	Reference to Standard or Specification
	Amount per mL[a]	Amount per Vial[b]		
Trastuzumab Emtansine	20 mg	(b) (4)	Active ingredient	Section 3.2.S.4.1
Sucrose			(b) (4)	NF and Ph. Eur.
Succinic Acid				NF
(b) (4)				NF and Ph. Eur.
Polysorbate 20				NF and Ph. Eur.
				(b) (4)

2.4 Comments on Novel Excipients

There are no Novel excipients.

2.5 Comments on Impurities or Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

T-DM1 is proposed as a single agent treatment of patients with HER2-positive (b) (4) metastatic breast cancer who have received both a trastuzumab- and lapatinib-containing chemotherapy regimen in the metastatic setting, but have progressed following their last treatment.

Dose 3.6 mg/kg
Route IV infusion
Schedule Once every three weeks

For comparison the following doses are approved for trastuzumab (Herceptin)

1) For the adjuvant treatment of HER2-overexpressing breast cancer administer either Initial dose of 4 mg/kg over 90 minute IV infusion, then 2 mg/kg over 30 minute IV infusion weekly for 52 weeks, or Initial dose of 8 mg/kg over 90 minutes IV infusion, then 6 mg/kg over 30−90 minutes IV infusion every three weeks for 52 weeks.

2) For metastatic HER2-overexpressing breast cancer an Initial dose of 4 mg/kg as a 90 minute IV infusion followed by subsequent weekly doses of 2 mg/kg as 30 minute IV infusions.

2.7 Regulatory Background

- 3/14/2005, 9/8/2005 Pre-IND Meetings
- 12/16/2005 New IND Submission
- 6-19/2006 Fast Track Denied
- 12/19/2007 EOP2 Pre Phase 2 Meeting
- 8/8/2008 EOP2 Meeting
- 4/3/2009 FDA agreement that reproductive toxicity testing would not be required
- 3/23/2010 Pre-BLA Meeting—requested further genotoxicity testing
- 7/6/2010 BLA Received
- BLA withdrawn
- BLA resubmitted July 2012

3 Studies Submitted

04-0962-1459	Response of BT474E1 Tumor to 3 Doses of Trastuzumab-MCC-DM1 Administered as an Every 3 Week Regimen in Beige Nude Mice
04-0962 A-1459	Dose Response of Trastuzumab-MCC-DM1 (Administered on a Weekly or Biweekly Basis) Against BT474EI Xenograft Tumors in Beige Nude Mice
05-1111	In Vitro Binding and Activity of Tmab-MCC-DM1
05-1508	Response of MMTV-HER2 Fo5 Tumor to Treatment of Trastuzumab and DM1 Approximately 30 Minutes Later in Nude Mice
07-1030 C	Response of MDA-MB-361.1 Mammary Tumor to Combination Therapy of GDC-0941 and Trastuzumab-MCC-DM1 in Taconic NCR Nude Mice
07-1629	Effect of Single Agent or Combinations of Pertuzumab and Trastuzumab-MCC-DM1 on KPL-4 Tumors Inoculated into the Mammary Fat Pad of SCID Beige Mice
10-0010	Binding of Trastuzumab-MCC-DM1 (T-DM1) to HER2 ECD by Surface Plasmon Resonance (Biacore)® Technology)
10-0046	In Vitro Biological Characterization of Trastuzumab-MCC-DM1 (T-DM1) for Fc Gamma Receptor Binding, Antibody-Dependent Cell-Mediated Cytotoxicity, and C1q Binding Activities
10-0825	In Vitro Characterization of Trastuzumab-MCC-DM1 in Breast Cancer Cell Lines
10-0826	Viability/Proliferation Assays on T-DM1 Clinical Qualification Lots
99-292 X-1459	Specificity of Trastuzumab-MCC-DM1 for HER2 Over-Expressing Mammary Tumors
99-292 ZC-1459	Response of MMTV-HER2 Fo5 Tumor to 3 Doses of Trastuzumab-MCC-DM1 Administered as an Every-3-Week Regimen
99-292 ZD-1459	Dose Response of MMTV-HER2 Fo5 Tumor to Trastuzumab-MCC-DM1 Administered on a Weekly Schedule in Mice

99-292 ZF-1469	Response of MMTV-HER2 Fo5 Tumor to Control Antibody-MCC-DM1
04-0901-4747	The Influence of HERCEPTIN on MMTV-HER2 Fo5 Tumor Response to Trastuzumab-DM1 Conjugates

Study #	Title
05-0754-1459	Validation of a Method for the Determination of DM1 in Sprague Dawley Rat LI-Heparin Plasma Samples by LC-MS/MS
05-0755-1459	Validation of a Method for the Determination of DM1 in Cynomolgus Monkey Li-Heparin Plasma Samples by LC-MS/MS
06-0809	Development and Validation of a Method for the Determination of DM1 in Cynomolgus Monkey Plasma
08-1059	Determination of the Stability of 4 Lots of Tmab-MCC-DM1 in Human and Cynomolgus Monkey Li-Heparin Plasma by LC-MS/MS
4-herc-1-avr-3-4.HERc.1	Total Trastuzumab (PRO132365) Antigen ELISA
4-herc-1-avr-3-4.HERc.2	Trastuzumab-MCC-DM1 (PRO132365) Antigen ELISA
4-herc-1-avr-3-4.HERc.3	Trastuzumab-MCC-DM1 (PRO132365) Antibody ECLA
vr0606	Determination of the Stability of Tmab-MCC-DM1 in Human, Cynomolgus Monkey and Sprague Dawley Rat L1-Heparin Plasma by LC-MS/MS
04-0380-1459	Characterization of the Pharmacokinetics of Her-SMCC-DM1 (Low Drug Load, 2.7 Drugs/Ab), Her-SMCC-DM1 (High Drug Load, 3.6 Drugs/Ab), and Her-MC-MMAE Conjugates in Male Rats
04-0645-1605	Pharmacokinetics of Herceptin ("Her", rhu4D5), Her-MC-vc-PAB-MMAF, Her-MC-MMAF, and Her-SMCC-DM1 Following IV Administration in Athymic (nu/nu) Mice
04-0975-1459	Evaluation of the Pharmacokinetics of PR0132365 (Trastuzumab-SMCC-DM1) Following a Single Intravenous Bolus Dose in Cynomolgus Monkeys with Preliminary Tolerability (Non-GLP)
05-0022-1459	Trastuzumab-SMCC-DM1 (PRO132365): Dose Range-Finding Study in Sprague-Dawley Rats
05-0278-1459	Trastuzumab-MCC-DM1 (PRO132365): Dose Ranging Pharmacokinetics Study in Non-Tumor Bearing Beige Nude Mice
05-282-1459	Trastuzumab-MCC-DM1 (PRO132365): Dose-Ranging Pharmacokinetics Study in Tumor-Bearing (BT474 E1) Beige Nude Mice
05-1104	DM1: Dose Range Finding Study in Male and Female Sprague-Dawley Rats
06-1068	A Study of the Pharmacokinetics of DM1 in Athymic Nude Mice
06-1270	Pharmacokinetics Study of MCC-DM1 in Male and Female Sprague-Dawley Rats
04-1022-1459	Tissue Distribution Study of Herceptin-MCC-[^{3}H] DM1 in Rats
05-0336-1459	Tissue Distribution of [^{125}I]Herceptin and [^{125}I]Herceptin-MCC-DM1 in Normal Rats
08-1136	Determination of Mass Balance and Route of Excretion Following Intravenous Administration of Free [^{3}H]-DM1 in Female Sprague-Dawley

Study #	Title
	Rats
04-1099-1459	Pharmacokinetics, Metabolism, and Excretion of Herceptin-sMCC-[^{3}H]-DM1 in Rat after IV Administration
05-1047-1459	In Vitro Plasma Protein Binding Study of DM1 (a Maytansinoid) in Sprague-Dawley Rat, Cynomolgus Monkey, and Human Plasma
05-0297-1459	Characterization of Plasma Stability of Trastuzumab-MCC-DM1, Trastuzumab-MC-vc-PAB-MMAF, and Trastuzumab-MC-MMAF In Vitro
05-0298-1459	Characterization of Plasma Stability of Trastuzumab-MCC-[^{3}H]DM1 In Vitro
08-1137	Determination of Radioactivity Excreted in the Bile Following a Single Intravenous Administration of [^{3}H]-DM1 or Unlabeled DM1 in Sprague-Dawley Rats
09-1060	Determination of Radioactivity in Plasma and Excreta Matrices following Intravenous Administration of Trastuzumab-MCC-[^{3}H]-DM1 or Unlabeled Trastuzumab-MCC-DM1 in Female Sprague Dawley Rats
09-1344	Inhibition of the Catalytic Activities of Cytochromes P450 in Human Liver Microsomes by DM-1
09-1929	Metabolite Radioprofiling and Identification in Plasma, Bile, and Urine from Rats Following IV Administration of Tmab-MCC-[^{3}H]DM1
09-2381	Metabolite Profiling and Identification of DM1 in Rat
09-2382	Evaluation of Cytochrome P450 Induction Potential by DM1 Using Human Cryopreserved Hepatocytes
09-2416	Reaction Phenotyping of DM1 Using Human Liver Microsomes and Recombinant Cytochrome P450 (rCYP) and Metabolite Profiling in Human
10-1207	In Vitro Interaction Studies of DM1 (Maytansine) with Human MDR1 (ABCB1/P-gp) ABC (Efflux) Transporter
10-1356	Exploratory Quantitative Determination of Lys-MCC-DM1, MCC-DM1, and DM1 in Plasma, Urine, and Bile from Rat following Intravenous Administration of Tmab-MCC-DM1
08-1136	Determination of Mass Balance ad Route of Excretion Following Intravenous Administration of Free [^{3}H]-DM1 in Female Sprague-Dawley Rats
08-1137	Determination of Radioactivity Excreted in the Bile Following a Single Intravenous Administration of [^{3}H]-DM1 or Unlabeled DM1 in Sprague-Dawley Rats
09-1060	Determination of Radioactivity in Plasma and Excreta Matrices following Intravenous Administration of Trastuzumab-MCC-[^{3}H]-DM1 or Unlabeled in Trastuzumab-MCC--DM1 Female Sprague-Dawley Rats
05-1359	Pharmacokinetic, Pharmacodynamic, Toxicokinetic, and Toxico-dynamic Modeling and Simulation Analysis of PRO32365 (Trastuzumab-MCC-DM1)
07-1474	Pharmacokinetic Comparability of Trastuzumab-MCC-DM1 (b) (4) in Female Cynomolgus Monkeys
07-1475	Pharmacokinetic Comparability of Trastuzumab-MCC-DM1 (b) (4) in Female Sprague Dawley Rats

Study #	Title
08-0800	Pharmacokinetic Comparability of Trastuzumab-MCC-DM1 (b) (4) in Female Cynomolgus Monkeys

Study #	Title
03-0140-1345	Safety Evaluation of Various Herceptin-DM-1 Lots; Herceptin-SPP-DM1 and Herceptin-DM1 with an Un-Cleavable Linker (Herceptin-SMCCDM1), and Free DM1 in Female Sprague-Dawley Rats
04-0976-1459	Single Dose Intravenous Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) in Cynomolgus Monkeys with a 3-Week Recovery Period
04-1214-1459	Single Dose Intravenous Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) in Sprague-Dawley Rats with a 3-Week Recovery Period
05-1157	A Dose Range-Finding Study of Intravenous DM1 in Female Sprague-Dawley Rats with a 3-Week Recovery Period
05-1191	Single Dose Intravenous Toxicity Study of DM1 in Sprague-Dawley Rats with a 3-Week Recovery Period
07-0389	A Single Dose 12-Day Intravenous Toxicity Study of Hu Tmab-MCC-DM1, Thio Hu Tmab-MPEO-DM1, Thio Hu Tmab-MC-MMAF, and Thio Hu Tmab-MC-vc-PAB-MMAE in Female Sprague-Dawley Rats (Crl:CD(SD))

Study #	Title
03-0674-1459	Trastuzumab-SMCC-DM1: Dose Range Finding Study in Cynomolgus Monkeys
04-0977-1459	Multiple Dose Intravenous Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) Administered to Cynomolgus Monkeys Once Every 3 Weeks for 4 Doses, with a 3- or 6-Week Recovery Phase
07-0653	An Intravenous Chronic Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) Administered to Cynomolgus Monkeys Once Every 3 Weeks for 8 Doses, with a 6-Weeks Recovery Phase
07-0655	A 6-Week Repeat Dose Intravenous Toxicity Study of Thio mab MC MMAF, Thio Tmab vc MMAE, Thio Tmab mpeo DM1, and Tmab-MCC-DM1 in Cynomolgus Monkeys
04-0669-1459	Safety Evaluation of Multiple-Dose Intravenous Herceptin-SMCC-DM1 Immuno-conjugates in Female Sprague-Dawley Rats

Study #	Title
09-2654	N2'-deacetyl-N2'-(3-mercapto-1-oxoproply) maytansine (DM1): Salmonella—Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay
09-2726	N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl) maytansine (DM1): *In Vivo* Rat Bone Marrow Micronucleus Assay
07-0653	An Intravenous Chronic Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) Administered to Cynomolgus Monkeys Once Every 3 Weeks for 8 Doses, with a 6-Weeks Recovery Phase

Study #	Title

Study #	Title
04-1031-1605	A Single Dose Intravenous Cardiovascular Safety Pharmacology Study of Trastuzumab-MCC-DM1 (PRO132365) Administered to Female Cynomolgus Monkeys with a 3 Week Recovery Period
04-1215-1605	Cross-Reactivity of Trastuzumab-MCC-DM1 (PRO132365) with Human and Cynomolgus Monkey Tissues In Vitro
04-1257-1459	Hemolytic Potential and Blood Compatibility of Trastuzumab-MCC-DM1 (PRO132365)
05-0617-1459	Safety Evaluation of Single-Dose Intravenous Herceptin-MCC-DM1 Immuno-conjugates in Female Sprague-Dawley Rats
05-0775-1459	Safety Evaluation of Single-Dose Intravenous MCC-DM1 in Female Sprague-Dawley
05-0848	Single Dose Intravenous Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) with 5-7% Unconjugated Maytansinoid in Cynomolgus Monkeys with a 3-Week Recovery Period
09-0234	Effects of N2'-deacetyl-N2'-(3-mercapto-1-oxoproply) maytansine (DM1) on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

3.1 Studies Reviewed

See Table of Contents

Abbreviations

BLQ – Below the limit of quantitation
ECD – Extracellular Domain
NSS – not statistically significant
ND – not done
NR – not relevant
SS – statistically significant

4 Pharmacology

4.1 Primary Pharmacology

1) *In Vitro* Binding and Activity of Tmab-MCC-DM1

Study number	05-1111
Filename	05-1111-i (b) (4) -final.pdf
Laboratory	Genentech, Inc., South San Francisco, CA 94080
Date	July 2005
GLP	No
Audited	No
Drug	PRO132365 (trastuzumab-MCC-DM1): Lot 1808-89 (Research Lot) Lot TMCCDM105-1 (Reference Lot), Lot FIL037D01 (Tox Lot), Lot L14897 (GMP Bulk Lot); Trastuzumab: Lot 41936-10 (Research Lot) Lot N29126AX, Lot 1097-3, Lot HER 401-4 MCC-DM1: Lot 44323-3, Lot G027797, Lot TNB46312-59 Free DM1: Lot 41869-66
Method	
Cell lines	Breast cancer cell lines SK-BR-3, BT-474, MCF7, and MDA-MB-468, Ovarian cancer line SK-OV-3, Non-small cell lung cancer (NSCLC) line Calu-3 Gastric carcinoma cell line MKN7 BT-474-EI cell line was established at Genentech by serial passage of BT-474 cells in nude mice in the absence of estrogen supplementation
Binding assay	Microtiter plate wells were coated with HER2 Extracellular Domain (ECD) at 0.05 M to test binding of trastuzumab or trastuzumab emtansine serial dilutions.
Binding assay	Trastuzumab-MCC-DM1 and Trastuzumab binding to a variety of human Fcγ receptors (FcγR) was evaluated using ELISA. These included FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa F158, and FcγRIIIa V158. Binding of Rituximab to these receptors was tested as an assay control. The human Fcγ receptors were expressed as recombinant fusion proteins of the ECD of the receptor alpha chains with Gly/His6/GST. Anti-GST-coated, BSA-blocked assay plates were used to capture the FcγR.

The investigators did not provide descriptive statistics or actual values in this report, only graphs and charts.

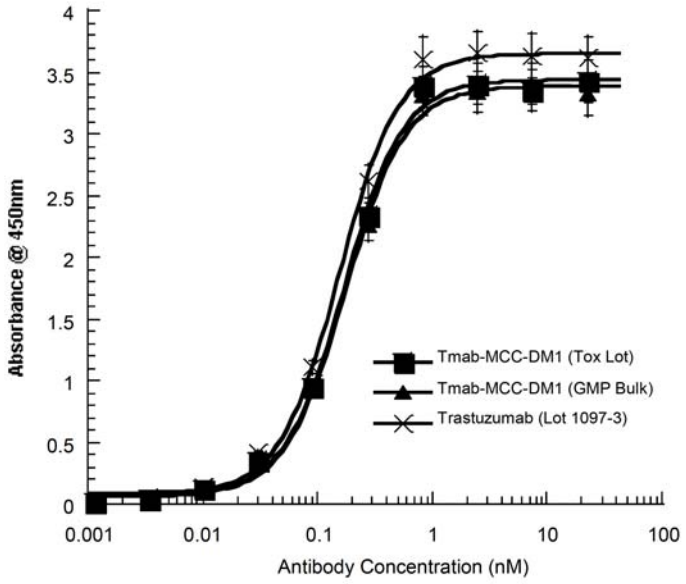
Results

Trastuzumab-MCC-DM1 Binding to HER2 ECD *in vitro*

The following figure from the study report shows the binding of TDM-1 used for the toxicology experiments (Tox Lot) to isolated HER2 extracellular domain. 'Tox Lot' is the lot used in the repeat dose study in monkeys given drug every three weeks for four doses (2005 *vide infra*). It

is not the lot used in the repeat dose study in monkeys given drug every three weeks for eight doses (2007).

Figure 2
Comparison of the Binding of Trastuzumab-MCC-DM1 and Trastuzumab to HER2 ECD



The Binding constants are:

Ligand	Lot	Ec_{50} (nM)
Trastuzumab-MCC-DM1	Tox Lot FIL037D01	0.170
Trastuzumab-MCC-DM1	GMP Bulk Lot L14897	0.174
Trastuzumab	Lot 1097-3	0.155

The report does not provide statistical comparisons, but the values are probably indistinguishable within the limits of the experiment.

Binding to off target immunological cell surface receptors

Gessner *et al.* demonstrated that the binding of the Fc region of IgG molecules to FcγR, a cell surface receptor found on a variety of immunological cells, can initiate a variety of immunological functions such as antibody-dependent cell-mediated cytotoxicity.[7] Effector cells that modulate this cytotoxicity include granulocytes, macrophages, and natural killer (NK) cells. There are two isoforms of FcγRII. One form, FcγRIIa, has an activating cytoplasmic domain while the second form, FcγRIIb, has an inhibiting cytoplasmic domain. FcγRIII has a polymorphism at amino acid residue 158. The phenylalanine allotype (FcγRIIIa F158) has a lower affinity for IgG1 than the valine allotype (FcγRIIIa V158). Monomeric IgG antibodies are capable of binding FcγRIa with high affinity. Binding of monomeric IgG with FcγRIIa, FcγRIIb,

[7] Gessner JE, Heiken H, Tamm A, Schmidt RE. The IgG Fc receptor family. Ann Hematol 1998;76:231−48.

and FcγRIIIa occurs with lower affinity. Multimeric IgG binds to the IIa, IIb, and IIIa receptors with increased binding affinity. Therefore, for the low-affinity receptor binding assays, test antibodies were cross-linked before testing. The investigators in this experiment evaluated the binding of trastuzumab emtansine (Reference Lot TMCCDM105-1) and trastuzumab (Lot Her401-4) to FcγRIa, FcγRIIa, FcγRIIb, FcγRIIIa V158, and FcγRIIIa F158 to determine whether covalent binding of trastuzumab to form trastuzumab emtansine results in altered binding. They included the binding of Rituximab to these receptors as an assay control.

The table below shows the EC_{50} values for these experiments:

Fcγ Receptor	Rituxan Control µg/mL	Trastuzumab µg/mL	Trastuzumab nM	TDM1 µg/mL	TDM1 nM
FcγRIa	0.0049	0.0056	0.038	0.0058	0.039
FcγRIIa	1.3	2.8[a]	19.2	0.8[a]	5.4
FcγRIIb	8.9	ND	ND	ND	ND
FcγRIIIa F158	11.6	ND	ND	1.5	10.1
FcγRIIIa V158	1.4	1.2	8.2	0.3	2.0

ND = not determined; binding curve did not show upper asymptote.
[a] Estimated value; binding curve did not well define an upper asymptote.

The EC_{50} values for the binding of rituxan, trastuzumab emtansine and trastuzumab to FcγRIa were similar and probably not statistically distinguishable. Binding is notably avid. These values in the nM range demonstrate tight binding to this surface receptor. This may explain some of the acute (day three) white cell toxicity seen in monkeys (*vide infra*).

The curves for type RII and RIII receptors did not demonstrate an upper asymptote, but did demonstrate a distinct upper sigmoid inflection; thus, investigators could estimate the EC_{50} values by inspection. The EC_{50} values for the binding of both trastuzumab emtansine and trastuzumab with type RII and RIII receptors was in the µg/mL range. This is consistent with lower binding affinities expected for these receptors. These concentrations are probably greater than those that are clinically relevant. The binding of curves for RIIb remained within the linear portion of the dose response curve, so an estimation of the EC_{50} was not possible. Nevertheless, the value is above 10 µM and thus clinically irrelevant.

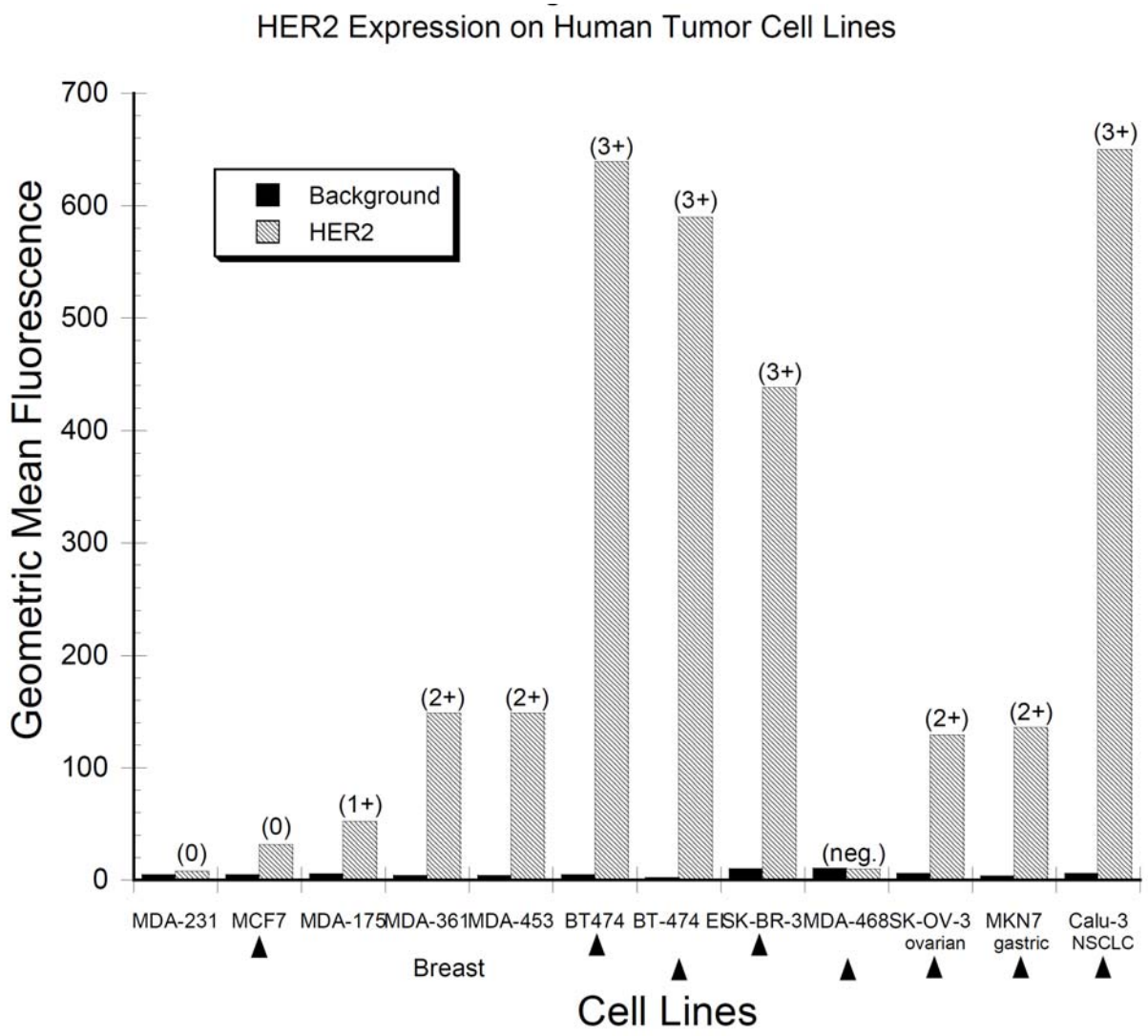
The binding curves for trastuzumab and trastuzumab emtansine are consistently different for all but the tight binding RIa receptor. In each case, Trastuzumab EC_{50} values are greater than those of trastuzumab emtansine by a factor of three. While this probably does not have any pharmacological implications at these concentrations, it does demonstrate that the binding of the antibody is different from the binding of the conjugate. The bound DM1 appears to increase the affinity of the antibody for these receptors.

In an assay to determine the binding of trastuzumab emtansine to complement protein C1q, the investigators determined an EC_{50} for trastuzumab emtansine of 1.4 µg/mL and an EC_{50} for trastuzumab of 1.2 µg/mL. The EC_{50} for the Rituxan control was 1.2 µg/mL. Again, these values are above those concentrations achieved clinically.

Anti-Proliferative Activity of Trastuzumab-MCC-DM1 in HER2-Positive Breast Cancer Cells

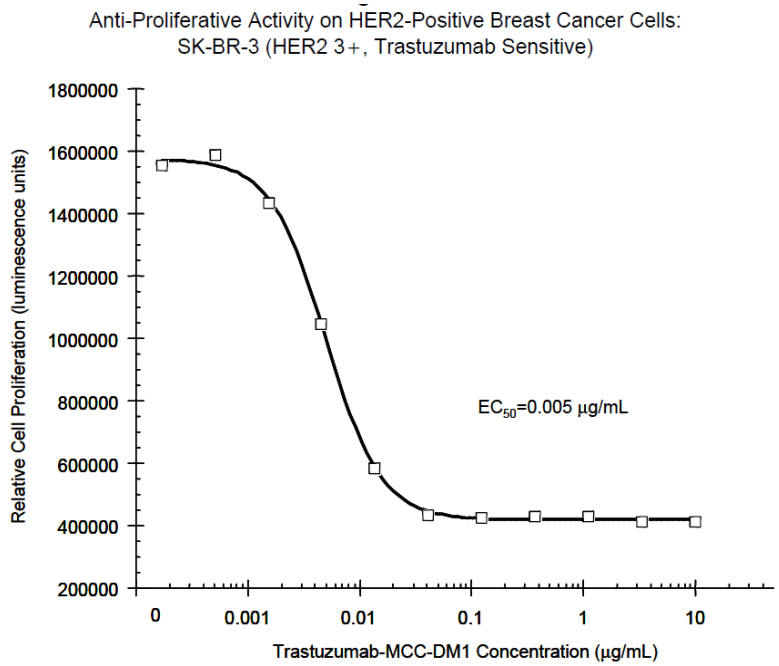
The investigators studied the ability of the different therapeutic antibodies to slow or stop the growth of in SK-BR-3, BT-474, MCF7, and MDA-MB-468 cells, *in vitro*. They selected these cell lines based on measurements of cell surface HER2-expression. The following graph

from the study report shows that HER2 3+ cells, SK-BR-3 and BT-474, are sensitive to trastuzumab. MCF7 and MDA-MB-468 cells are HER2 normal and HER2 negative, respectively.



Decrease in cellular viability at 72 hours as a function of antibody concentration

The following curve shows the decrease in the number of viable SK-BR-3 HER2 3^+ positive cells at 72 hours after treatment with increasing concentrations of trastuzumab emtansine.



The following table shows the EC_{50} values calculated for the rest of the cell lines the investigators tested.

Cell Line	HER2 sensitivity	EC_{50} µg/mL	Tumor Tissue
SK-BR-3	3+	0.005	Breast
BT-474	3+	0.145	Breast
MCF7	0 (normal)	>5	Breast
MDA-MB-468	Negative	>2	Breast

These results demonstrate that cells that over express HER2 are more sensitive to the toxicity of trastuzumab emtansine than cells that do not. The study report does not present comparable data for trastuzumab or for the cell lines derived from other cancers.

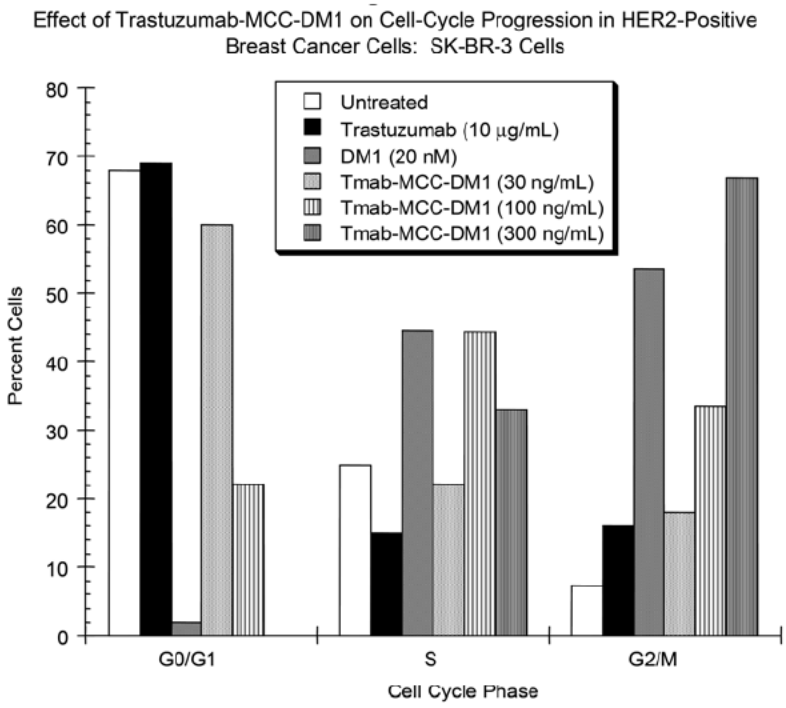
Induction of Apoptosis by Trastuzumab-MCC-DM1 as Measured by Caspase-3 and -7 Activation

The investigators studied the ability of trastuzumab emtansine to induce apoptosis *in vitro* by measuring caspase-3 and -7 activity in SK-BR-3 and BT-474 cells. Trastuzumab-MCC-DM1 induced increasing caspase-3 and -7 activation with increasing dose in both cell types. The EC_{50} in SK-BR-3 cells was 0.028 µg/mL (~ 0.19 nM) and the EC_{50} in BT-474 cells was approximately 2 µg/mL (13 nM). Trastuzumab did not induce caspase-3 and -7 activation (data not shown). These data suggest that, after internalization, DM-1 causes sufficient cell damage to initiate apoptosis in these cancer cell types, *in vitro*.

Effect of Trastuzumab-MCC-DM1 on Cell-Cycle Progression in HER2-Positive Breast Cancer Cells

Once DM1 is cleaved from trastuzumab emtansine, it can bind to β-tubulin. In doing so it inhibits microtubule assembly (*vide supra*). Microtubules must assemble in a precise manner

in order for a cell division to progress through the G_2/M stage of the cell cycle. Thus, binding of DM1 to β-tubulin blocks cell division. When a dividing cell is arrested in G_2/M, it initiates apoptosis and dies. On the other hand, trastuzumab blocks a cell surface signal responsible for the initiation of mitosis. Inhibition of this signal results in cells remaining in at the G0/G1 transition. The investigators in this study used flow cytometry to determine the *in vitro* effects of trastuzumab emtansine, trastuzumab and DM-1 on cell cycle arrest in human tumor cells after 48 hours of treatment. The following chart from the study report shows the results of this experiment for SK-BAR-3 breast cancer cells.



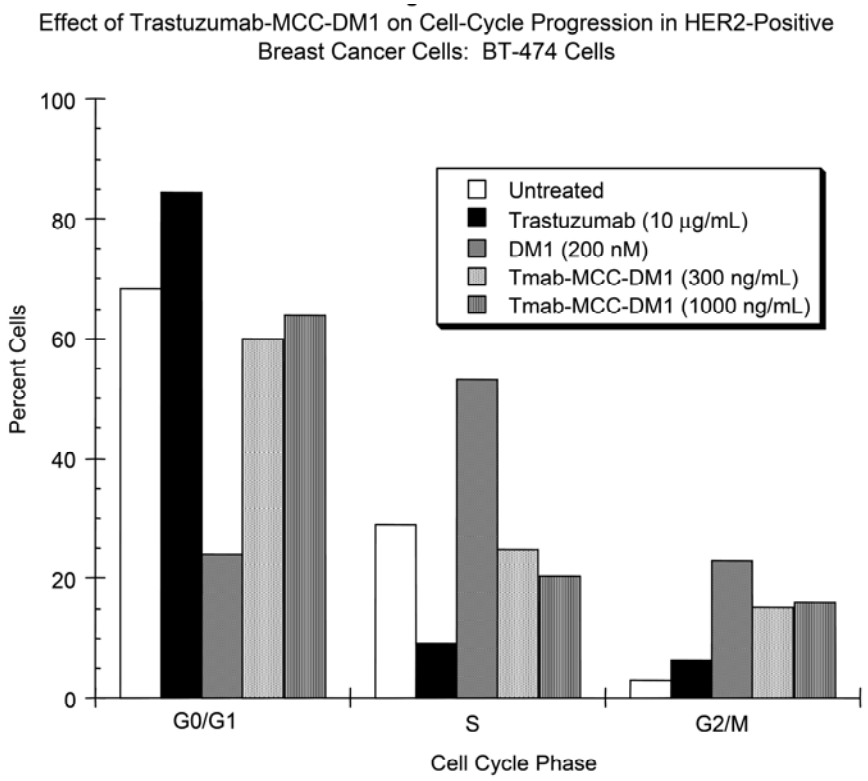
The chart shows results that are somewhat consistent with the postulated mechanisms. With trastuzumab alone or low dose trastuzumab emtansine most of the cells remain quiescent in G_0/G_1, but this result is similar to control suggesting that these treatments have little effect on cell division. With DM1 alone, few cells remain in G_0/G_1 while most of them are either in S transition or stopped at G_2/M. Similar results appertain for the two higher doses of trastuzumab emtansine, particularly with the highest concentration of trastuzumab emtansine where no cells are detected in G_0/G_1 and nearly 70% are stopped at the G_2/M transition.

The following table shows estimates of the molar concentrations of trastuzumab emtansine in the experiment above using a FW of 148781 g/mole and a molar ratio of DM-1 to trastuzumab of 3.8.

Concentration TDM1			DM-1
ng/ml	ng/L	nM	nM
30	30000	0.20	0.77
100	100000	0.67	2.55
300	300000	2.02	7.66

The calculated value of the concentration of DM-1 in the HD trastuzumab emtansine experiment is less that the concentration of DM-1 in the analogous experiment, but not as much as one might expect.

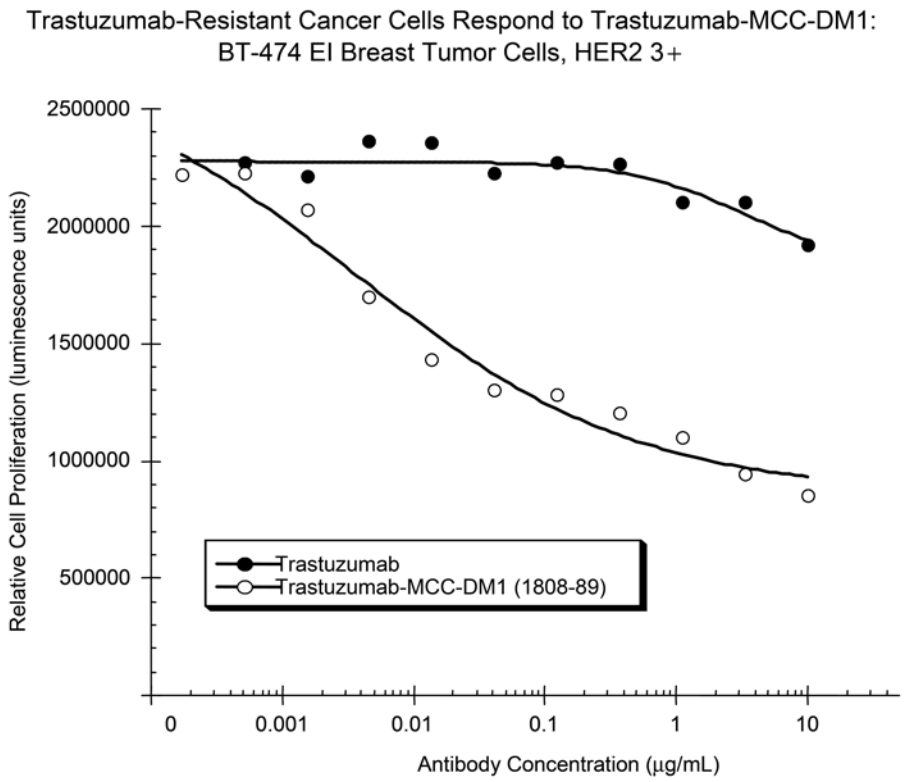
In BT-474 cells, HER2 is amplified and they express a mutated PI3kinase. The following graph shows the results of treatment of these cells with the different drugs. The investigators did not provide descriptive statistics



Trastuzumab treatment of these cells inhibited progression of the cells out of G_0/G_1; few cells advanced to S Phase or G_2/M. These cells were relatively insensitive to DM-1 or trastuzumab emtansine.

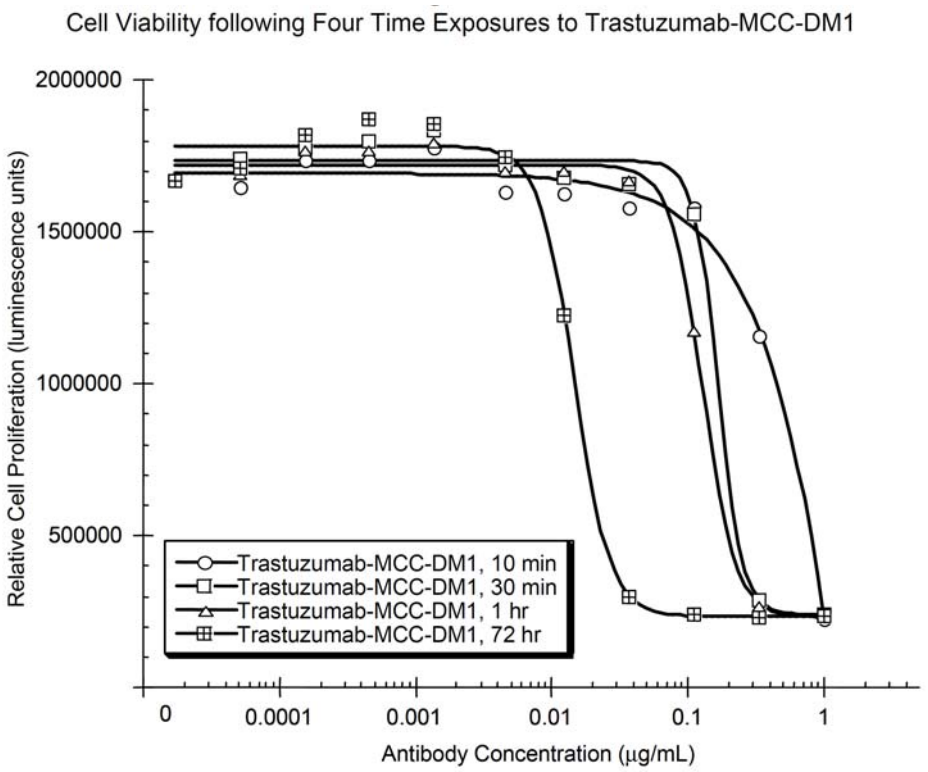
Response of HER2-Positive, Trastuzumab-Resistant Cancer Cells to Trastuzumab-MCC-DM1 and Trastuzumab

The investigators evaluated the ability of trastuzumab emtansine to inhibit proliferation in HER2 cell lines that were selected to be resistant trastuzumab, Calu-3, SK-OV-3, MKN7, and BT-474-EI. Trastuzumab did not inhibit cell growth in any of these cell lines appreciably at concentrations up to 10 μg/mL. The following graph is illustrative of the results for all four cell lines. The other cell lines behaved more or less similarly. The investigators did not provide any quantitation of the difference in inhibition for these cell lines.



Time dependence of the effect of trastuzumab emtansine on SK-BR-3 cells

The investigators state that exposure of SK-BR-3 cells to cytostatic concentrations of trastuzumab is reversible once the antibody is washed off but did not present the data. In this study they incubated SK-BR-3 cells with trastuzumab emtansine for 10 minutes, 30 minutes, one hour or 72 hours at different concentrations. After this exposure they washed the cells and incubated them in medium for 72 hours. The following graph from the study report shows the results of this study.



Inhibition of cell growth increased with increasing time of exposure, but the effect was relatively small in the first 60 minutes and the results are not adequately quantified. Exposure for the full 72 hours inhibits proliferation at least one order magnitude more than in the other three exposure times. The experiment does not necessarily demonstrate a lasting effect as one cannot determine whether the effect seen is due to the length of exposure or the reversal of the effect. It is possible that all groups were inhibited to the same extent as the 72 hour exposure, but the inhibition was reversible once the antibody was washed off.

In Vitro Potency Bioassay in BT-474 Cells: Reduced Potency Observed for Stability Samples Stored at 40°C for 1 Month Shows that Potency Assay is Stability-Indicating

The investigators developed an assay to demonstrate equivalent potency across lots of trastuzumab emtansine using BT-474 cells. This assay met the applicant's acceptance criteria and was adequate to demonstrate a significant decrease in potency when a sample of the trastuzumab emtansine Toxicology Lot was stored at 40°C for one month.

Comparison of Anti-Proliferative Activity of Trastuzumab-MCC-DM1 with Trastuzumab, Anti-IL8-MCC-DM1, Free DM1, or Free MCC-DM1 in BT-474 Cells

The investigators used the above assay to compare the inhibition of proliferation among five different treatments: trastuzumab emtansine, trastuzumab, a control DM1 conjugate (anti-IL8-MCC-DM1), free DM1, or free MCC-DM1. The following table from the study report shows the results of this experiment.

EC_{50} Values (ng/mL) for Anti-Proliferative Activity

Molecule	04JUN05 Assay	09SEP05 Assay				Mean	% Activity vs. Trastuzumab-MCC-DM1
		Assay 1	Assay 2	Assay 3	Assay 4		
Trastuzumab-MCC-DM1 EC_{50} (nM) [a]	0.5	0.5	0.4	0.5	0.5	0.5	100.0
Trastuzumab EC_{50} (nM) [a]	1.6	1.9	NT	NT	1.9	1.8	28.2
DM1 Bound (trastuzumab-MCC-DM1) EC_{50} (nM) [b]	NT	1.6	1.5	1.8	1.6	1.6	100.0
DM1 Free EC_{50} (nM)	NT	32.3	32.7	35.4	NT	33.5	4.8
MCC-DM1 Free EC_{50} (nM)	NT	NT	NT	114.2	185.2	149.7	1.1

Notes: EC_{50} values for each assay are the average of two measurements.
[a] Molar concentration of antibody.
[b] Molar concentration of bound DM1 in trastuzumab-MCC-DM1.

Comparison of Anti-Proliferative Activity of Free DM1 or Free MCC-DM1 in BT-474 and SK-BR3 Cells

The investigators studied the dose response for cell growth inhibition of the free maytansinoids (DM1 and MCC-DM1) in BT-474 and SK-BR3 cells. TDM-1 hydrolyzes to yield these compounds *in vivo*. There is evidently some hydrolysis during storage. The following table shows the EC50 values determined in this experiment.

Drug	BT-474	SK-BR3
DM1 nM	32	~ 5
MCC-DM1 nM	114	~ 50

The assays for the two cell types were somewhat different, so direct comparison is not possible. Nevertheless, the assays suggest that MCC-DM1 is less toxic than DM1.

2) Binding of Trastuzumab-MCC-DM1 (T-DM1) to HER2 ECD by Surface Plasmon Resonance (Biacore Technology)

Study number	10-0010
Filename	10-0010-(b) (4)-final.pdf
Laboratory	Genentech, Inc., South San Francisco, CA 94080
Date	October 2009
GLP	No
Audited	No
Drug	T-DM1 Reference Material, Lot TMCCDM1208-2
	HER2 ECD, Lot 002
	T-DM1 Phase II, Lot B25
	T-DM1 Phase III Qualification Lots B107, B108,and B109
	Trastuzumab Reference Material, Lot HER401-4
	Bevacizumab Reference Material, Lot antiVEGF408-3
Method	
Assay	Ligand immobilized in flow cells
Analysis	surface plasmon resonance (SPR)
Control	Bevacizumab
Instrument	Biacore 3000 (GE Healthcare; Piscataway, NJ)
	Biacore 3000 BIAevaluation Software, version 4.1, assuming 1:1 binding

Results

The following table from the study report shows that all of the preparations of TDM-1 demonstrated similar binding affinity with HER2 ECD. There were no statistically significant differences among any of the preparations.

Kinetic Analysis and Binding Affinity of HER2 ECD to Indirectly Bound Ligand Trastuzumab or T-DM1 Lots from SPR Analysis Using a Biacore 3000 Instrument

Analyte	Fitting Model [a]	Ligand	Mean (±SD)	k_a (1/Ms)	k_d (1/s)	K_D (M)	χ^2 (RU^2)
HER2 ECD (n=4)	1:1	trastuzumab	Mean	2.16E+05	2.21E−04	1.01E−09	0.667
			SD	1.94E+04	5.42E−05	1.78E−10	0.165
		T-DM1 Ref. Mat.	Mean	2.19E+05	2.31E−04	1.06E−09	0.704
			SD	1.67E+04	3.38E−05	1.44E−10	0.216
		T-DM1 Lot B25	Mean	1.93E+05	2.44E−04	1.27E−09	0.552
			SD	6.56E+03	7.63E−06	6.85E−11	0.059
		T-DM1 Lot B107	Mean	2.11E+05	2.07E−04	9.86E−10	0.542
			SD	2.46E+04	4.44E−05	2.25E−10	0.158
		T-DM1 Lot B108	Mean	2.06E+05	2.20E−04	1.08E−09	0.642
			SD	1.15E+04	2.90E−05	1.67E−10	0.184
		T-DM1 Lot B109	Mean	2.08E+05	2.13E−04	1.03E−09	0.753
			SD	1.18E+04	4.59E−05	2.58E−10	0.506
		T-DM1 (all lots)	Mean [b]	2.07E+05	2.23E−04	1.08E−09	0.638
			SD [b]	1.62E+04	3.40E−05	1.93E−10	0.255

χ^2=measure of the average squared residual (difference between the experimental data and the fitted curve); ECD=extracellular domain; k_a=association rate constant; k_d=dissociation rate constant; K_D=dissociation equilibrium constant; Ref. Mat.=reference material; RU=response unit; SPR=surface plasmon resonance; T-DM1=trastuzumab-MCC-DM1.

Note: The Biacore 3000 instrument was manufactured by GE Healthcare (Piscataway, NJ).

[a] Fitting model with built-in mass transport limitation correction.

[b] Mean and SD of raw data.

The average KD values were determined to be 1.08 nM for T-DM1 (all lots) and 1.01 nM for trastuzumab. HER2 ECD bound to T-DM1 lots with average k_a and k_d values of $2.07*10^5$ 1/Ms and $2.23*10^{-4}$ 1/s, respectively. The same protein bound to trastuzumab with average k_a $2.16*10^5$ 1/Ms and k_d of $2.21*10^{-4}$ 1/s.

3) *In Vitro* Characterization of Trastuzumab-MCC-DM1 in Breast Cancer Cell Lines

Study number	10-0825
Filename	10-0825-(b) (4)-final.pdf
Laboratory	Genentech, Inc., South San Francisco, CA
Date	July 2007 to March 2010
GLP	No

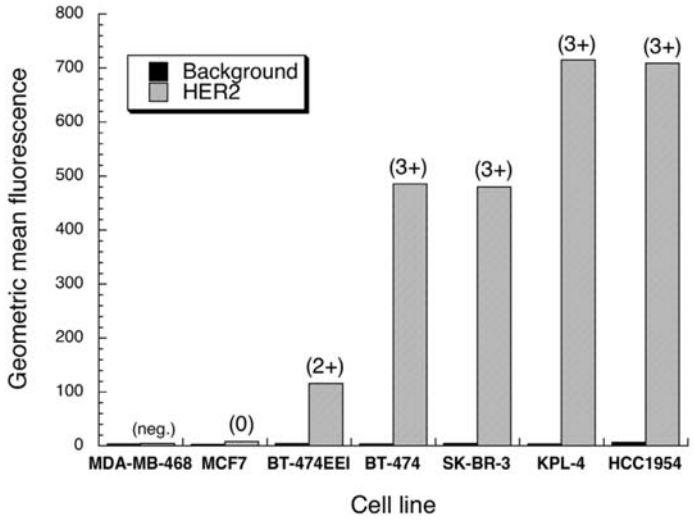
Audited	No
Drug	Trastuzumab-MCC-DM1, Lots 1808-89, FIL037F01 and TMCCDM1208-2 (pool of Phase III Clinical Lots 101 and 102) Trastuzumab, Lots N9126AX, M59316, and reference material HER401-4 L-DM1, Lot 1696-194 L-DM1-methyl, Lot 2454.06 MCC-DM1, Lot G-027797.1-2 L-lysine-MCC-DM1, Lot 2283.20 SMCC: M5525, Lot 048K5007
Method	
Cells	MDA-MB-468 Breast cancer (PTEN mutation, HER2 negative) MCF7 (ER positive, HER2 overexpression) BT-474 (HER2 overexpression, ER positive) SK-BR-3 Breast cancer (HER2 overexpression) HCC1954 Breast cancer (HER2 overexpression, ER and PR negative) KPL-4 Breast cancer (HER2 over expression) BT-474EEI (an exogenous estrogen-independent tumorigenic subline of BT-474 developed at Genentech). BT-474-M1 (a tumorigenic subline of the BT-474 breast cancer cell line)
N	≥ 3
Analysis	Fluorescence Activated Cell Sorting (FACS) Flow Cytometry ELISA for HER2 extra cellular domain (ECD) using BT-474-M1 cells

Results

Anti-Proliferative Activity of T-Dm1 in Breast Cancer Cell Lines

The following graph from the study report show that the different cell lines used in this experiment expressed HER2 to a different degree. The MDA-MB-468 cell line does not overexpress HER2 and so serves as a control.

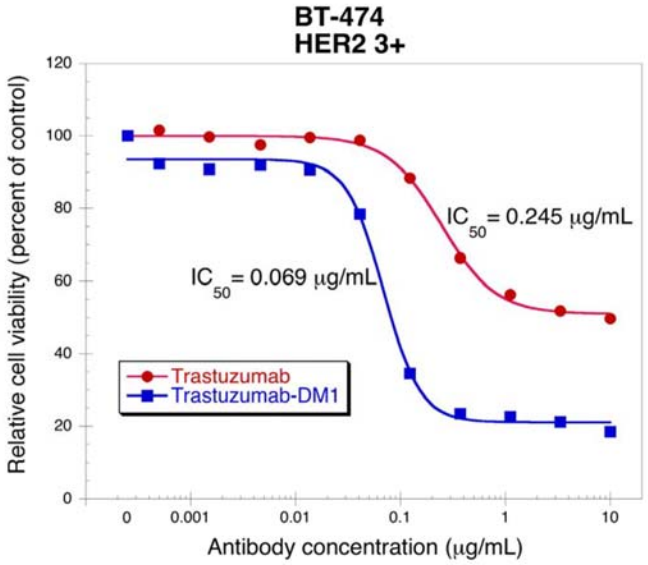
Figure 2
FACS Analysis for HER2 Expression



FACS = fluorescence-activated cell sorting.

The investigators assessed the diminution of normal proliferation *in vitro* caused by trastuzumab emtansine or herceptin for each cell line. The following graph shows a typical graph of the results. Graphs for other cell lines were similar.

Figure 3
Anti-Proliferative Activity of T-DM1



The following table shows the results for all the cell lines tested.

Cell Line	HER2 Expression	IC_{50} TDM1 µg/mL	IC_{50} Trastuzumab µg/mL	IC_{50} TDM1 nM	IC_{50} Trastuzumab nM
BT-474	3+	0.069	0.245	0.46	1.68
SK-BR-3	3+	0.006	0.059	0.04	0.41
KPL-4	3+	0.009	>10	0.06	>70
HCC1954	3+	0.043	>10	0.29	>70
BT-474EE1	2+	0.018	>10	0.12	>70
MCF7	0	>10	>10	>70	>70
MDA-MB-468*	0	2	>10	13.44	>70

* I estimated the value for trastuzumab emtansine from the graph. There were too few data points for the program the investigators were using to calculate an IC_{50} value.

These results raise the question of why trastuzumab inhibits cell growth in some cell lines that overexpress HER2 but not others.

Induction of Apoptosis by T-Dm1: Activation of Caspases 3 and 7

The investigator estimated the ability of T-DM1 to induce apoptosis *in vitro* by measuring caspase 3/7 activity in SK-BR-3, BT-474, and KPL-4 breast cancer cells after exposure to the drug for 48 hours. Caspases initiate a cascade of enzymatic reaction that culminates in apoptosis. Caspases 3 and 7 activities increased with increasing dose of TDM1 in three cell lines. Trastuzumab did not induce caspase 3/7 activity in trastuzumab-sensitive (SK-BR-3 and BT-474) or trastuzumab-resistant (KPL-4) cell lines. The report states that this is consistent with the reported cytostatic mechanism for trastuzumab *in vitro*, but did not cite a reference. The experiment did not avail the use of a non-HER2 expressing cell line as a control. The following table shows that TDM1 induced caspase activity at nM concentrations while trastuzumab did not at concentrations less than 70 nM.

Cell Line	HER2 Expression	IC_{50} TDM1 µg/mL	IC_{50} Trastuzumab µg/mL	IC_{50} TDM1 nM	IC_{50} Trastuzumab nM
BT-474	3+	0.761	>10	5.11	>70
SK-BR-3	3+	0.028	>10	0.19	>70
KPL-4	3+	0.016	>10	0.11	>70

Effect of T-Dm1 Individual Components and Identified Catabolites on Cell Viability

The investigators designed this experiment to assess the contribution of the individual components of TDM-1 to the ability of that compound to affect retardation of cell proliferation. They exposed SK-BR-3 cells to T-DM1, L-DM1, L-DM1-methyl, MCC-DM1, L-lysine-MCC-DM1, SMCC (linker), or unconjugated trastuzumab at concentrations of 0.0005 to 10 nM for five days. L-DM1 contains a free sulfhydryl (SH) moiety which binds covalently to the antibody. Sulfhydryl groups can react with other sulfhydryl-containing molecules in cells or tissue culture medium (e.g., glutathione or albumin) thus modifying the concentration of free compound. Therefore, the investigators included an experimental group using L-DM1-methyl, containing a methyl-protected SH, for comparison. Studies using radiolabeled T-DM1 in cultured tumor cells and in xenograft tumors and in rats have shown that T-DM1 is catabolized to L-lysine-MCC-DM1 and

MCC-DM1 (*vide infra*). Thus, the investigators included L-lysine-MCC-DM1 and MCC-DM1 in this experiment.

Only T-DM1 and unbound DM1 (L-DM1 and L-DM1-methyl) diminished cell growth substantially at concentration less than 10 nM. The IC_{50}s for trastuzumab emtansine and LDM-1-methyl were 0.02 nM. The IC_{50} for unmethylated L-DM1 was 0.4 nM. Trastuzumab showed signs of inhibiting cell growth at concentrations greater than 1 nM but the experiment was not done at concentrations sufficiently high to define this toxicity. Likewise, MCC-DM1 showed signs of toxicity at concentrations of 5 and 10 nM. Nevertheless, the experiment is not informative of the *in vivo* situation because the C_{max} for trastuzumab emtansine after a dose of 3.6 mg/kg is about 87 μg/mL, or about 587 nM. This concentration is much higher than the concentrations used in this experiment. In similar experiments at higher concentrations, the IC_{50} of trastuzumab for SK-BR-3 cells was greater than 338 nM.[8]

Effects of T-Dm1 on Cell Cycle Progression in Her2-Positive Breast Cancer Cells

In this set of experiments the investigators determined the effects trastuzumab, L-DM1, and T-DM1 on cell cycle progression in SK-BR-3 and BT-474 breast cancer cell. Cells were exposed to the different drugs for 48 hours and DNA was stained with propidium iodide for quantification of the different cell cycle phases. In previous experiment, a concentration of trastuzumab of 10 µg/mL (69 nM) trastuzumab caused cells to arrest in the G1 phase of the cell cycle.[9] In this experiment, SK-BR-3 cells were incubated with 30, 100, or 300 ng/mL (0.2, 0.67 or 6.7 nM) T-DM1; BT-474 cells were incubated with 300 or 1000 ng/mL (2 nM or 6.7 nM) T-DM1. Exposure to trastuzumab emtansine resulted in a dose-dependent increase in G_2/M phase cells in both cell lines, as would be predicted by a drug that disrupts microtubule function. L-DM1 exposure (20 nM for SK-BR-3 and 200 nM for BT-474) also caused an increase in cells in the G_2/M cell cycle phase. Exposure to L-DM1 also resulted in increased S-phase cells and decreased G_0/G_1 cells, indicating that cells were still traversing S-phase. These cell cycle alterations were also observed in SK-BR-3 cells exposed to trastuzumab emtansine. In BT-474 cells, the predominant effect of trastuzumab emtansine was an increase in cells in G_2/M, with little change in the proportion of cells in the G_0/G_1 or S-phases. The trastuzumab-insensitive line, KPL-4, was also analyzed for trastuzumab emtansine cell cycle effects. In this cell line, trastuzumab emtansine caused a dose-dependent increase in the proportion of cells in the G_2/M phase as well as in cells undergoing apoptosis (sub-G_0/G_1). The lowest concentration of trastuzumab emtansine tested, 25 ng/mL, had no effect on cell cycle progression. Nevertheless, exposure to 50 ng/mL resulted in a decline in G_1 cells and increased G_2/M and apoptotic cells. KPL-4 cells exposed to 100 or 200 ng/mL trastuzumab emtansine showed substantial changes in all cell cycle phases: decreased G_1 and S-phase cells, with pronounced increases in the proportion of G_2/M-phase and apoptotic cells. These results show that in trastuzumab-insensitive breast cancer cells, trastuzumab emtansine induces a G_2/M-phase cell cycle arrest and apoptosis.

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[8] J.-L. Merlin et al., *In vitro* comparative evaluation of trastuzumab (Herceptin) combined with paclitaxel (Taxol) or docetaxel (Taxotere) in HER2-expressing human breast cancer cell lines. *Annals of Oncology* **13**: 1743–1748, 2002

[9] M.X. Sliwkowski, et al. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Sem Oncol* 1999;26(S12):60−70.

Figure 6
Cell Cycle Effects of T-DM1

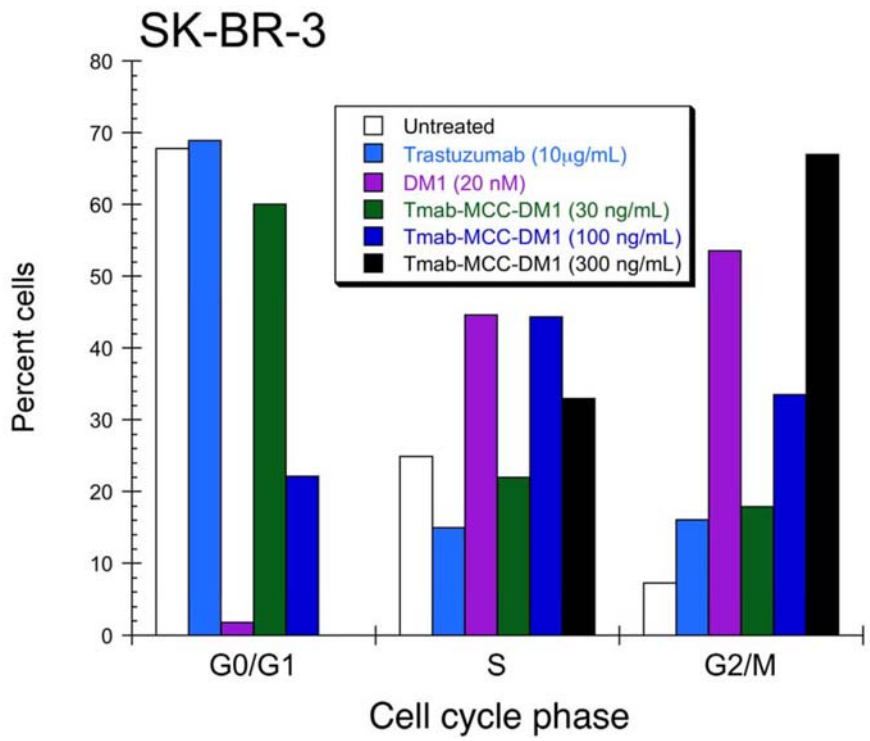


Figure 6 (cont'd)
Cell Cycle Effects of T-DM1

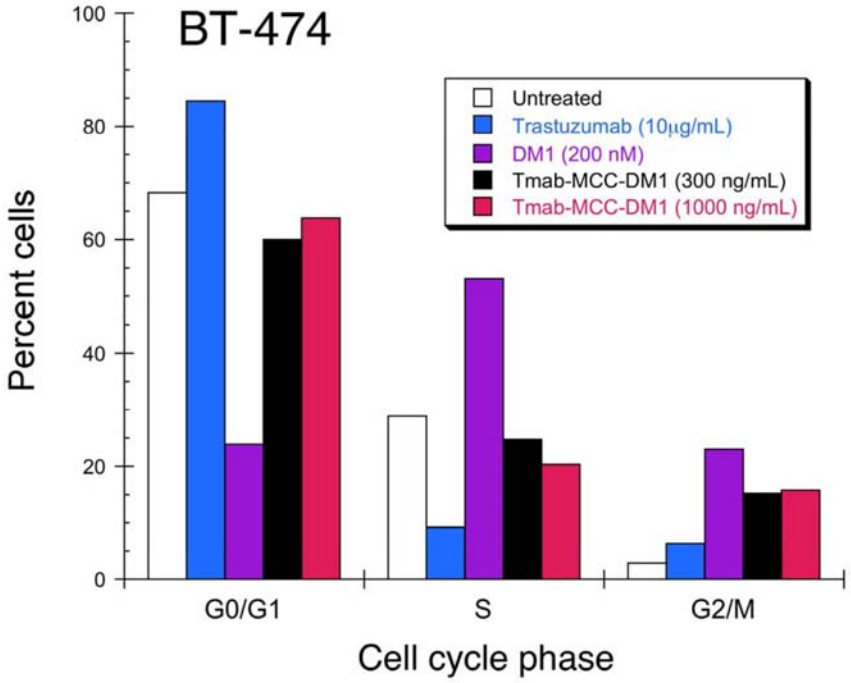
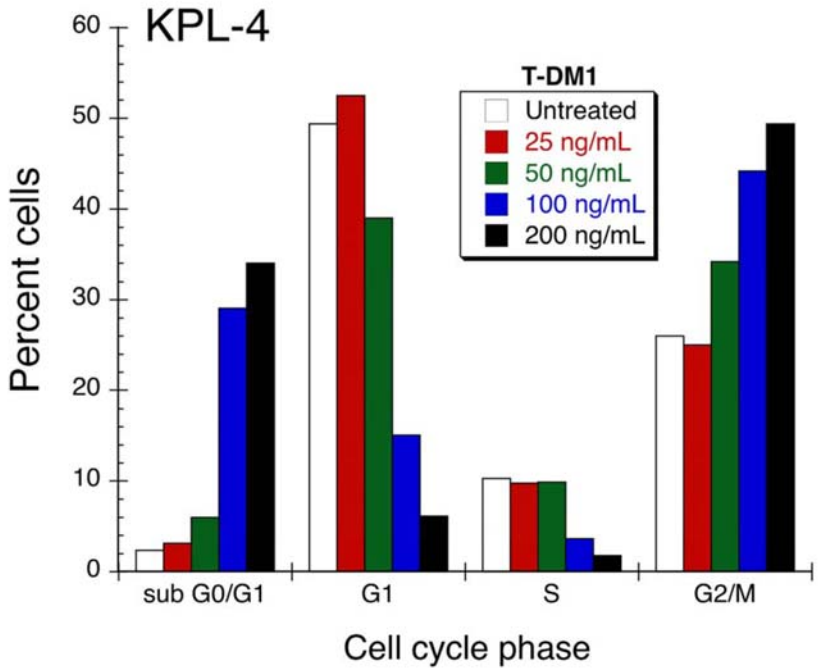


Figure 6 (cont'd)
Cell Cycle Effects of T-DM1

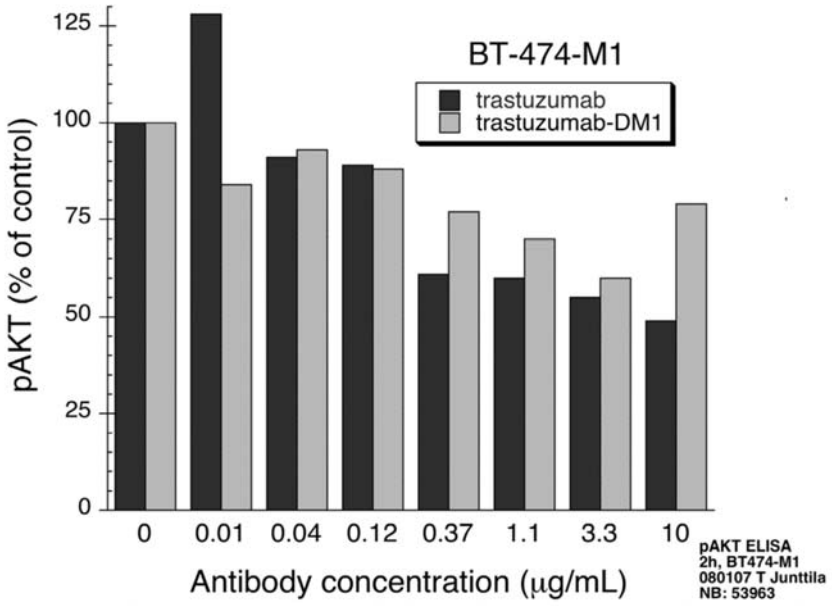


Analysis of Signaling Pathways and Cell Cycle Markers

In this set of experiments, the investigators compared the effects of trastuzumab emtansine and trastuzumab on the phosphorylation of AKT *in vitro*. They incubated BT-474-M1 cells for two hours with varying concentrations of TDM1 and trastuzumab. The phosphorylation state of Ser473 of AKT1 (pAKT) was detected using ELISA. The following graph from the study report shows that addition of either antibody caused a decrease in AKT phosphorylation and that this effect increased with increasing concentration. The decreases for both compounds were comparable, though the decrease caused by trastuzumab was consistently greater than that caused by trastuzumab emtansine.

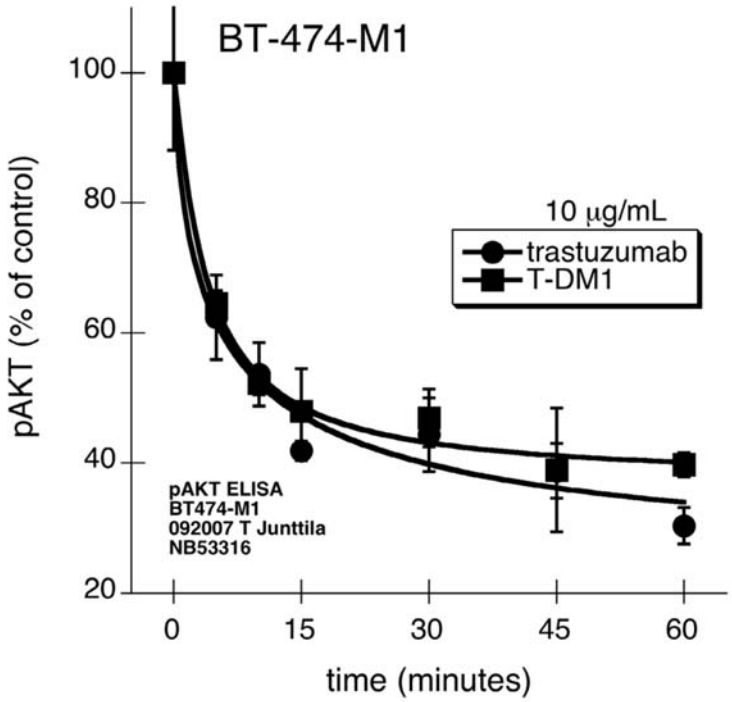
Figure 7
T-DM1 and Trastuzumab Inhibit AKT Phosphorylation

A



The investigators further showed that the decrease in phosphorylation was rapid, having a half-life of approximately 15 minutes for both compound when the cells were treated with 10 μg/mL (about 67 nM TDM1 and 69 nM trastuzumab). The following graph form the study report shows that the rapid decrease was not complete, with at least 30% of AKT remaining phosphorylated after 60 minutes.

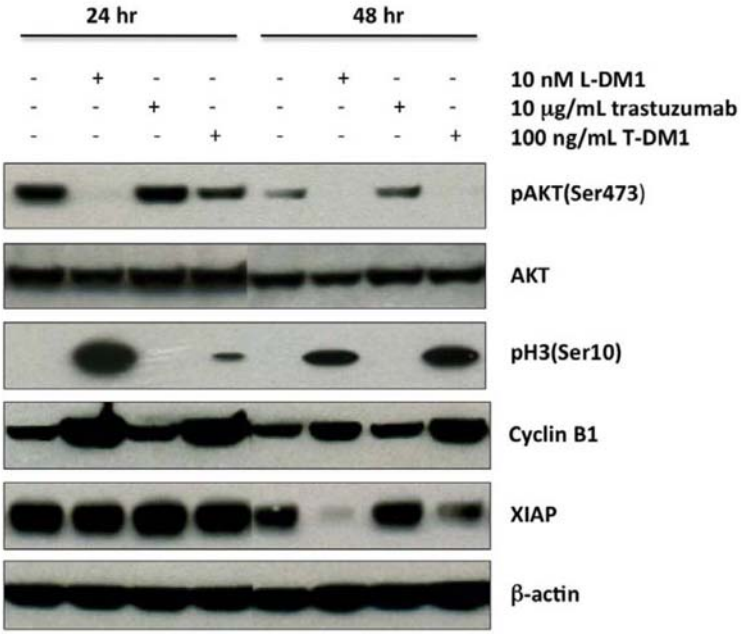
Figure 7 (cont'd)
T-DM1 and Trastuzumab Inhibit AKT Phosphorylation



Prolonged exposure for up to 16 hours with concentration of 1 µg/mL (about 6.7 nM TDM1 and 6.9 nM trastuzumab) did not decrease phosphorylation beyond those levels seen at four hours (about 40% of control, not shown).

The investigators also treated trastuzumab-insensitive KPL-4 cells to distinguish the effects of trastuzumab from T-DM1, which would demonstrate effects mediated by cytosolic DM1 exposure. KPL-4 cells were exposed for 24 or 48 hours to 10 nM L-DM1, 10 µg/mL trastuzumab (about 69 nM), or 100 ng/mL T-DM1 (about 0.67 nM) and lysed for Western blot analysis. The following figure from the study report show that both trastuzumab emtansine and L-DM1 inhibited phosphorylation of AKT at Ser473, while trastuzumab had no effect.

Figure 8
Western Analysis of Signaling and Cell Cycle Markers in KPL-4 Cells

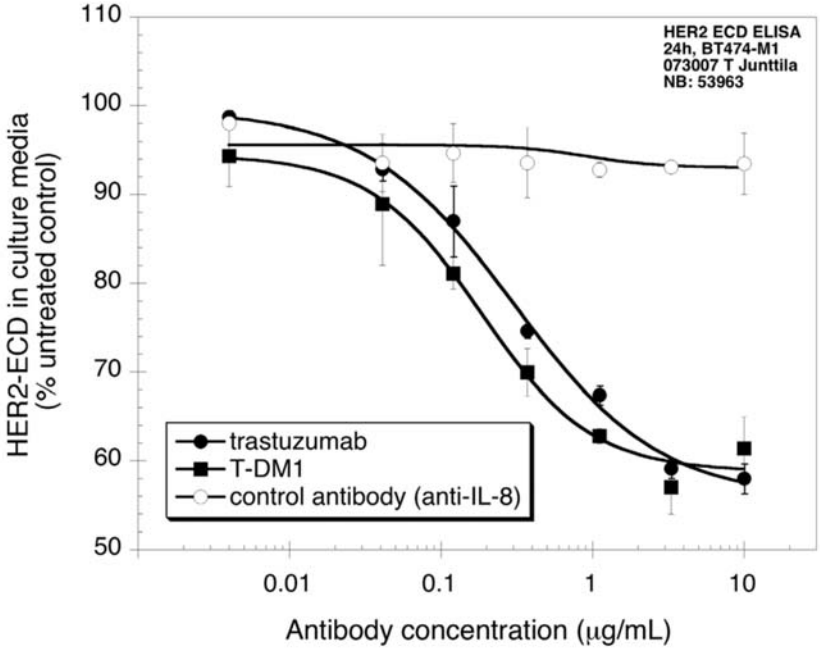


Here pH3(ser10) is phospho-histone H3 phosphorylated at serine 10 and XIAP is x-linked inhibitor of apoptosis. The pattern of inhibition or stimulation of these other markers is consistent with the cell cycle arrest and initiation of apoptosis expected with DM1. In further experiments (not shown) treatment of KPL-4 cells with DM1 or TDM1 caused cleavage of PARP, a further indicator of the initiation of apoptosis. Nevertheless, the diminution of phosphorylation of AKT, a protein far upstream from the nucleus, suggests feedback inhibition of the mitotic signal of phosphorylated AKT. This inhibition of AKT phosphorylation almost certainly follows the inhibition of tubulin function by DM1 and so probably does not contribute directly to TDM1 efficacy.

Effect of T-Dm1 on Shedding of Her2 Ectodomain

In this experiment, the ability of TDM1 to inhibit HER2-ECD shedding was determined by measuring the amount of soluble HER2-ECD in BT-474-M1-conditioned growth media following a 24-hour incubation with TDM1, trastuzumab or control antibody (anti-IL-8). Maximal inhibition of HER2-ECD shedding was 42% with trastuzumab exposure and 43% with TDM1 exposure. The IC_{50} values for the antibodies were 0.30 ± 0.05 µg/mL (about 2 nM) and 0.18 ± 0.04 µg/mL (about 1.2 nM), respectively. To exclude the possibility that the reduction of HER2-ECD in the media was due to decreased cell number in the treated wells, the number of viable cells was analyzed in parallel with media collection. The treatments did not affect cell number at 24 hours (data not shown), confirming that the reduction of HER2 ectodomain in the media was due to inhibition of HER2-ECD shedding.

Figure 10
T-DM1 and Trastuzumab Inhibit Shedding of HER2-ECD



4) Shedding of the HER2 Extracellular Domain – a Literature Review

A search of MEDLine using the terms "HER2 and shedding and extracellular domain" returned 10 articles. A search on "HER2 and kinase activity and extracellular domain" yielded 64 references. The following is a review of the most relevant of these articles and articles discovered as citations therein. While this review is thorough, it is not completely comprehensive.

J. R. Zabrecky *et al*. provided the first literature report to document the cleavage of the HER2 extracellular domain from SK-BR-3 cells *in vitro*.[10] They first proposed that the release of the extracellular domain may have implications in oncogenesis and its detection could prove useful as a cancer diagnostic.

T. A. Christianson *et al.* identified an NH_2 terminally truncated HER2 product that migrated at 95 kD by Western blotting and immuno-precipitations using domain-specific antibodies.[11] The truncated p95 membrane fragment of HER2 was phosphorylated in an immune complex when incubated with ^{32}P-ATP *in vitro*. Many people have inferred from these results that the p95 kinase was constitutively active. This is not necessarily the case as intact HER2 also demonstrated kinase activity under the conditions of this experiment. The results of this paper do not demonstrate as many have claimed that the truncated p95 membrane fragment is activated by the cleavage of the ECD. The full length HER2 receptor migrates with a molecular weight of about 185 kD. The extracellular domain fragment is about 105 to 110 kD. The investigators determined that two normal breast epithelial cell lines had no detectable p95. In addition, the SKOV3 ovarian carcinoma cells, which overexpress HER2, had a

[10] J. R. Zabrecky, J. R., The extracellular domain of p185/neu is released from the surface of human breast carcinoma cells, SK-BR-3. J. Biol. Chem., *266:* 1716–1720, 1991.
[11] Christianson TA, Doherty JK, Lin YJ, et al: NH2-terminally truncated HER-2/neu protein: Relationship with shedding of the extracellular domain and with prognostic factors in breast cancer. Cancer Res 58:5123-5129, 1998

disproportionately low amount of p95. The authors suggest that the cleavage of the ECD is a regulatory process.

Codony-Servat *et al*. reported the proteolytic cleavage of the HER2 extracellular domain.[12] They demonstrated that this cleavage was slow, reaching maximal concentrations only after 48 hours. It is also inefficient in the absence of inducers as only 5% of the labeled HER2 molecules released their ECD over this period. Protein kinase C did not activate this process. Nevertheless, pervanadate, a general inhibitor of protein-tyrosine phosphatases, induces a rapid shedding of HER2 ECD. This shedding of HER2 ECD is inhibited by the broad-spectrum metalloprotease inhibitors EDTA, TAPI-2, and batimastat. The concentrations required to achieve this inhibition were relatively high (> 5 μM). These results suggested that a heretofore unidentified protease mediates HER2 cleavage.

Evidence that trastuzumab prevents the shedding of the extracellular domain of HER2 comes from the work by M.A. Molina *et al*. and references therein.[13] Most authors on the subject cite this work and it is the basis for the experiments the Applicant's investigators did to show that trastuzumab emtansine also prevented shedding of the ECD. Molina et al. demonstrated that 4-aminophenylmercuric acetate (APMA), a matrix metalloprotease activator, increased shedding of the ECD in HER2-overexpressing breast cancer cells. The HER2 p95 fragment, which appears after APMA-induced cleavage, was phosphorylated. The activation of HER2 shedding by APMA in cells was blocked with batimastat, a broad-spectrum metalloprotease inhibitor. This provides evidence that the ECD may be cleaved by a metalloproteinase. Trastuzumab inhibited basal and APMA-induced HER2 cleavage as evidenced by the diminished generation of phosphorylated p95 *in vitro*. 2C4, an antibody against a different epitope of the HER2 ectodomain, did not reduce formation of the p95 fragment. These investigators also analyzed 24 human breast cancer specimens, and a phosphorylated M(r) 95,000 HER2 band could be detected in 14 of them. Nevertheless, these experiments were done with frozen tissue samples and the results were highly variable and not reported with quantitative detail.

P.C.C. Liu *et al.* described the use of transcriptional profiling, pharmacological and *in vitro* approaches to identify the major source of HER2 proteolytic activity.[14] They used real-time PCR to identify those ADAM (A Disintegrin And Metalloproteinase) family members which were expressed in HER2 shedding cell lines. siRNAs that selectively inhibited ADAM10 expression reduced HER2 shedding.

Many articles and reviews refer to the membrane bound HER2 fragment as constitutively active as a kinase but tracing the references back in these papers leads to an article by H.-J. S. Huang *et al.* that demonstrates kinase activity not in a fragment of HER2 but in truncated mutant of EGFR (HER1).[15] One mutant EGFR which occurs frequently in human cancers lacks a portion of the extracellular ligand-binding domain due to genomic deletions that eliminate exons 2 to 7 and increases tumorigenicity *in vivo*. The mutant receptors were expressed on the cell surface and constitutively autophosphorylated at a significantly decreased level compared with wild-type EGFR activated by ligand treatment. Authors have inferred that the cleavage of the

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[12] J. Codony-Servat et al., Cleavage of the HER2 Ectodomain Is a Pervanadate-activable Process That Is Inhibited by the Tissue Inhibitor of Metalloproteases-1 in Breast Cancer Cells. *Cancer Res* 1999;59:1196-1201.

[13] M.A. Molina et al. Trastuzumab (Herceptin), a Humanized Anti-HER2 Receptor Monoclonal Antibody, Inhibits Basal and Activated HER2 Ectodomain Cleavage in Breast Cancer Cells. *Cancer Res* 2001;**61**:4744-4749.

[14] P.C.C. Liu et al., Identification of ADAM10 as a Major Source of HER2 Ectodomain Sheddase Activity in HER2 Overexpressing Breast Cancer Cells, Cancer Biology & Therapy 5:6, 657-664, June 2006

[15] H.-J. S. Huang et al. The Enhanced Tumorigenic Activity of a Mutant Epidermal Growth Factor Receptor Common in Human Cancers Is Mediated by Threshold Levels of Constitutive Tyrosine Phosphorylation and Unattenuated Signaling. J. Biol. Chem., 272(5):2927–2935, 1997

ECD from the full receptor confers constitutive activity on the p95 fragment. I found in this review of the literature no evidence to substantiate this hypothesis though this view remains widely held and frequently asserted.

P.P. Di Fiore *et al.* have shown that when HER2 complimentary DNA was expressed in NIH/3T3 cells under the control of the SV40 promoter, the gene lacked transforming activity despite expression of detectable levels of the HER2 protein.[16] But a further five to tenfold increase in its expression was associated with activation of HER2 as a potent oncogene. These investigators did not further identify the mechanism of this activation but numerous subsequent authors have inferred that it is by auto-activation of HER2 kinase activity. O. Segatto *et al.* have observed autokinase activity by generating mutant forms of HER2.[17] Auto-activation in these mutants resulted in oncogenic transformation.

G. C Ghedini *et al.* examined biological effects of overexpressed HER2/ECD on cancer growth and in response to trastuzumab. They stably transfected SKOV3 tumor cells to release a recombinant HER2-ECD molecule (rECD). Transfectants that released high concentrations of 110-kDa rECD, identical in size to native HER2/ECD (nECD), grew significantly slower than did controls, which released only basal levels of nECD. While transmembrane HER2 and HER1 were expressed at equal levels by both controls and transfected cells, activation of these molecules and of downstream ERK2 and Akt was significantly reduced only in rECD transfectants. Surface plasmon resonance analysis revealed heterodimerization of the rECD with HER1, -2, and -3. In cell growth bioassays *in vitro*, shed HER2-ECD significantly blocked HER2-driven tumor cell proliferation. In mice, high levels of circulating rECD significantly impaired HER2 driven SKOV3 tumor growth, but not that of HER2-negative tumor cells. *In vitro* and in mice, trastuzumab significantly inhibited tumor growth due to the rECD-facilitated accumulation of the antibody on tumor cells. These findings are quite contrary to the prevailing hypotheses about the function of HER2-ECD.

A Vazquez-Martin *et al.*[18] have shown have shown that the dual HER1/HER2 tyrosine kinase inhibitor lapatinib, which works intracellularly and binds at the TK domain of HER2, significantly augments basal shedding of HER2-ECD to inhibit HER2 driven cancer cell growth. Lapatinib treatment significantly increased the concentration of the inactive (unphosphorylated) form of HER2 protein at the tumor cell membrane and exacerbated HER2-ECD shedding to the extracellular milieu of HER2 overexpressing cancer cells. In these experiments, trastuzumab-resistant cancer cells contained nearly undetectable levels of soluble HER2 ECD when compared with trastuzumab-sensitive parental cells. lapatinib treatment fully restored high levels of basal HER2-ECD shedding and trastuzumab sensitivity. This increased HER2-ECD shedding that occurs upon treatment with lapatinib was fully suppressed in lapatinib-refractory HER2-positive cells. Again these results are contrary to many prevailing hypotheses. Taken with those results of Ghedini *et al.* they suggest that the cleavage of the HER2-ECD is a regulatory process.

In 2010, the U.S. Food and Drug Administration approved a fluorescence *in situ* hybridization test (Dako HER2 FISH pharmDx™) for gene amplification in human cancers. The test is used to screen patients for eligibility for treatment with herceptin, particularly in patients with gastric cancer and requires a tumor sample. In 2000 the FDA approved the Oncogene Science/Siemens Healthcare Diagnostics HER-2 ECD enzyme-linked immunosorbent assay

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[16] P. P. DiFiore *et al*. ErbB-2 is a potent oncogene when overexpressed in NIH3T3 cells. Science. 237: 178-182. 1987.

[17] O. Segatto et al. Different Structural Alterations Up-regulate In Vitro Tyrosine Kinase Activity and Transforming Potency of the erbB-2 Gene. Molecular and Cellular Biology, Dec. 1988, 8(12);5570-5574

[18] A Vazquez-Martin et al. Lapatinib, a Dual HER1/HER2 Tyrosine Kinase Inhibitor, Augments Basal Cleavage of HER2 Extracellular Domain (ECD) to Inhibit HER2-Driven Cancer Cell Growth. J. Cell. Physiol. 226: 52–57, 2011.

(Siemens Healthcare Diagnostics Inc, Tarrytown, NY), which is available as both a manual microtiter plate assay and as an automated platform. The currently approved cutoff for an elevated HER2-ECD is greater than 15 ng/mL, which results in a positive test in approximately 5% of healthy controls.[19] R.C. Payne *et al.* have shown that circulating trastuzumab does not interfere with the assay because the antibodies used in the test recognize different and non-overlapping epitopes on the ECD from those recognized by trastuzumab. The FDA has approved a number of other tests and the value of such testing and the choice of which test to use has generated considerable controversy.[20]

S. Lennon *et al.* analyzed sequential ECD determinations on 322 patients treated with six different treatment regimens in four clinical trials.[21] They had baseline values of serum ECD in 296 patients, and of these, 205 (69%) had raised concentrations above 15 ng/mL. They discovered no clear relationship between baseline ECD levels and tumor response. After initiating combination therapy, ECD concentrations declined irrespective of treatment received and tumor response. For trastuzumab monotherapy, some trend between changes in ECD levels in early cycles and best response was discernable, but the overlap was too broad to be clinically useful. Disease progression was not reliably predicted by rising ECD levels in the majority of patients. These authors concluded that "[o]verall, the data presented do not support the clinical utility of measuring serum ECD levels in patients with HER2-positive advanced breast cancer. Baseline ECD levels were not reliably predictive of clinical outcome. ECD levels declined irrespective of treatment or regimen received; for combination therapy, similar reductions occurred at the commencement of therapy irrespective of treatment outcome. In most patients, PD was not preceded or accompanied by an increase in ECD. A similar percentage of patients experienced an increase or a decline in ECD before or at PD." They further concluded that "based on our data, we cannot recommend using serum HER2-ECD levels to make trastuzumab or other treatment decisions for individual patients with advanced/metastatic breast cancer."

A.F. Leary *et al.* have reviewed the extensive clinical literature by examining every report concerning HER2 status in women with breast cancer that included more than 50 patients between 1992 and 2007.[22] They focused on the value and limitations of serum ECD in both early and advanced breast cancer in the following clinical contexts: as a marker of HER2 tumor tissue status; clinical implications of raised levels in women who have a tumor not overexpressing HER-2; as a prognostic indicator and as a predictor of response to treatment; and as a monitoring tool for early recurrence. Their analysis shows that the prevalence of high ECD in women with primary breast cancer ranged from 3.1 to 34%. Part of this high variability was due to differing testing methodologies, but the results clearly indicated that high serum HER2-ECD was not a reliable marker for primary breast cancer. When they correlated high serum ECD with HER2 status at diagnosis the sensitivity of ECD as a predictor was low and variable, ranging from only 16 to 55% of patients. They concluded that "The sensitivity of HER-2 ECD for the diagnosis of HER2–positive primary breast cancer is poor. In addition, a small proportion of HER2–negative tumors produce HER2 ECD. Therefore, there is no evidence to support the use of HER2-ECD to determine the HER2 status of a primary tumor." Similarly the prevalence of high serum HER2-ECD in women with metastatic disease ranged from 23 to 62%.

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[19] R.C. Payne, et al: Automated assay for HER-2/neu in serum. Clin Chem 46:175-182, 2000

[20] M. Allison, The HER2 testing conundrum, Nature Biotechnology 28, 117–119 (2010)

[21] S. Lennon *et al.*, Utility of serum HER2 extracellular domain assessment in clinical decision making: pooled analysis of four trials of trastuzumab in metastatic breast cancer J Clin Oncol. 2009 Apr 1;27(10):1685-93.

[22] A.F. Leary et al., J Clin Oncol. Value and limitations of measuring HER-2 extracellular domain in the serum of breast cancer patients. 2009 Apr 1;27(10):1694-705.

The authors further concluded that "[a]lthough there is a consensus that high HER2-ECD is associated with poor TTP and OS, correlations with RR are conflicting, which supports HER2 ECD as an indicator of poor prognosis rather than a predictor of response. There are no data to support the value of HER2-ECD in guiding treatment decisions regarding the selection of specific cytotoxic or endocrine therapies." Ultimately they stated that on "the basis of our review of the literature, we conclude that there is currently insufficient evidence to support the use of serum HER2-ECD in the routine management of individual patients with breast cancer. This conclusion is in agreement with the 2007 American Society of Clinical Oncology guidelines on the use of biomarkers in breast cancer."

C. Tse and P-J. Lamy have objected to aspects of the methodology of the two clinical reviews cited above.[23] They advocate the utility of graphing the levels of HER2-ECD with time as a predictor of progression and have called for a large prospective study to settle the issue of serum ECD as a prognostic indicator.

From this brief review of the literature it is clear that the role of HER2-ECD in the development and progression of breast cancer has not been resolved at the molecular level. Likewise, the clinical situation remains quite muddled. As ECD shedding is evidently a regulated process (*vide supra*) actively controlled by cell surface proteases, some of the evidence above suggests that it may be reasonable to postulate that shedding is a natural control mechanism by which the cell diminishes the HER2 down-stream signal when ERB2 is amplified and HER2 is overexpressed. This would be counter to some of the current hypotheses and would suggest that the inhibition of HER2 shedding is antithetical to the purpose of trastuzumab. The concept that inhibition of shedding acts mechanistically to diminish the mitogenic signal depends on the notion that truncation of the HER2 receptor results in constitutive activation, a phenomena for which there is as yet no good evidence.

5) In Vitro Biological Characterization of Trastuzumab-MCC-DM1 (T-DM1) for Fc Gamma Receptor Binding, Antibody-Dependent Cell-Mediated Cytotoxicity, and C1q Binding Activities

Study number	10-0046
Filename	10-0046-[redacted] (b) (4) -final.pdf
Laboratory	Genentech, Inc., South San Francisco, CA 94080
Date	September 2009
GLP	No
Audited	No
Drug	T-DM1 Reference Material, Lot TMCCDM1208-2 T-DM1 Phase II Lot B25 T-DM1 Phase III Qualification Lots B107, B108, and B109 Trastuzumab Reference Material, Lot HER401-4
Method	
Cell lines	BT-474 cells, a human breast cancer cell line that overexpresses HER2
ADCC cells	Human peripheral blood mononuclear cells (PBMCs)
Assay	The investigators added clacein AM to the BT-474 cells. This dye permeated the cells which then converted it to green-fluorescent calcein. Release of green-fluorescent calcein into the cell culture supernatant is indicative of cell lysis and can be measured with a fluorescent plate reader. T-DM1 samples and trastuzumab were assayed in three

[23] C. Tse and P-J. Lamy. Clinical Utility of Serum Human Epidermal Growth Factor Receptor 2 Extracellular Domain Levels: Stop the Shilly-Shally—It Is Time for a Well-Designed, Large-Scale Prospective Study, J. Clin. Oncology, 2009, 27(36).

independent experiments using PBMCs from different donors. Percentage ADCC was plotted against the concentration of the antibodies, and the data were fitted with a four-parameter model. In separate experiments the investigators determined cell lysis by the four different antibodies in the absence of PBMCs.

This report contains other experiments with the antibodies that do not directly pertain to the safety and efficacy of trastuzumab emtansine. I have not reviewed these studies.

Results

The following table from the study report shows the results of the experiment with both antibody and PBMCs. The investigators did not do a statistical analysis on the results. I did a Student's t-test on the results for trastuzumab (reference material) verses those for trastuzumab emtansine (reference material). The mean for trastuzumab was 13.5 and that for trastuzumab emtansine was 9.13, $p = 0.355$. The analysis confirmed there is no difference between the results for trastuzumab and those for trastuzumab emtansine. The data is presented as percentage ADCC and the materials and methods section says that these percentages were calculated against the values for spontaneous lysis and antibody independent cytotoxicity. Spontaneous lysis is that cell disruption seen in wells containing BT-474 cells plus antibody but no PBMCs. Antibody independent cytotoxicity is the lysis seen in wells with PBMCs but no antibodies. The study report does not provide the values for spontaneous lysis or antibody independent cytotoxicity so there is no information on the relative importance of ADCC in the mechanism of trastuzumab emtansine. TE Kute *et al.* appear to have done more comprehensive studies of this interaction between PBMCs and BT-474 cells bound to trastuzumab or trastuzumab emtansine.[24]

In the experiments with the antibodies alone the investigators did not provide the primary data instead presenting the results of but one experiment graphically. The graph (not shown) shows that antibody alone had no effect on the integrity of the breast cancer cells. The investigators postulate that the incubation period was too short to demonstrate DM1 cytotoxicity. This is likely correct.

[24] TE Kute *et al.* Breast tumor cells isolated from in vitro resistance to trastuzumab remain sensitive to trastuzumab anti-tumor effects in vivo and to ADCC killing. Cancer Immunol Immunother. 2009 Nov;58(11):1887-96.

Table 4
Summary of EC_{50} Values and Relative Activity of T-DM1 and Trastuzumab Samples from ADCC Assay

	EC_{50} (ng/mL)				
Sample	Test 1	Test 2	Test 3	Range	
Trastuzumab	20.5	11.2	8.69	8.69	20.5
T-DM1 Reference Material	13.2	6.39	7.81	6.39	13.2
T-DM1 Qual. Lot B107	17.7	9.17	7.65	7.65	17.7
T-DM1 Qual. Lot B108	14.3	6.56	9.62	6.56	14.3
T-DM1 Qual. Lot B109	18.8	10.2	8.58	8.58	18.8
T-DM1 Phase II Lot	19.6	8.84	6.99	6.99	19.6
	Relative Activity [a]				
Sample	Test 1	Test 2	Test 3	Range	
Trastuzumab	0.644	0.571	0.899	0.571	0.899
T-DM1 Reference Material	1.00	1.00	1.00	1.00	1.00
T-DM1 Qual. Lot B107	0.746	0.697	1.02	0.697	1.02
T-DM1 Qual. Lot B108	0.923	0.974	0.812	0.812	0.974
T-DM1 Qual. Lot B109	0.702	0.626	0.910	0.626	0.910
T-DM1 Phase II Lot	0.673	0.723	1.12	0.673	1.12

ADCC = antibody-dependent cell-mediated cytotoxicity; EC_{50} = concentration of test antibody at which 50% of its maximal binding activity is observed; Qual = qualification; T-DM1 = trastuzumab-MCC-DM1.

[a] Relative Activity = EC_{50} T-DM1 reference/EC_{50} sample.

4.3 Safety Pharmacology

1) Effects of $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl) maytansine (DM1) on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

Study number	Sponsor – 09-0234, (b) (4) Study Number: 090303.DPW
Filename	09-0234- (b) (4) -final.pdf
Laboratory	(b) (4)
Date	November 2009
GLP	Yes (with exceptions in quality of product determinations)
Audited	Yes
Drug	DM1, Immunogen Lot Number: N088-08-02-01 Purity 95.4%
Method	
Cells	Human embryonic kidney cells transfected with the human ether-a-go-go gene (hERG)
N	≥ 3
Vehicle	DMSO. 0.3% v/v
Doses	2.6, 8.8, and 29.5 µM actual 0.35, 0.61 and 1.90 µM actual
Positive control	Terfenadine, 60 nM
Reference	E-4031 (Sigma-Aldrich) IC_{50} = 12 nM

Results

DM1 inhibited hERG potassium current by 0.3 ± 0.6% at 2.6 µM, 1.0 ± 0.5% at 8.8 µM and 2.5 ± 0.4% at 29.5 µM versus 0.6 ± 0.3% in control. Under identical conditions, the positive control (60 nM terfenadine) inhibited hERG potassium current by 82.0 ± 3.0%. The IC_{20} and IC_{50} for the inhibitory effect of DM1 on hERG potassium current could not be determined because the hERG inhibition at the highest concentration tested, 29.5 µM, was only 2.5%. Therefore, both the IC_{20} and IC_{50} were estimated to be greater than 29.5 µM. DM1 does not inhibit the cardiac slow rectifier current at physiologically relevant concentrations.

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2) A Single Dose Intravenous Cardiovascular Safety Pharmacology Study of Trastuzumab-MCC-DM1 (PRO132365) Administered to Female Cynomolgus Monkeys with a 3 Week Recovery Period

Study no	Sponsor – 04-1031-1605; (b) (4) – 6281-605
File name	04-1031-1605- (b) (4) -final.pdf
Laboratory	(b) (4)
Study Date	April 2005
GLP	Yes
Audited	Yes
Drug	Tmab-MCC-DM1, Lot number FIL037D01, purity 98.0%
Method	

Species	Female naïve cynomolgus monkeys (*Macaca fascicularis*) approximately 3 to 6 years old, and body weights ranged from 2.6 to 4.2 kg
Doses	The following table from the study report shows the dose groups, doses, dose volume and N for the study.

Group	Test Article	Number of Animals Female	Dose Tmab-MCC-DM1[a] (mg/kg)	Dose DM1[b] (μg/m2)	Concentration (mg/mL)
1 (control)	Vehicle	4	0	0	0
2 (low)	Tmab-MCC-DM1	4	3	612	0.5
3 (mid)	Tmab-MCC-DM1	4	10	2040	1.67
4 (high)	Tmab-MCC-DM1	4	30	6120	5

a Dose expressed as mg total Tmab-MCC-DM1/kg body weight; the dose volume was 6 mL/kg.

b Dose expressed as DM1 ($\mu g/m^2$) estimated as follows: [Dose Level (mg/kg Tmab-MCC-DM1) x 12 x 0.017 (fraction of DM1 to trastuzumab in Tmab-MCC-DM1c) x 1000]. The conversion factor of 12 is taken from US Department of Health and Human Services Food and Drug Administration, CDER/CBER, Guidance for Industry and Reviewers: Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, December 2002.

c The fraction of DM1 to trastuzumab in Tmab-MCC-DM1 is determined using the molecular weights (MW) of DM1 (MW 738) and trastuzumab (MW 145167), and the measured drug/Antibody ratio (DAR) 3.4 and is calculated as follows: (MW DM1 x DAR)/MW trastuzumab).

Schedule	Single doses
Route	IV, saphenous vein
Body Weight	Weekly
Food Cons.	Daily
Toxicokinetics	Blood was collected for serum concentration levels once prior to treatment, on Day 2 after the 24 hour telemetry data collection was completed for all animals, and on Day 22.
Cardiac enzymes	Blood was collected for determination of troponins I and T and creatine kinase (CK) Isoenzyme once prior to initiation of treatment (presurgery), on Day 2 after the 24 hour telemetry data collection was completed for all animals, and on Day 22.
Antibody test	End of study
Cage Side Obs.	Observed at least once daily, twice daily for mortality or distress
Procedure	Surgically implanted telemetrical sensors for electrocardiographic parameters were place in the abdomen 3 weeks prior to dosing. Two baseline ECG and pressure recordings were made prior to dosing.
Termination	Animals were returned to the colony

Cardiac parameters

ECG and pressure measurements were recorded for at least 60 minutes prior to dosing on Day 1, continuously through at least 8 hours postdose, and then for a 10-minute period each hour through at least 24 hours postdose. ECG and pressure measurements were also recorded for at least a 10-minute interval at approximately the same time of day as dosing on Days 3, 4, 5, 8, 15, and 22. Quantitative evaluation included ECG measurements including RR interval, QT, and rate corrected QTcf (Fridericia). Blood pressure measurements included heart rate and

systolic, diastolic, mean arterial, and pulse pressures (systolic-diastolic). Intrathoracic pressure measurements included respiratory rate and a qualitative assessment of respiratory depth.

Results

Drug Analysis Within specifications
Mortality None

Clinical Observations No toxicologically significant changes

Body Weight No toxicologically significant changes

ECG No toxicologically significant changes

Antibody analysis Negative

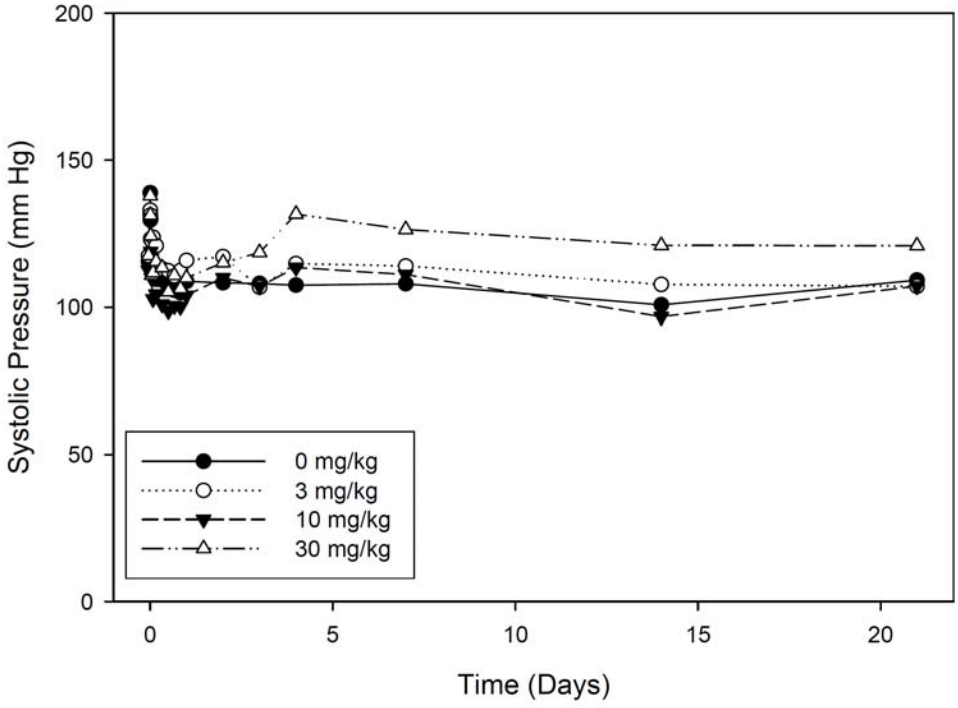
Troponin No toxicologically significant changes

Toxicokinetics The table below shows that this experiment achieved significant exposure to trastuzumab emtansine and total trastuzumab.

	TDM1 µg/mL		Total Tras µg/mL	
Dose mg/kg	Day 1	Day 22	Day 1	Day 22
0	BLQ	BLQ	BLQ	BLQ
3	33.7	0.133	38.4	1.36
10	136	2.59	142	17.7
30	323	17.8	332	90.9

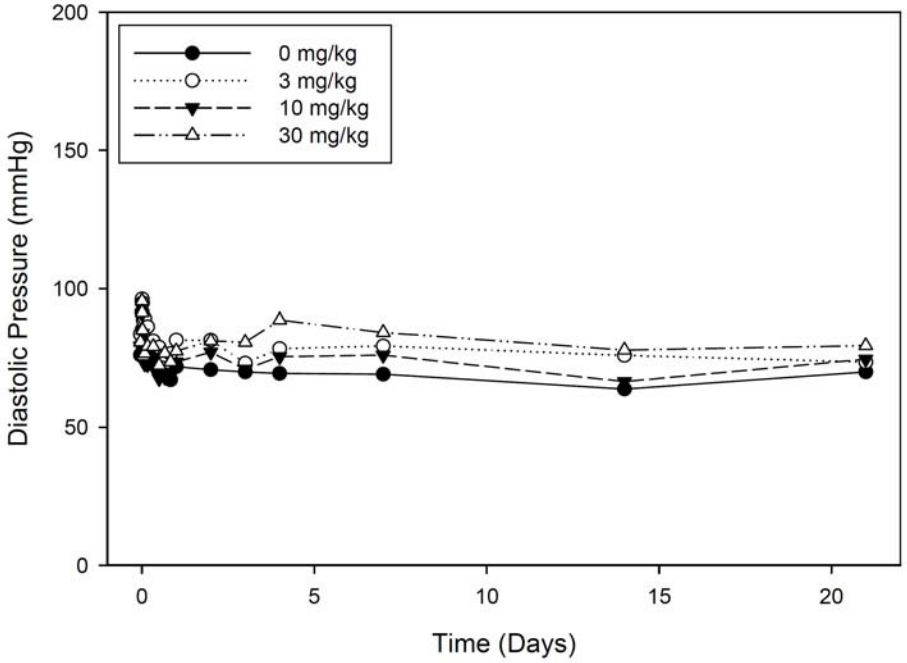
Blood Pressure The following graph from the study report shows that aortic systolic pressure was consistently elevated in the HD group over the period of 1 to 22 days. This difference relative to control did not reach significance by ANOVA but its persistence in the HD group suggests that it is possibly drug related. In diastole, the LD and MD groups pressures are also slightly elevated above control but again the effect does not reach statistical significance (graph from the report below). Mean arterial pressure also slightly elevated (not shown). Because of its greater variability, pulse pressure and heart rate show no consistent pattern (not statistically significant, not shown).

Figure 2
Aortic Systolic Pressure (Days 1 - 22)



Note: No statistically significant differences at $p \leq 0.05$

Figure 4
Aortic Diastolic Pressure (Days 1 - 22)

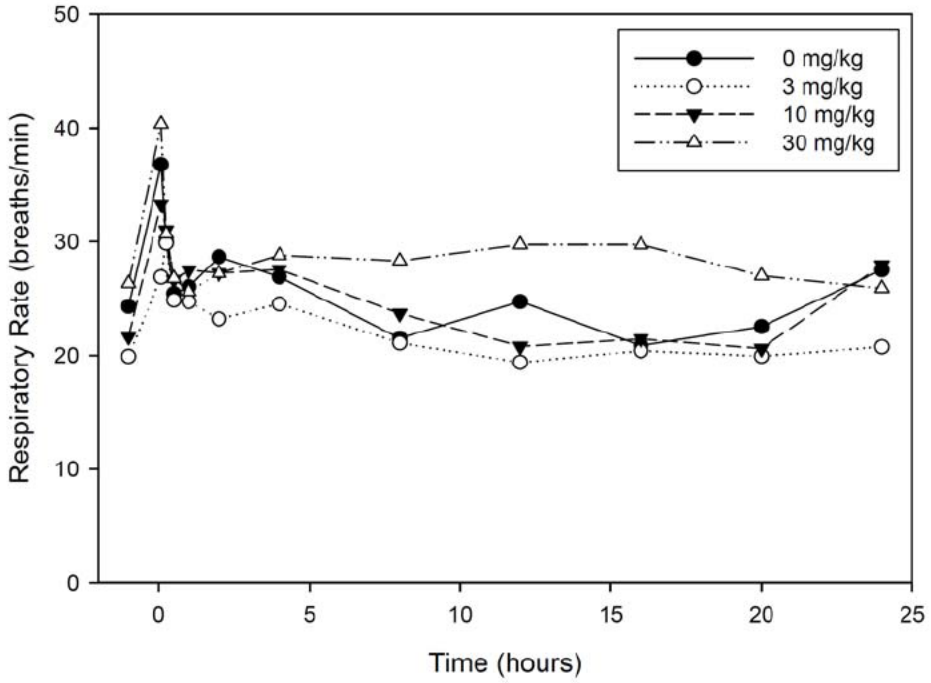


Note: No statistically significant differences at $p \leq 0.05$

Respiration

On day 1 the graph below from the study report shows that respiratory rate in the HD group is higher than controls and the other dose groups on the first day. This difference does not reach significance but is possibly drug related. This effect is not seen on days 1 through 22 (not shown).

Figure 11
Respiratory Rate (Day 1)



Note: No statistically significant differences at $p \leq 0.05$

3) Cross-Reactivity of Trastuzumab-MCC-DM1 (PRO132365) with Human and Cynomolgus Monkey Tissues *In Vitro*

Study no	Sponsor number 04-1215-1605, [redacted] (b) (4) No. OCA00058
File name	04-1215-1605 (b) (4) final.pdf
Laboratory	[redacted] (b) (4)
Study Date	May 2005
GLP	Yes but with exceptions of unaudited immunohistochemistry methodology and concentration analysis
Audited	No
Drug	Trastuzumab-SMCC-DM1, Lot No. FIL037D01
Methods	
Tissues	Human and cynomolgus monkey tissues and cell lines from a variety of major organs. The human tissue from three individuals was harvested at autopsy. The monkey tissue from three individuals was harvested at necropsy. See results below.
Analysis	Binding of Tmab-MCC-DM1 was identified in the tissue samples with a mouse anti-DM1 secondary antibody followed by a biotinylated anti-mouse IgG tertiary antibody. Bound antibody was detected with a streptavidin-biotin-horseradish peroxidase complex (ABC)/3-3'diamino-benzidine HCl (DAB) reaction.
Doses	1.0 μg/mL or 10.0 μg/mL trastuzumab emtansine

Positive Controls	The sponsor determined that two cell lines, MDA-231 (aka MDA-MB-231) and MDA-175 (aka MDA-MB 175-VII), were positive for HER2 protein expression.
Negative Control	Antibody human IgG1, kappa at doses of 1.0 μg/mL or 10.0 μg/mL
Negative Control	The sponsor determined that MDA-468 (aka MDA-MB-468) cells were negative for HER2 protein expression.
Grading scale	The investigators used the following grading scale to determine the extent of the trastuzumab emtansine binding reaction with individual tissues under the microscope. They did not maintain a photographic record of the results.

Staining Intensity		Staining Distribution	
0 or Blank	No labeled cells	0 or Blank	No labeled cells
1 or +	Light stain	1 or +	Occasional cells
2 or ++	Light-medium stain	2 or ++	Small number of cells
3 or +++	Moderate	3 or +++	Medium number of cells
4 or ++++	Dark or Strong stain	4 or ++++	Large number of cells
NA	Not applicable or Not available	NA	Not applicable or Not available
Staining Pattern	Cytoplasmic or Membranous or both		

Results

Positive controls

MDA-231 cells express the HER2 protein receptor in numbers comparable to expression on normal human and cynomolgus monkey HER2 receptor expressing tissues. MDA-175 cells overexpress the HER2 receptor at a low level. Specific Tmab-MCC-DM1 binding in the cell membrane of both cell lines was detected at 1.0 and 10.0 μg/mL of Tmab-MCC-DM1. The percentage of positive staining cells was 100% for both cell lines at both concentrations. The staining intensity varied from 2-3+ for MDA-231 and was consistently 4+ for MDA-175 cells.

Negative controls

Neither MDA-468 (aka MDA-MB-468) cells isotype control antibody (Human IgG1κ) stained positive.

Human Tissues

The following table summarizes the frequency of binding of trastuzumab emtansine to the human tissues examined in this study. The values in bold red are those that demonstrated a greater frequency of binding in humans than in monkeys.

Human Tissues	Epithelial		Spindle		Glial		Mononuclear	
	1.0 µg/mL	10.0 µg/mL	1.0 µg/mL	10.0 µg/mL	1.0 µg/mL	10.0 µg/mL	1.0 µg/mL	10.0 µg/mL
Adrenal	-	-	2/3	3/3	-	-	-	-
Bladder	3/3	3/3	3/3	3/3	-	-	-	-
Blood	-	-	-	-	-	-	-	-
Bone Marrow	-	-	-	-	-	-	-	-
Breast	3/3	3/3	**-**	**1/3**	-	-	-	-
Cerebellum	-	-	**3/3**	3/3	3/3	3/3	-	-
Cerebral Cortex	-	-	**3/3**	3/3	3/3	3/3	-	-
Colon	**3/3**	3/3	**3/3**	3/3	-	-	-	-
Endothelium	-	-	**3/3**	**3/3**	-	-	-	-
Eye	**3/3**	**3/3**	**3/3**	**3/3**	-	**-**	-	-
Fallopian Tube	3/3	3/3	**3/3**	**3/3**	-	-	-	-
GI tract	**3/3**	3/3	3/3	2/3	-	-	-	-
Heart	-	-	**3/3**	**3/3**	-	-	-	-
Kidney	**3/3**	**3/3**	**3/3**	**3/3**	-	-	-	-
Liver	3/3	3/3	**3/3**	**3/3**	-	-	-	-
Lung	3/3	3/3	**2/3**	**3/3**	-	-	-	-
Lymph Node	-	-	**2/2**	**2/3**	-	-	2/2	3/3
Ovary	3/3	3/3	**1/3**	**3/3**	-	-	-	-
Pancreas	3/3	3/3	3/3	3/3	-	-	-	-
Parathyroid	**1/1**	-	-	-	-	-	-	-
Pituitary	**3/3**	**3/3**	**3/3**	3/3	3/3	3/3	-	-
Placenta	-	-	-	-	-	-	-	-
Prostate	3/3	3/3	**3/3**	**3/3**	-	-	-	-
Skin	3/3	3/3	**3/3**	**3/3**	-	-	-	-
Spinal Cord	-	-	**3/3**	**3/3**	3/3	3/3	-	-
Spleen	-	-	**3/3**	**3/3**	-	-	3/3	3/3
Striated Muscle	-	-	**3/3**	**3/3**	-	-	-	-
Testes	2/3	1/3	**3/3**	**3/3**	-	-	-	-
Thymus	2/3	2/3	**3/3**	**3/3**	-	-	-	-
Thyroid	**3/3**	**3/3**	**3/3**	**3/3**	-	-	-	-
Tonsil	3/3	3/3	**2/3**	**3/3**	-	-	-	**2/3**
Ureter	3/3	3/3	**2/3**	**2/3**	-	-	-	**-**
Uterus (cervix)	**3/3**	3/3	**3/3**	**3/3**	-	-	-	**-**
Uterus (endometrium)	**3/3**	**3/3**	**3/3**	**3/3**	-	-	-	**1/3**

The investigators identified specific Tmab-MCC-DM1 binding in all human tissues at 1.0 and 10.0 µg/mL except the placenta, blood and bone marrow. Binding only occurred weekly in the parathyroid and paradoxically only at the low dose. The staining intensity and character of Tmab-MCC-DM1 binding at 1.0 µg/mL was similar to that at 10.0 µg/mL but it was less intense and membrane staining was slightly more extensive at the higher concentration.

In human tissues trastuzumab emtansine bound to both granular membrane and cytoplasm and elements of the cytoplasm depending on the cell type. In epithelial cells binding was primarily at the membrane while in cells identified by the investigators as spindle cells, which they presume to be Schwann cells, was primarily in the cytoplasm. Glial and mononuclear cells were stained in both the membrane and membrane and the cytoplasm. In general, epithelial and glial cell staining was greater in intensity than spindle cell staining. Positive staining epithelial cells were identified in the urinary bladder and ureter (urothelium), mammary gland (duct and gland epithelium), colon (mucosal epithelium), fallopian tubes

(mucosal epithelium), eye (conjunctiva, ciliary body, cornea and retinal epithelium), pituitary (subset of cells in pars distalis and intermedia), thyroid (follicular epithelium), parathyroid (epithelium, chief cells), kidney
(convoluted and collecting duct tubule epithelium), small intestine, (mucosal epithelium), exocrine pancreas (duct epithelium), lung (bronchiolar epithelium and Type 1 pneumocytes), ovary (follicular and surface epithelium), prostate (glandular epithelium), liver (hepatocytes and bile ducts), skin (epidermis, hair follicles, apocrine and sebaceous glands), testes (duct epithelium), thymus (epithelium), tonsil (mucosal epithelium), cervix (mucosal epithelium) and uterus (mucosal and glandular epithelium). Ductal and apocrine gland epithelium stained with the greatest intensity (4+) and squamous epithelium with the least. Basal and basolateral cell surfaces stained more intensely than the apical cell surface.

In several tissues, staining was seen in the cytoplasm in nerve spindle cells (presumptive Schwann cells) and perivascular spindle. Positive staining spindle cells were identified in the supporting connective tissue stroma of the adrenal gland, urinary bladder, colon, gastrointestinal tract, heart, cerebellum, cerebral cortex, kidney, liver, lung, ovary, pancreas, pituitary, lymph node, prostate, skin, spleen, skeletal muscle, testes, thymus, thyroid, tonsil, ureter, cervix and endometrium. Positive spindle cells were closely associated with peripheral nerve bundles and ganglia, located in the perineurium and epineurium. Positive staining glial cells were identified in the cerebellum, cerebral cortex, pituitary gland (pars nervosa) and spinal cord (white and gray matter). In the liver, in addition to the bile duct staining, sporadic hepatocyte staining was identified in 1 of 3 individuals. Small numbers of randomly distributed mononuclear cells in the lymph node (medulla), spleen (red pulp) and tonsil stained positive. Morphologically, these cells resembled lymphocytes and macrophages, but definitive identification was not possible without the aid of additional stains. TDM1 binding was identified in a subset of granulocytes (presumptive neutrophils) in the peripheral blood and hematopoietic cells in the bone marrow at 1.0 μg/mL. In the placenta, trophoblasts, mesenchymal cells and decidua had moderate to dark staining. Smooth muscle in the urinary bladder, colon, fallopian tube, GI tract endometrium and heart (vascular smooth muscle cells) was faintly (1+) positive and breast was (2+) positive at 10.0 μg/mL and not present at 1.0 μg/mL. Cardiac myocytes had faint (1+) positive membrane and intercalated disk staining with Tmab-MCC-DM1. Staining in human myocardiocytes was limited to 1 of 3 individuals at 1 μg/mL and 2 of 3 individuals at 10 μg/mL. The significance of this sporadic cell staining was considered uncertain due to its random nature of occurrence and failure to stain at both binding concentrations. Additional tissue components were identified that bound TDM1 at 10.0 μg/mL only including pia mater (brain), epicardium (mesothelium) and the GI tract serosal surface (mesothelium).

Monkey Tissue Binding

The table below shows the frequency of antibody binding in monkeys.

Cynomolgus Tissues	Epithelial		Spindle		Glial		Mononuclear	
	1.0 µg/mL	10.0 µg/mL	1.0 µg/mL	10.0 µg/mL	1.0 µg/mL	10.0 µg/mL	1.0 µg/mL	10.0 µg/mL
Adrenal	-	-	3/3	3/3	-	-	-	-
Bladder	3/3	3/3	-	3/3	-	-	-	-
Blood	-	-	-	-	-	-	-	-
Bone Marrow	-	-	-	-	-	-	-	-
Breast	3/3	3/3	3/3	3/3	-	-	-	-
Cerebellum	-	-	-	3/3	2/3	3/3	-	-
Cerebral Cortex	-	-	-	3/3	3/3	3/3	-	-
Colon	-	3/3	-	3/3	-	-	-	-
Endothelium	-	-	-	-	-	-	-	-
Eye	-	1/3	-	-	-	1/3	-	-
Fallopian Tube	3/3	3/3	-	-	-	-	-	-
GI tract	1/3	3/3	3/3	3/3	-	-	-	-
Heart	-	-	-	-	-	-	-	-
Kidney	2/3	2/3	-	1/3	-	-	-	-
Liver	2/3	2/3	-	-	-	-	-	-
Lung	2/3	3/3	-	-	-	-	-	-
Lymph Node	-	-	-	-	-	-	2/3	2/3
Ovary	3/3	1/3	-	-	-	-	-	2/3
Pancreas	3/3	2/3	-	2/3	-	-	-	-
Parathyroid	-	-	-	-	-	-	-	-
Pituitary	-	-	-	3/3	3/3	3/3	-	-
Placenta	-	-	-	-	-	-	-	-
Prostate	3/3	3/3	-	-	-	-	-	-
Skin	3/3	3/3	-	1/3	-	-	-	-
Spinal Cord	-	-	-	-	3/3	3/3		
Spleen	-	-	-	-	-	-	3/3	3/3
Striated Muscle	-	-	1/3	1/3	-	-	-	-
Testes	-	-	-	2/3	-	-	-	-
Thymus	3/3	3/3	-	-	-	-	-	-
Thyroid	-	2/3	-	-	-	-	-	-
Tonsil	3/3	3/3	-	-	-	-	-	-
Ureter	3/3	3/3	-	1/3	-	-	-	-
Uterus (cervix)	-	3/3	-	-	-	-	-	-
Uterus (endometrium)	-	1/3	-	-	-	-	-	-

The cellular distribution of Tmab-MCC-DM1 binding in the cynomolgus monkey tissues differed in intensity and location within some tissues. Binding was localized in tissues similar to that seen in humans. Staining more frequent in the high dose than the low dose in some cases but the staining intensity was similar at both concentrations in all positive staining cell types. Positive staining epithelial cells were identified in the urinary bladder and ureter (urothelium), mammary gland (duct and gland epithelium), colon (mucosal epithelium), fallopian tubes (mucosal epithelium), eye (conjunctiva), thyroid (follicular epithelium), kidney (collecting duct epithelium only), small intestine (mucosal epithelium), exocrine pancreas (duct epithelium), lung (bronchiolar epithelium), ovary (surface epithelium), prostate (glandular epithelium), liver (bile duct), skin (epidermis, hair follicles, apocrine and sebaceous glands), thymus (epithelium), tonsil (mucosal epithelium), cervix (mucosal epithelium) and uterus (mucosal and glandular epithelium). In general, ductal and apocrine gland epithelium stained with the greatest intensity and squamous epithelium with the least. Basal and basolateral cell surfaces stained more intensely than the apical cell surface. In several tissues, nerve spindle cells (presumptive Schwann cells) and perivascular spindle cells had cytoplasmic staining with Tmab-MCC-DM1.

Positive staining spindle cells, often closely associated with peripheral nerve bundles and ganglia, were identified in the supporting connective tissue stroma of the adrenal gland, urinary bladder, breast, colon, gastrointestinal tract, cerebellum, cerebral cortex, kidney, pancreas, pituitary, skin, striated muscle, testes and ureter. Positive staining glial cells were identified in the cerebellum, cerebral cortex, pituitary gland (pars distalis), spinal cord and optic nerve (eye). Small numbers of randomly distributed mononuclear cells/macrophages in lymph node (medulla), spleen (red pulp), tonsil and lung (alveolar macrophages) stained positive. Tmab-MCC-DM1 binding was identified in a subset of hematopoietic cells in the bone marrow. In the placenta, mesenchymal cells had strong staining.

Isotype Control Staining of Human and Cynomolgus Monkey Tissue Samples with Human IgG1 kappa

Non-specific background staining occurred in multiple tissue sections. This included macrophages and neutrophils, red blood cells, various glandular secretions, intravascular protein, intracellular and extracellular pigments, connective tissue, mucus, hepatocytes, sinusoidal epithelium, Kupffer cells, adrenal cortical epithelium and cortical tubular epithelium in the kidney. The investigators considered this staining in these cell types background only when the distribution, character and frequency of staining were the same with both Tmab-MCC-DM1 and Human IgG1κ antibody treatments.

Validation of Human and Cynomolgus Monkey Tissue Samples with an Anti-CD31 (PECAM-1) Antibody

Anti-CD31 Binding in Tissue Samples

Control Cell Lines	Anti-CD31 binding was not observed in the MDA-231, MDA-175 or MDA-468 cell lines. These cells lines are not known to express CD31 antigen.
Human Tissue	Specific staining of endothelial cells or megakaryocytes occurred in all tissues examined, confirming the validity of the tissues for immunohistochemistry.
Cynomolgus Monkey	Specific staining of endothelial cells or megakaryocytes occurred in all tissues examined, confirming the validity of the tissues for immunohistochemistry.

Isotype Control Staining of Human and Cynomolgus Monkey Tissue Samples with Mouse IgG

Same as seen in humans (vide supra).

4) Hemolytic Potential and Blood Compatibility of Trastuzumab-MCC-DM1 (PRO132365)

Study no	04-1257-1459
File name	04-1257-1459- (b) (4) -final.pdf
Laboratory	(b) (4)
Study Date	March 2005

GLP	Yes, except that Genentech did the control article characterization, stability, and dose solution stability
Audited	Yes
Drug	trastuzumab-MCC-DM1, Lot No. FIL037D01 Purity 98% monomer Drug to antibody ratio = 3.4, free maytansine = 2.4%
Methods	
Species	Three cynomolgus monkeys and one human volunteer
Concentrations	1.25, 2.5, and 5 mg/mL
Vehicle	10 mM succinate, 100 mg/mL trehalose, 0.1% polysorbate 20, pH 5.0)
Positive control	Saponin 1%
N	Single test per dose group
Incubation	29 minutes at 25 ºC

PRO132365 did not cause hemolysis in either cynomolgus monkey or human blood and was compatible with cynomolgus monkey and human serum and plasma.

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5 Pharmacokinetics/ADME/Toxicokinetics

1) *In Vitro* Plasma Protein Binding Study of DM1 (a Maytansinoid) in Sprague-Dawley Rat, Cynomolgus Monkey, and Human Plasma

Study number	Sponsor – 05-1047-1459,
Filename	05-1047-1459- (b) (4) -final.pdf
Laboratory	Genentech, South San Francisco, CA
Date	September 2005
GLP	No
Audited	No
Drug	DM1, DM1, Lot 41869-66 Purity not specified
Method	
Test tissue	Pooled male and female plasma from Sprague-Dawley rats, cynomolgus monkeys and humans (b) (4)
Concentrations	1000, 100, and 20 ng/mL in rat, monkey, and human plasma
Instrumentation	Spectrum Dialyzer 20 semi-micro chamber equilibrium dialysis system (Spectrum Labs; Rancho Dominquez, CA) regenerated cellulose discs with a molecular weight cut-off of 12 kDa for equilibrium dialysis
Solution	0.5% BSA/PBS

Results

The following table from the study report shows that rats bind a greater percentage of DM1 than do monkeys or humans. Monkeys and humans are comparable.

DM1 Protein Binding in Plasma from Sprague-Dawley Rats, Cynomolgus Monkeys, and Humans

Species	Nominal Concentration (ng/mL)	% Bound (Mean±SD) [a]	Species % Bound (Mean±SD) [b]
Sprague-Dawley rat	20	97.5±0.0399	97.1±0.430
	100	97.2±0.243	
	1000	96.6±0.0671	
Cynomolgus monkey	20	92.2±1.10	91.5±1.03
	100	91.9±0.753	
	1000	90.5±0.325	
Human	20	93.2±0.815	92.5±0.824
	100	91.8±0.702	
	1000	92.5±0.203	

[a] Mean and standard deviation values were based on n=3.

[b] Mean and standard deviation values were based on n=9 and represent the % Bound of DM1 for the species.

Metabolism

1) Evaluation of Cytochrome P450 Induction Potential by DM1 Using Human Cryopreserved Hepatocytes

Study no Sponsor reference number 09-2382, (b) (4) Study number 6281-603
File name 09-2382- (b) (4) -final.pdf
Laboratory Genentech, Inc., South San Francisco, CA 94080
Study Date May 2009
GLP No
Audited No
Drug DM1, Lot 833-833-03-004
Methods
Tissue Cryopreserved human hepatocytes from 3 male donors
Analysis HPLC
Test substrate

Cytochrome P450	Substrate	Product	Inducer (control)
1A2	phenacetin	acetaminophen	omeprazole
2B6	bupropion	hydroxybupropion	phenobarbital
3A4 and 3A5	testosterone	6β-hydroxytestosterone	rifampicin

Incubation 2X24 hours with fresh medium and inducers including DM1 in test wells
[DM1] 0.1, 1, 10, 100, 500, or 1000 nM in 0.1% DMSO in triplicate
mRNA Determined for each isoenzyme
Cell viability 24 and 48 hours

Results

The cells maintained their viability during the assay. DM1 did not induce Cytochromes P450 1A2, 2B6 or 3A4 or 5. The lack of induction potential indicates that DM1 at concentrations up to 1000 nM will probably not interact with PXR, CAR, or AhR.

2) Inhibition of the Catalytic Activities of Cytochromes P450 in Human Liver Microsomes by DM-1

Study no Sponsor reference number 09-1344,
(b) (4) Study number 300942690
File name 09-1344- (b) (4) -final.pdf
Laboratory (b) (4)
Study Date August 2009
GLP No
Audited No
Drug DM1, Lot 833-833-03-004, MT07-0147S
Methods
Tissue This study used 150 donor pooled human liver microsomes (HLM) as the enzyme source (BD UltraPool™ HLM 150)
Analysis

Test substrate

P450 Isoform	Substrate	Substrate Concentration µM	Metabolite	Internal standard (mass transition)	HLM Concentration mg/mL	Incubation Time Minutes
Cytochrome P450 1A2	Phenacetin	40	Acetamidophenol	Acetamidophenol-[13C215N]	0.2	10
Cytochrome P450 2B6	Bupropion	80	OH-Bupropion	OH-Bupropion-[D6]	0.1	5
Cytochrome P450 2C8	Amodiaquine	1.5	Desethylamodiaquine	Desethylamodiaquine-[D3])	0.02	5
Cytochrome P450 2C9	Diclofenac	5	4´-OH Diclofenac	4´-OH Diclofenac-[13C6]	0.05	5
Cytochrome P450 2C19	(S)-Mephenytoin	40	4´-OH S-Mephenytoin	4´-OH S-Mephenytoin-[D3]	0.3	10
Cytochrome P450 2D6	Dextromethorphan	5	Dextrorphan	Dextrorphan-[D3]	0.1	5
Cytochrome P450 3A4	Midazolam	3	1´-OH Midazolam	1´-OH Midazolam-[13C3]	0.02	5
Cytochrome P450 3A4	Testosterone	50	6ß-OH Testosterone	6ß-OH Testosterone-[D7]	0.05	10

[DM1] 0, 1.4, 4.1, 14, 41, 136, 407 or 678 nM
1.0, 3.0,10, 30, 100, 300 or 500 ng/mL
Reaction volume 400 µL
Activation system NADPH-generating system (1.3 mM NADP+, 3.3 mM glucose-6-phosphate, 0.4 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride)
Positive Controls

	Reversible Inhibitors (IC_{50})		Time dependent inhibitors (IC50 Shift)	
P450 Isoform	Positive Control	Acceptable range IC_{50} value (µM)	Positive Control	Acceptable range IC_{50} value[1] (µM)
Cytochrome P450 1A2	7,8-Benzoflavone	0.0020 – 0.040	NA	NA
Cytochrome P450 2B6	Ketoconazole	0.60 – 9.0	NA	NA
Cytochrome P450 2C8	Montelukast	0.0050 – 0.090	NA	NA
Cytochrome P450 2C9	Sulfaphenazole	0.10 – 2.5	NA	NA
Cytochrome P450 2C19	S-Benzylnirvanol	0.10 – 1.6	NA	NA
Cytochrome P450 2D6	Quinidine	0.015 – 0.25	NA	NA
Cytochrome P450 3A4 Testosterone	Ketoconazole	0.0050 – 0.10	NA	NA
Cytochrome P450 3A4 Midazolam	Ketoconazole	0.0050 – 0.10	Azamulin	0.0010 – 0.010

Analysis LCMS
IC_{50} Shift Cytochrome P450 3A4/5 only with midazolam as test substrate, with DM1 concentrations as above, DM1 pre-incubated with HLM for 30 minutes in the absence of NADPH

Results

The following table from the study report shows the results of this study.

Table 6. Summary of Inhibition results for DM-1 using Human Liver Microsomes as the Enzyme Source

P450 Isoform	DM-1			Positive Control			
	Reversible IC_{50}		Time-Dependence IC_{50}	Inhibitor	Reversible IC_{50} (µM)	Time-Dependence IC_{50} (µM)	
	(nM)	(ng/mL)	30 min			Inhibitor	30 min
CYP1A2	>678	>500	--	7,8-Benzoflavone	0.015	--	--
CYP2B6	>678	>500	--	Ketoconazole	3.0	--	--
CYP2C8	>678	>500	--	Montelukast	0.040	--	--
CYP2C9	>678	>500	--	Sulfaphenazole	0.50	--	--
CYP2C19	>678	>500	--	S-Benzylnirvanol	0.58	--	--
CYP2D6	>678	>500	--	Quinidine	0.080	--	--
CYP3A4 (M)	>678	>500	155 nM (114 ng/mL)	Ketoconazole	0.028	Azamulin	0.0029
CYP3A4 (T)	>678	>500	--	Ketoconazole	0.025	--	--

DM-1, in the presence of NADPH-generating system, inhibited cytochrome P450 1A2, cytochrome P450 2B6, cytochrome P450 2C8, cytochrome P450 2C9, cytochrome P450 2C19, cytochrome P450 2D6 and cytochrome P450 3A4 catalytic activities by less than 50 % at the highest concentration (678 nM; 500 ng/mL)) examined. Pre-incubation of DM-1 for 30 min with human liver microsomes, in the presence of NADPH, resulted in inhibition of cytochrome P450 3A4 (midazolam 1'hydroxylase) activity with an IC_{50} value of 155 nM (114 ng/mL). These results suggest that DM-1 did not cause reversible inhibition at the concentrations examined; however, it may be a potential time-dependent inhibitor of cytochrome P450 3A4.

3) Metabolite Radioprofiling and Identification in Plasma, Bile, and Urine from Rats Following IV Administration of Tmab-MCC-[^{3}H]DM1

Study no	Genentech number 09-1929, (b) (4) number 09686
File name	09-1929- (b) (4) -final.pdf
Laboratory	(b) (4)
Study Date	September 2009
GLP	No
Audited	No
Drug	Trastuzumab-MCC-[^{3}H]-DM1, Lot 2564-053, 3.33 mg/mL, 0.009 µCi/µg, radiolabel at DM1 Trastuzumab-MCC-DM1, Lot FIL037F01 Purity 98% monomer Drug to antibody ratio = 3.4, free maytansine = 2.4%
Methods	
Animals	Six female Sprague-Dawley rats (cannulated), weight 250 to 300 g
Study Design	The samples in this study are those collected in Genentech study 09-1060 (*vide supra*). The samples analyzed were those of group three in that study.
Vehicle	PBS
Blood collection	Jugular cannula 15 minutes and 24, 120, and 168 hours post-dose
Urine collection	Urine was collected predose and at the following intervals after dosing: 0 to 4, 4 to 8, 8 to 24, 24 to 34, 34 to 48, 48 to 58, 58 to 72, 72 to 82, 82 to 96, 96 to 106, 106 to 120, 120 to 130, 130 to 144, 144 to 154, and 154 to 168 hours.
Feces collection	Same as urine

Bile samples	Groups 3 and 4 at predose and at the following intervals after dosing: 0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 5, 5 to 6, to 7, 7 to 8, 8 to 24, 24 to 34, 34 to 48, 48 to 58, 58 to 72, 72 to 82, 82 to 96, 96 to 106, 106 to 120, 120 to 130, 130 to 144, 144 to 154, and 154 to 168 hours.
Analysis	Liquid chromatography coupled with liquid scintillation as well as solid scintillation counting using a Packard TopCount® NXT™ Microplate Counter. The catabolites/metabolites in each matrix were characterized by LC/MS/MS. Standards of Lys-MCC-DM1 (MW=1104), MCC-DM1 (MW=974), and DM1 (MW=737) were used to confirm the identity of catabolites/metabolites. Samples from 6 rats were pooled.

Results

Plasma

DM1 contains a free sulfhydryl thus some portion of DM1 released from T-DM1 will dimerize or react with other thiol-containing molecules in the circulation. The investigators added a reductant, *tris*(2-carboxyethyl) phosphine (TCEP), to the samples to prevent disulfide formation but a substantial portion of the radioactivity, more than 95%, was already protein bound (see study 09-1060). They treated the pooled plasma samples with or without TCEP and extracted them with acetonitrile. Less than 5% of the plasma radioactivity was in the acetonitrile soluble fraction both in the presence and absence of TCEP.

In the acetonitrile soluble fraction in the absence of TCEP, the LC/MS/MS detected no DM1. There was only one peak, M18. M18 coeluted M17 at the solvent front. In the presence of TCEP, in addition to the HPLC solvent front peaks, M17 and M18, the analysis detected M16, identified as DM1, as 0.3% of the $AUC_{0\text{-}168hr}$ of total plasma radioactivity. M17 and M18 accounted for about 1.7% to 3.8% of the $AUC_{0\text{-}168}$ hr of total plasma radioactivity in the absence and presence of TCEP. The investigators identified M17 as tritiated water; M18 remains unidentified. Lys-MCC-DM1 and MCC-DM1 were not detected.

Bile

Rats excreted 47.6% of total trastuzumab emtansine radiolabel in the bile over the 0 to 144 hr collection period (study 09-1060). Samples from six bile-duct cannulated rats were pooled by time intervals (0 to 48 hr and 48 to 144 hr) for radio-profiling. An aliquot of the pooled bile sample was treated with or without TCEP and then centrifuged. In the absence of TCEP, the supernatant contained five metabolite peaks. Two peaks were isomers of Lys-MCCDM1 (21.5% of the administered dose); two peaks were isomers of MCC-DM1 (4.6% of the administered dose). Tritiated water accounted for 3.3% of the administered dose. In the presence of TCEP, the DM1 accounted for about 1% of the administered dose, suggesting that most DM1 was eliminated as a disulfide bound conjugate. No other single component exceeded 1.0% of the administered dose. The metabolite profiles between 0 to 48 hr and 48 to 144 hr were similar except that more of the MCCDM1 was excreted during the 0 to 48 hr interval than in the 48 to 144 hr interval. The remainder of the radioactivity, 18.5%, was distributed in various peaks, each representing <5% of the dose. The investigators did not characterize these peaks.

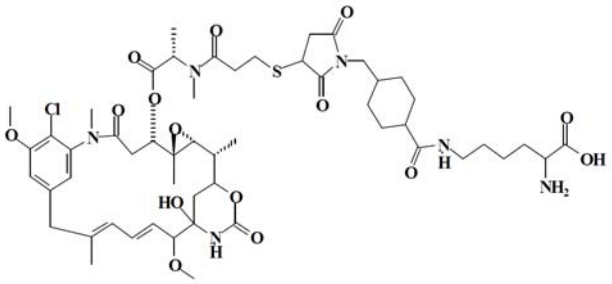
Urine

The rats eliminated only 7.7% of the radiolabeled dose in urine over the 0 to 144 hour interval. (Genentech study 09-10601). Samples from six bile-duct intact rats were pooled by time intervals 0 to 48 hr and 48 to 144 hr. The pooled urine sample was treated with or without

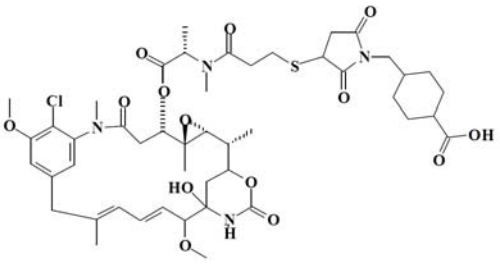
TCEP and then centrifuged. In urine, the same catabolites were identified as in bile. In the absence of TCEP, two isomers of Lys-MCCDM1 accounted for 2.2% of the dose and two isomers of MCC-DM1 accounted 0.2% of the administered dose during the 0 to 144 hr interval. Tritiated water accounted for 2.4%. In the presence of TCEP, only trace amounts of DM1, about 1% of the dose, were present. No other single component exceeded 1.0% of the administered dose and these unidentified peaks accounted for the remainder of the total radiation in urine. The metabolite profiles between 0 to 48 hr and 48 to 144 hr were similar except that more MCC-DM1 was excreted during the 0 to 48 hr interval.

The study identified the following compounds as catabolites of DM1.

Catabolites M12 and M13 (Lys-MCC-DM1)



Catabolites M14 and M15 (MCC-DM1)



M16 = DM1

Characterization of Plasma Stability of Trastuzumab-MCC-[^{3}H]DM1 In Vitro

Study no	Genentech number 05-0298-1459, (b) (4) number 09686
File name	05-0298-1459-(b) (4)-final.pdf
Laboratory	Genentech, Inc., South San Francisco, CA 94080
Study Date	March 2010
GLP	No
Audited	No
Drug	Trastuzumab-MCC-[^{3}H] DM1 (PRO132365), Lot 2044-92 Drug to antibody ratio 3.43, 10.3 μCi/mg
Concentration	50 µg/mL
Solutions	Human Plasma Cynomolgus Monkey Plasma

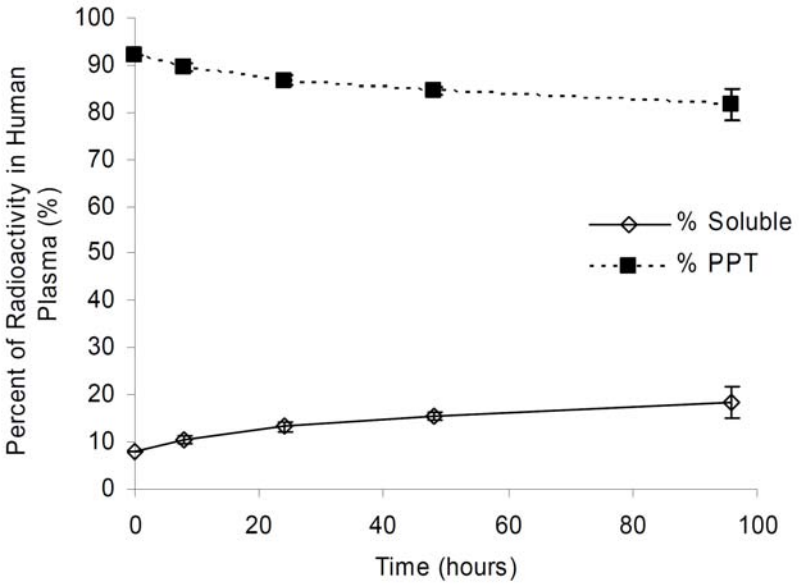
	Rat Plasma (not reviewed)
	PBS with 0.5% BSA
Precipitation	1% trifluoroacetic acid (TFA)/acetonitrile (ACN)
Duration	4 days at 37°C
Analysis	Liquid scintillation, ELISA and size exclusion HPLC (SE-HPLC)

Results

Liquid scintillation counting showed total radioactivity did not change over the 4-day study period following incubation of Tmab-MCC-[^{3}H] DM1 in plasma or buffer control. The following graph from the study report shows that most of the radioactivity in human plasma is associated with the precipitated proteins but that fraction decreases with time.

Figure 2

Percent (±SD) of ACN-Soluble and Precipitable Radioactivity to Total Radioactivity in Human Plasma

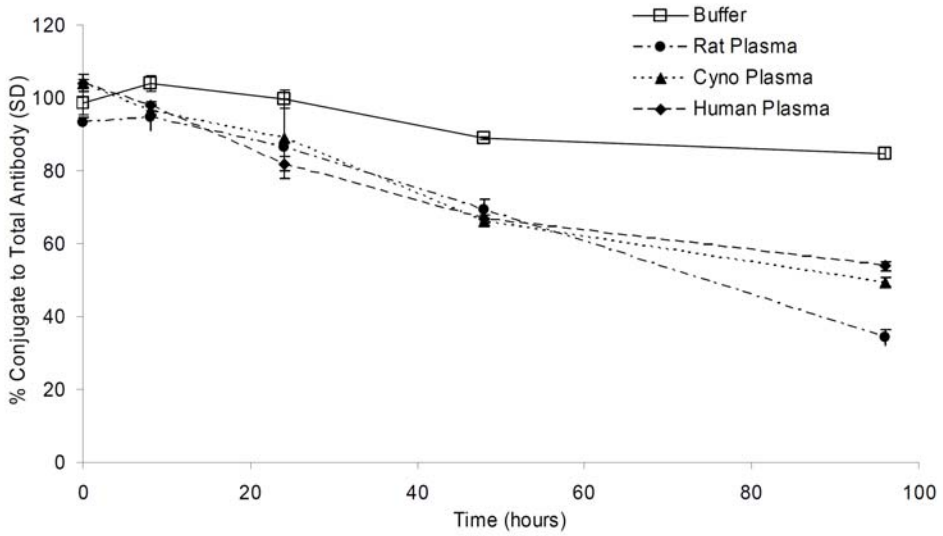


PPT = precipitated protein (pellet).

At 0 hour, the percentage of ACN-soluble radioactivity was 7.95 ± 0.05 in human plasma, 8.2 ± 0.2 in cynomolgus monkey plasma, and 8.1 ± 0.5 in rat plasma. By 96 hours, the percentage of ACN-soluble radioactivity was 18.4 ± 3.2 in human plasma, 21.8 ± 0.3 in monkey plasma and 21.3 ± 1.3 in rat plasma, respectively.

The following graph from the study report shows that using ELISA analysis the concentration of intact Tmab-MCC-[^{3}H] DM1 decreased in plasma over time.

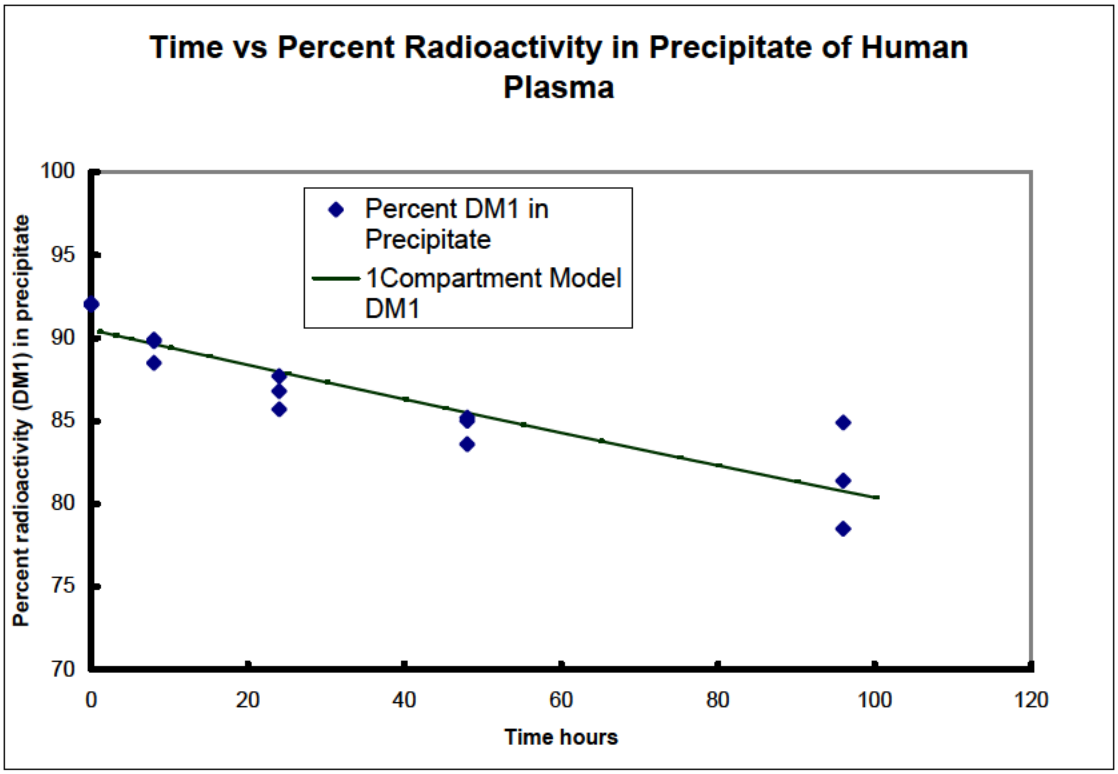
Figure 5
Mean (±SD) Percent of Tmab-MCC-[^{3}H] DM1 to Total Antibody in Plasma and Buffer Control



The percentage of ADC to total antibody at 0 hour was 104 ± 1 in human plasma, 104 ± 22 in cynomolgus monkey plasma, and 93 ± 1 in rat plasma. At 96 hours, the percentage of ADC to total antibody was 54 ± 1 in human plasma, 49 ± 1 in monkey plasma and 34 ± 2 in rat plasma.

SE-HPLC analysis showed that the radioactivity peak that co-migrated with the Tmab-MCC-[^{3}H] DM1 standard decreased at a rate and extent similar to that measured by ELISA. A concomitant increase in both a higher and lower molecular weight radioactive species was observed following *in vitro* incubation in plasma from all species studied. In conclusion, Tmab-MCC-[3H] DM1 underwent a time-dependent biotransformation following *in vitro* incubation in human, cynomolgus monkey, and rat plasma at 37°C.

I fit the data from the figure 5 above (data not shown) for the amount of radioactivity in the precipitate to a one exponential decay model using Microsoft Excel Solver module assuming that the hydrolysis of DM1 in plasma is a first order process. The following graph shows that the data fit this model well though the intercept is lower than expected. This implies the presence of a significant amount of free DM1 in this radioactive drug lot. With this model the calculated half-life for the hydrolysis of DM1 from trastuzumab emtansine in human plasma *in vitro* is estimated to be about 585 hours. The graph in figure 5 above suggests shows that the half-life in monkeys will be similar or indistinguishable from that of humans.



Elimination

1) Tissue Distribution of ^{125}I-Herceptin and ^{125}I-Herceptin-MCC-DM1 in Normal Rats

Study number	Sponsor – 05-0336-1459,
Filename	05-0336-1459-[redacted] (b) (4) -final.pdf
Laboratory	Genentech, South San Francisco, CA
Date	April 2005
GLP	No
Audited	No
Drug	^{125}I-Herceptin,Lot # M03945, purity not specified ^{125}I-Herceptin-MCC-DM1, Lot # PRO132365, purity not specified
Method	
Animals	Sprague-Dawley rats average 75g
Dose groups	^{125}I-Herceptin, 13 mg/kg, 200 μCi/kg ^{125}I-Herceptin-MCC-DM1, 13 mg/kg, 200 μCi/kg
Pre-treatment	Sodium Iodide to prevent sequestration in the thyroid
N	8 female per dose group
Route	IV
Schedule	Single dose
Time points	Two animals from each dose group were killed humanely and analyzed for radioactive antibody on days 1, 4, 7, and 14.

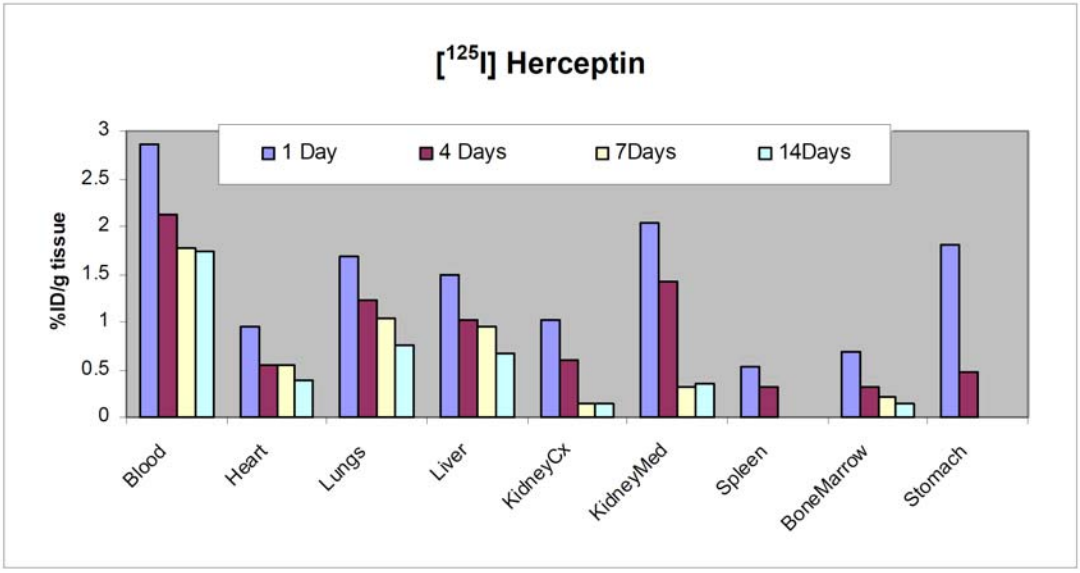
Analysis	Three sagittal sectioning levels were used from each rat to obtain a representation of all major organs. Three sections, 20µm thick, were obtained from each level, yielding approximately 9 sections per rat.
LOQ	0.25 µCi/g

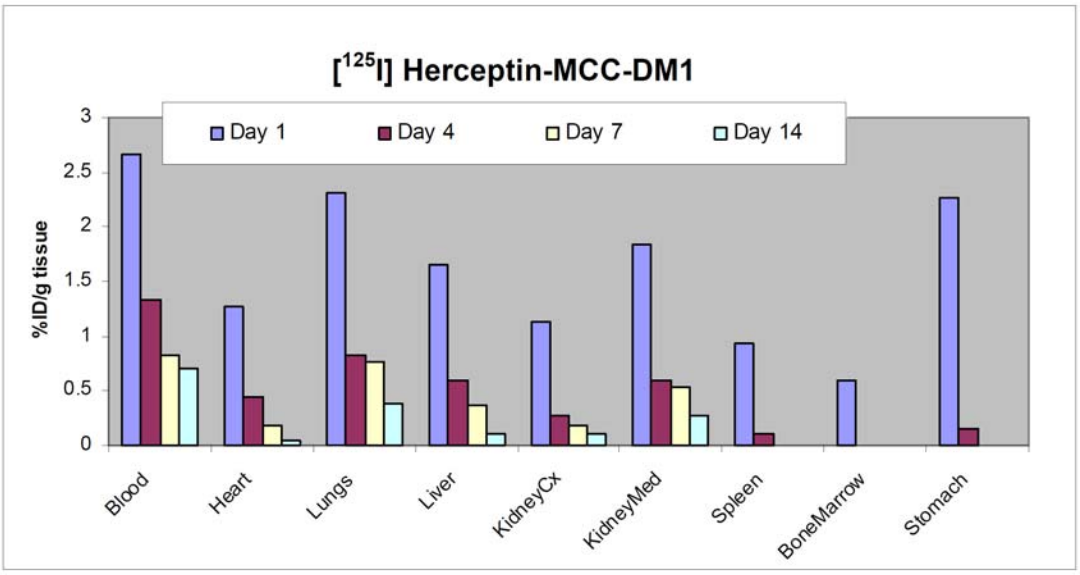
Results

The addition of trichloroacetic acid to precipitate the plasma protein showed that 100% of the radioactivity was associated with the resulting precipitate at 4, 7, and 14 days post-dose. On day 1 85% of the radioactivity was associated with the precipitate indicating a lack of complete distribution at the time of necropsy or possibly the presence of free iodine. The dose solutions were stored for 14 days prior to dosing.

The following charts from the study report show that the antibodies appear to distribute with total blood flow to each of the major organs studies. The study report does not provide actual numerical values for the radioactivity only the charts. The study report says that highest percentage of the radioactive dose was in the blood compartment at 1 day post-dose (2.85 % of injected drug [ID]) followed by the highly perfused organs in the order; lungs > live > kidney > heart > bone marrow > spleen. No radioactivity was detected in the brain at any time. Radioactivity did not appear to accumulate in any major organ during the four days study period, but rather decreased in proportion to the decrease in the plasma concentration.

Quantitative Whole Body Autoradiography of [^{125}I] Herceptin and [^{125}I] Herceptin-MCC-DM1 in normal rats





Quantitation of concentration of radioactivity in tissues and blood (from heart pool) was performed with [^{125}I] radioactive standards and MCID software (Imaging Research Inc., version 7). The concentration of radioactivity per gram of tissue was converted to the percent of the injected dose per gram of tissue (%ID/g). Data is expressed as the mean values of two animals per time point in each group.

2) Determination of Radioactivity in Plasma and Excreta Matrices following Intravenous Administration of Trastuzumab-MCC-[^{3}H]-DM1 or Unlabeled Trastuzumab-MCC-DM1 in Female Sprague Dawley Rats

Study no	Genentech number 09-1060
File name	09-1060-[redacted] (b) (4) -final.pdf
Laboratory	Genentech, Inc., South San Francisco, CA 94080
Study Date	May 2009
GLP	No
Audited	No
Drug	Trastuzumab-MCC-[^{3}H]-DM1, Lot 2564-053, 3.33 mg/mL, 0.009 μCi/μg, radiolabel at DM1 Trastuzumab-MCC-DM1, Lot FIL037F01 Purity 98% monomer Drug to antibody ratio = 3.4, free maytansine = 2.4%
Methods	
Animals	Female Sprague-Dawley rats (12 cannulated and 12 not cannulated), weight 250 to 300 g
Study Design	The following table from the study report shows the dose groups in this study

Group	No./Sex	Route	Non-Cannulated or Bile Duct–Cannulated Rat	Dose Level T-[^{3}H]-DM1 (mg/kg)	Dose Level T-DM1 (mg/kg)	Dose Volume (mL)
1 [a]	6/F	IV	Non-Cannulated	10	—	0.8
2 [a]	6/F	IV	Non-Cannulated	—	10	0.8
3 [b]	6/F	IV	Bile Duct–Cannulated	10	—	0.8
4 [b]	6/F	IV	Bile Duct–Cannulated	—	10	0.8

IV = intravenous; T = trastuzumab.

[a] Blood, urine, and feces were collected from these rats, which were housed in metabolism cages.

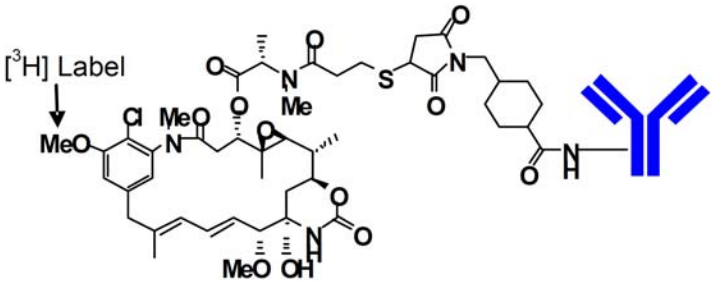
[b] Blood, bile, and feces were collected from these rats, which were housed in metabolism cages.

Vehicle	PBS
Blood collection	Tail vein for groups 1 and 2 Jugular cannula for groups 3 and 4 15 minutes and 24, 120, and 168 hours post-dose
Urine collection	Urine was collected predose and at the following intervals after dosing: 0 to 4, 4 to 8, 8 to 24, 24 to 34, 34 to 48, 48 to 58, 58 to 72, 72 to 82, 82 to 96, 96 to 106, 106 to 120, 120 to 130, 130 to 144, 144 to 154, and 154 to 168 hours.
Feces collection	Same as urine
Bile samples	Groups 3 and 4 at predose and at the following intervals after dosing: 0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 5, 5 to 6, to 7, 7 to 8, 8 to 24, 24 to 34, 34 to 48, 48 to 58, 58 to 72, 72 to 82, 82 to 96, 96 to 106, 106 to 120, 120 to 130, 130 to 144, 144 to 154, and 154 to 168 hours.
Analysis	Since only rats in Groups 1 and 3 were dosed with [^{3}H]-labeled material, only samples from those two groups were analyzed for radioactivity. The samples from animals given radiolabeled trastuzumab emtansine were analyzed for total radiation, radiolabel in the acetone soluble fraction and

	radiolabel in the precipitable fraction. Cell pellets from blood were also analyzed.
Analysis	The samples from animals given unlabeled trastuzumab emtansine were analyzed by HPLC.

The following figure from the study report shows the position of the tritium label on DM1.

Structure of Trastuzumab-MCC-[^{3}H]-DM1 and Site of [^{3}H] Label

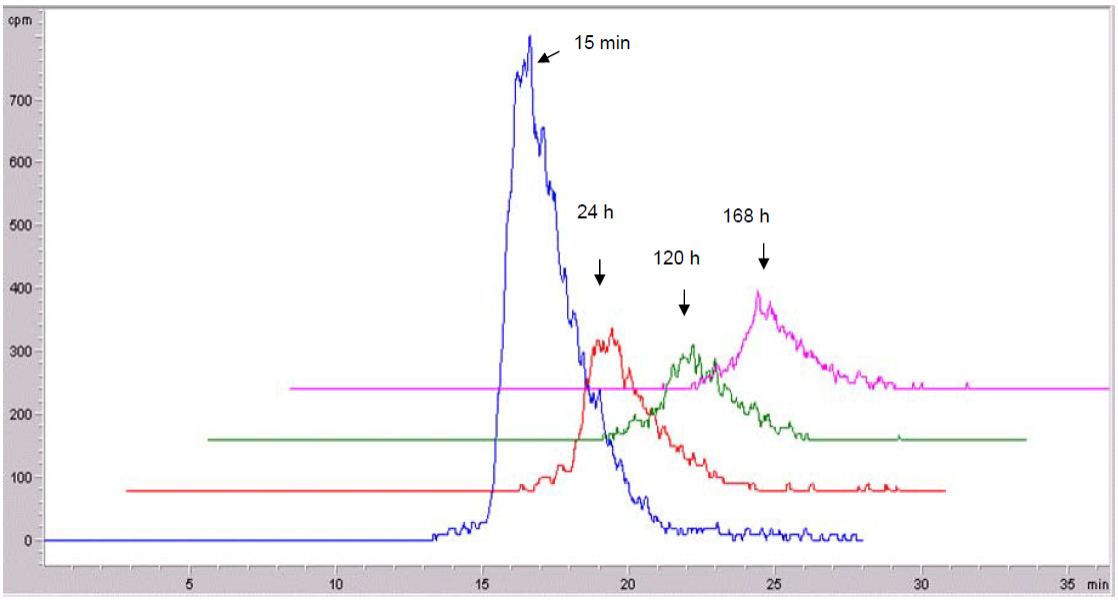


Results

Blood

Blood sampling was sparse but the graphs of time vs concentration was similar to those seen in other studies. Most of the radioactivity, nearly 100% at all time points, in the plasma was associated with the acetonitrile fraction. HPLC analysis detected only a single peak that migrated at the same retention time as trastuzumab-MCC-[3H]-DM1. This finding is consistent with most of the radioactivity being precipitable in these samples. This suggests that little free DM1 or metabolites were circulating due to the slow hydrolysis and fast clearance of those compounds. Hydrolysis is rate limiting (vide infra). For all rats the amount of radioactivity associated with the pellet was less than 3% of the injected dose (ID). Little trastuzumab emtansine appears to bind to RBCs.

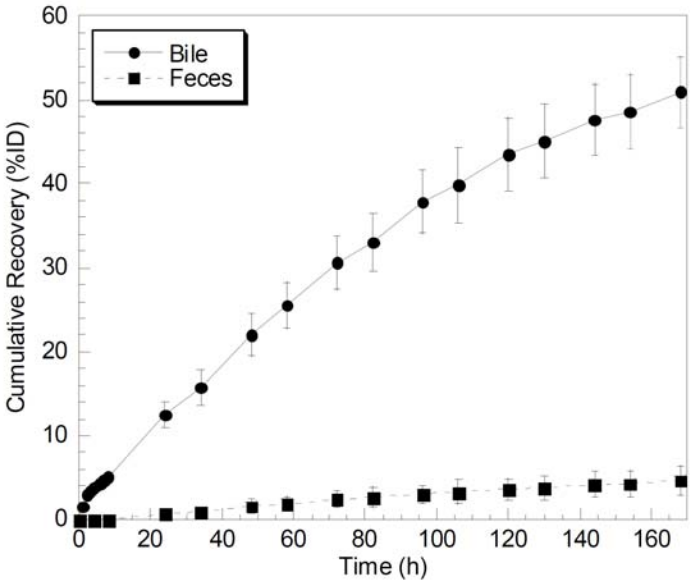
SE-HPLC Analysis of the Plasma Samples



Bile and Feces from Cannulated Rats

The following graph from the study report shows the cumulative recovery of radiolabel in the bile and feces. The radioactivity in the bile was measurable within hours post-dose and steadily increased with time. By the end of the 7-day study, 50.9% ± 4.2% ID had been eliminated in the bile. Minimal amounts of radioactivity were detected in the feces from bile duct−cannulated rats with 4.7% ± 1.7% ID recovered in the feces at the end of the 7-day study. Direct excretion of radioactivity into the gastrointestinal tract is a minor elimination pathway.

Mean (±SD) Cumulative Recovery of Radioactivity in Bile and Feces from Bile Duct–Cannulated Rats



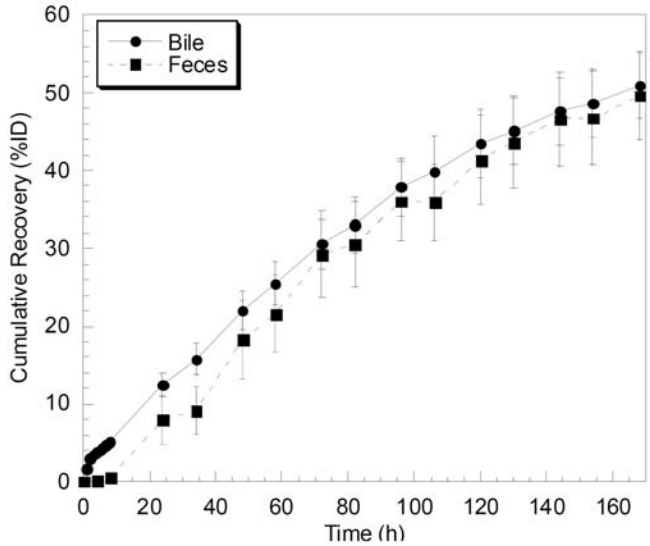
Most of the radioactivity in the bile and feces was associated with the acetonitrile fraction. For the bile, the percent soluble radioactivity ranged from 97.0 ± 1.46 to 99.5 ± 0.1 of

the total at each sample time. By HPLC, trastuzumab-MCC-[^{3}H]-DM1 was not detected in the bile, but several low molecular-weight peaks were. Some peaks were around the same retention time as free DM1. For the feces, the percent soluble radioactivity ranged from 74.4 ± 3.4 to 79.3 ± 3.7 of the total at each sample time. These values are lower probably because feces is more difficult to extract completely than is bile.

Feces and Urine from Non-Cannulated Rats

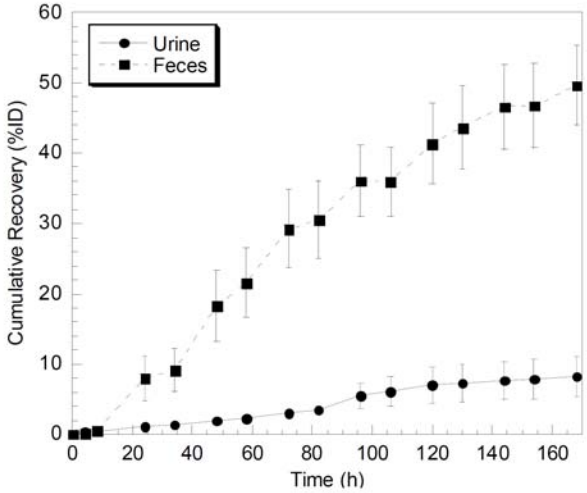
The following figure from the study report shows that the cumulative radioactivity recovered in the feces samples from non-cannulated rats was comparable to that detected in the bile samples from the bile duct−cannulated rats. Radioactivity in feces was first detected in the 8 to 24 hours after dosing. At the end of the study period, 50.9% ± 4.2% of total radioactivity was recovered in the bile and 49.6% ± 5.7% was recovered in the feces.

Figure 6
Mean (±SD) Cumulative Recovery of Radioactivity in Bile from Bile Duct–Cannulated Rats and Feces from Non-Cannulated Rats



The following figure from the study report shows that most of the dose given to non-cannulated rats was excreted in the feces.

Figure 5
Mean (±SD) Cumulative Recovery of Radioactivity in Urine and Feces from Non-Cannulated Rats



In the urine of non-cannulated rats, the total mean recovered radioactivity was 8.2% ± 2.8% of the injected dose. Of this, 98 ± 1% was recovered in the soluble fraction. The investigators did not measure total radioactivity in the carcass, but it is likely that the remainder of the radiolabel remains bound at 168 hours.

5.2 Toxicokinetics

1) Reaction Phenotyping of DM1 Using Human Liver Microsomes and Recombinant Cytochrome P450 (rCYP) and Metabolite Profiling in Human

Study no	Sponsor number 09-2416, (b) (4) Study number 6281-603
File name	09-2416- (b) (4) -final.pdf
Laboratory	Genentech, Inc. South San Francisco, CA 94080
Study Date	June 2009
GLP	No
Audited	No
Drug	DM1, Lot 833-833-03-004
Methods	
Tissue	Pooled male and female human liver microsomes (HLM)
	Human rCYP1A2 — Human rCYP2A6
	Human rCYP2B6 — Human rCYP2C8
	Human rCYP2C9 — Human rCYP2C18
	Human rCYP2C19 — Human rCYP2D6
	Human rCYP2E1 — Human rCYP3A4
	Human rCYP3A5 — Human rCYP4A11

Procedure for HLM study

In the controls for this study, HLM (0.5 mg/mL protein) were incubated with or without NADPH reduction-regeneration system in the presence of 1 μM DM1. Each reaction was done in triplicate. The reaction was initiated by the addition of DM1. The reaction was stopped by adding an aliquot to aqueous acetonitrile. In the experiments with inhibitors, the inhibitor was added to the microsomal preparation with regenerating system 5 or 15 minutes before the reaction was started with the addition of DM1. In each case, a sample of the reaction mixture was taken immediately after the addition of DM1 and after 60 minutes of incubation. The samples were analyzed by LC/MS/MS.

Test inhibitors The following table shows the cytochrome 450 isoenzymes and their corresponding specific inhibitor.

Targeted cytochrome P450 Isoenzyme	Chemical Inhibitor	Concentration of Inhibitor (μM)
1A2	Furafylline [a]	10
2A6	tranylcypromine	1
2B6/2C19	Ticlopidine	10
2C8/2C9	Quercetin	10
2C9	Sulfaphenazole	10
2D6	Quinidine	1
3A4/5	Ketoconazole	1
3A4/5	troleandomycin [a]	20
All Cytochromes P450	1-aminobenzotriazole [a]	1000

a – inhibitors were pre-incubated with HLM for 15 minutes, all others were pre-incubated for five minutes

Procedure for Human recombinant human cytochrome P450 (rCYP) Incubations

For each assay, 40 pmol/mL rCYP was incubated with 1mM NADPH for 5 minutes at 37°C. Each reaction was done in triplicate. The reaction was started with the addition of DM1 (1 µM). Again, samples were collected from each reaction at the 0- and 60-minute time-points and transferred to aqueous acetonitrile to stop the reaction containing 7.5 nM of Maytansine as an internal standard. The samples were then analyzed by LC/MS/MS for disappearance of DM1.

Results

The following table presents the results of the experiments with human liver microsomes rounded for significant figures.

Targeted Cytochrome P450 Isoform	Chemical Inhibitor	Percentage of DM1 Remaining [a]	SD	Percentage of DM1 metabolized
Control	none with NADPH	27	3	73
Control	none without NADPH	82	16	18
All	1-aminobenzotriazole [b]	81	10	19
1A2	Furafylline [c]	25	3	75
2A6	tranylcypromine	25	5	75
2B6/2C19	Ticlopidine	26	3	74
2C8/2C9	Quercetin	28	6	72
2C9	Sulfaphenazole	25	5	75
2D6	Quinidine	22	7	78
3A4/5	Ketoconazole	76	17	24
3A4/5	troleandomycin [c]	84	9	16

In the control with NADPH, the HLM metabolized 73% of the DM1; in the absence of NADPH the HLM system metabolized only 27% of the DM1. Thus, most of hepatic DM1 metabolism must be NADPH dependant. In the presence of 1-aminobenzotriazole, an inhibitor of most forms of human cytochrome P450 metabolism, the microsomes metabolized only 29% of the available DM1. This is consistent with the amount of metabolism that occurred in the absence of NADPH and suggests that cytochromes P450 mediate most of the human hepatic metabolism of DM1. Except for ketoconazole and troleandomycin, all the inhibitors fail to prevent baseline cytochrome P450 metabolism with remarkable consistency. Again the amount of metabolism is similar to baseline metabolism in the presence of NADPH. Conversely, ketoconazole and troleandomycin inhibit DM1 metabolism at a level remarkably similar to that seen in the absence of NADPH, suggesting that cytochromes P450 3A4 or 3A5 or both may be responsible for much of the human hepatic metabolism of DM1.

The following table presents the results for the experiments with human recombinant cytochrome P450 enzymes.

Recombinant human Cytochrome P450 Isoenzyme	Percentage of DM1 remaining	SD	Percentage of DM1 metabolized
1A2	86	6	14
2A6	104	11	0
2B6	112	22	0
2C8	93	8	7
2C9	76	4	24
2C18	102	17	0
2C19	99	4	1
2D6	92	12	8
2E1	81	4	19
3A4	22	4	78
3A5	54	5	46
4A11	99	N = 1	1

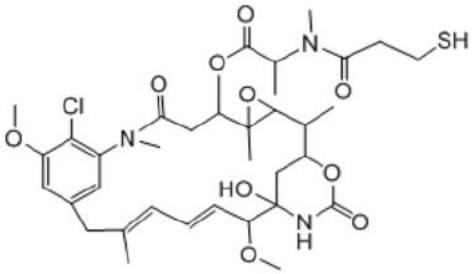
These experiments corroborate the results with HLM, again showing that DM1 is metabolized by cytochromes P450 3A4 and 3A5, but also to a lesser extent 2C9 and 2E1.

The following figure shows the metabolites that the investigators identified by mass spectroscopy in these experiments. The thioethyl group is the most labile group in the molecule. The sulfonation metabolites M3 and M2 will be significantly more water soluble than the parent compound and thus more easily eliminated in the urine. The M7 metabolite is potentially less soluble and potentially more toxic than the parent.

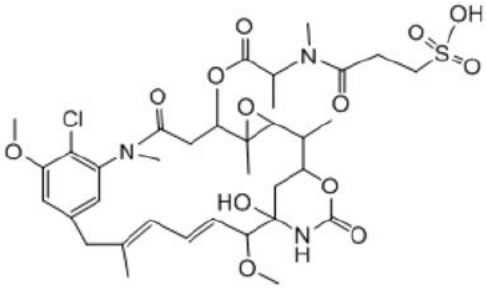
Figure 1

Structure of DM1 and Proposed Structures of Its Metabolites

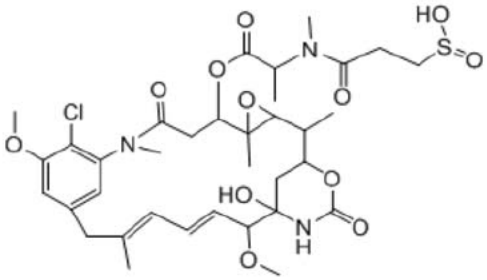
DM1



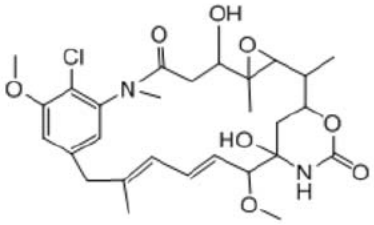
M3



M2



M7



2) *In Vitro* Interaction Studies of DM1 (Maytansine) with Human MDR1 (ABCB1/P-gp) ABC (Efflux) Transporter

Study no	Sponsor reference number 10-1207, (b) (4) Protocol Number: XT-162-Genentech-02-31Mar2010
File name	10-1207 (b) (4) -final.pdf
Laboratory	(b) (4)
Study Date	March 2010
GLP	No
Audited	No
Drug	DM1 – Lot number 139504-50-0/12213 ^{3}H-DM1 – Lot number E001451-08 516 mCi/mmol

This study evaluated, whether DM1 is a substrate and/or inhibitor of the human efflux transporter MDR1 in MDCKII-MDR1 cells.

This study was a series of standard assays to determine the interaction of DM1 with MDR1 (p-glycoprotein). The studies show that DM1 is probably a substrate for MDR1 membrane transport, but that it does not appear to inhibit the transport of other substrates.

3) Pharmacokinetic Comparability of Trastuzumab-MCC-DM1 (b) (4) in Female Cynomolgus Monkeys

Study no	Sponsor reference number 07-1474, (b) (4) number 6281-875
File name	07-1474- (b) (4) -final
Laboratory	(b) (4)
Study Date	March 2005
GLP	No
Audited	No
Drug	Trastuzumab-MCC-DM1 (b) (4) Lot FIL037F01 antibody to drug ratio 3.5, 5 mg/mL (10 mL/vial) purity 98% (ELISA), 4% free maytansinoids Trastuzumab-MCC-DM1 (b) (4) Lot 07p12-01-001 antibody to drug ratio 3.8, Lyophilized cake purity 99% (ELISA), free maytansinoids not specified
Methods	
Species	Naïve female cynomolgus monkeys (Macaca *fascicularis*) Initial age not specified, weighing between 2.0 and 2.9 kg
Dose	Group 1 (b) (4) 10 mg/kg Group 2 (b) (4) 10 mg/kg
N	13 per dose group
Schedule	Single dose
Formulation	Not specified for either drug product. The sponsor supplied the vehicle for each drug product pre-mixed to (b) (4).
Route	IV infusion, saphenous vein
Clinical signs	Twice daily
Body weights	Weekly
Food cons.	Qualitative daily
Antibody analysis	Day 43
Toxicokinetics	Blood was collected predose and at 5 minutes, 6, 12, 24, (Day 2), 48 (Day 3), 72 (Day 4), 120 (Day 6), 168 (Day 8), 336 (Day 15), 504 (Day 22), 672 (Day 29), 840 (Day 36), and 1008 (Day 43) hours postdose.
Analysis	ELISA (total trastuzumab and TDM1) and Electrospray LC/MS/MS (DM1)

The monkeys were retuned to the colony after the completion of the study.

Results

The Applicant had (b) (4) do these experiments to establish the pharmacokinetic equivalence of two lots of trastuzumab emtansine (vide supra). The Applicant made several changes (b) (4) In the new process, the Applicant conjugates DM1 to trastuzumab (b) (4) and formulates T-DM1 as a lyophilized product.

Dose Analysis	The measured concentration of the dose in Group 1 (b) (4) was 107% of the target concentration. The measured concentration of the dose in Group 2 (b) (4) was 99.7% of the target concentration.
Mortality	None
Clinical signs	No toxicologically significant signs or symptoms

Antibody analysis One monkey of 13 in the Group 2 (b) (4) developed antibodies to trastuzumab emtansine during the course of the experiment. No monkeys Group 1 (b) (4) developed antibodies to trastuzumab emtansine.

Body weight No toxicologically significant changes

Food Consumption No toxicologically significant changes

Toxicokinetic Analysis

The following tables from the study report show the calculated toxicokinetic parameters using non-compartmental analysis in WinNonLin Professional.

Table F1
Mean (±SD) Non-Compartmental Pharmacokinetic Parameters for DM1 (n=13/group)

Group No.	Test Material	Lot No.	$t_{1/2}$ (day)	C_{max} (ng/mL)	AUC_{last} (ng•day/mL)	CL (mL/day/kg)	V_{ss} (mL/kg)
1	Trastuzumab-MCC-DM1 (b) (4)	FIL037F01	2.14±0.498	16.1±3.01	8.43±±1.68	1030000±187000	2700000±505000
2	Trastuzumab-MCC-DM1 (b) (4)	07p12-01-001	1.92±0.385	12.8±1.81	6.03±1.49	1370000±202000	3410000±737000

Table F2
Mean (±SD) Non-Compartmental Pharmacokinetic Parameters for Total Trastuzumab (n=13/group)

Group No.	Test Material	Lot No.	$t_{1/2}$ (day)	C_{max} (ng/mL)	AUC_{last} (ng•day/mL)	CL (mL/day/kg)	V_{ss} (mL/kg)
1	Trastuzumab-MCC-DM1 (b) (4)	FIL037F01	6.85±1.96	281000±27600	1340000±371000	8.26±3.73	74.3±4.22
2	Trastuzumab-MCC-DM1 (b) (4)	07p12-01-001	8.26±1.73	248000±26000	990000±177000	10.3±2.66	103±8.44

The following three graphs from the study report show the time course for the elimination of trastuzumab emtansine, total trastuzumab and DM1.

Figure 1
Group Mean (±SD) Serum T-DM1 Concentration–Time Data
for Groups 1 (b) (4) and 2 (b) (4)

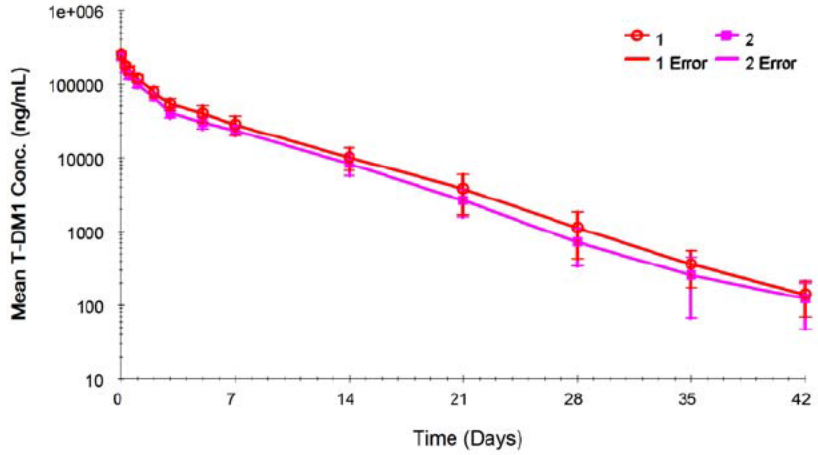


Figure 5
Group Mean (±SD) Serum Total Trastuzumab Concentration–Time Data
for Groups 1 (b) (4) and 2 (b) (4)

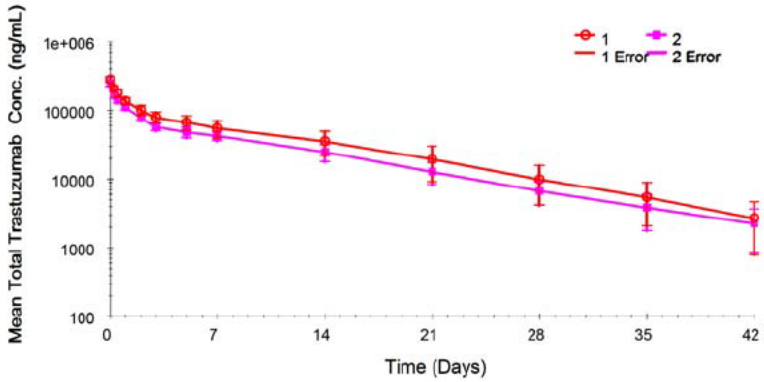
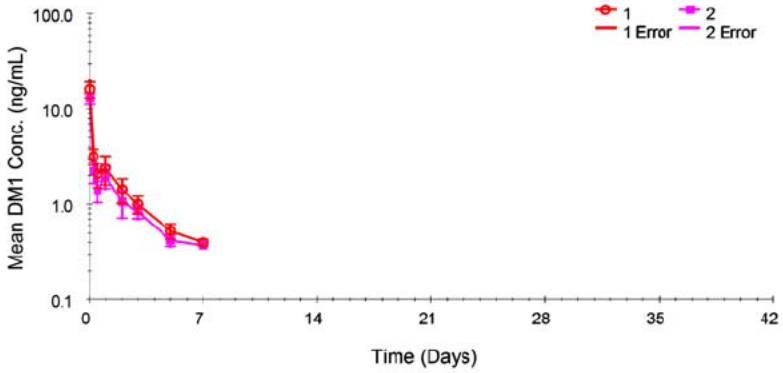


Figure 4
Group Mean (±SD) Plasma DM1 Concentration–Time Data
for Groups 1 (b) (4) and 2 (b) (4)



The following tables from the study report show the statistical comparison for bioequivalence between the two different lots of drug product.

Table 2

AUC_{last} Geometric Means and Ratio of Individual Animal Data (n = 13/group) for T-DM1

Group	Test Material	Lot No	AUC_{last} (day • ng/mL) Mean ± SD	Geometric Mean	95% LCL	95% UCL	Geometric Mean Ratio (CI) [a]
1	Trastuzumab-MCC-DM1 (b) (4)	FIL037F01	701000 ± 134000	687000	601000	786000	
2	Trastuzumab-MCC-DM1	07p12-01-001	575000 ± 74400	570000	523000	620000	0.830 (0.732, 0.940)

AUC_{last} = area under the curve from time 0 to the time of last measurable concentration; CI = 90% confidence interval; LCL = lower confidence limit; UCL = upper confidence limit.

[a] The ratio of geometric means and confidence interval for AUC_{last} comparing Group 2 with Group 1.

Table 3

C_{max} Geometric Means and Ratio of Individual Animal Data (n = 13/group) for T-DM1

Group	Test Material	Lot No.	C_{max} (ng/mL) Mean ± SD	Geometric Mean	95% LCL	95% UCL	Geometric Mean Ratio (CI) [a]
1	Trastuzumab-MCC-DM1 (b) (4)	FIL037F01	253000 ± 28500	251000	234000	269000	
2	Trastuzumab-MCC-DM1	07p12-01-001	241000 ± 28300	239000	223000	257000	0.952 (0.880, 1.03)

C_{max} = maximal concentration from time 0 to the time of last measurable concentration; CI = 90% confidence interval; LCL = lower confidence limit; UCL = upper confidence limit.

[a] The ratio of geometric means and confidence interval for C_{max} comparing Group 2 with Group 1.

The tables show that the geometric mean ratio for C_{max} is 0.95 with 90% confidence limits (CL) of 0.880 to 1.03. The investigators state that this satisfies the criteria for bioequivalence though they do not explicitly state what those criteria were. Nevertheless, this seems reasonable since the CI includes 1. The tables also show that the mean geometric ratio for AUC_{last} is 0.830, a 17% difference, with confidence limits of 0.732 to 0.940. The investigators state that this result fails to demonstrate bioequivalence. This is reasonable since the CL excludes 1.

In the graphs of time vs concentration above, the values for Group 1 is consistently greater than that of Group 2 giving rise to a significant difference in AUC. There were at least two confounding factors that contributed to this result. First, the measured concentration of the dose in Group 1 (b) (4)) was 107% of the target concentration. The measured concentration of the dose in Group 2 (b) (4) was 99.7% of the target concentration. Secondly, one monkey in Group 2, number I04904, developed antibodies to trastuzumab emtansine. The serum concentrations of trastuzumab emtansine in this monkey were consistently the lowest or second lowest in this dose group. This monkey was not excluded from the analysis. These two factors combined are probably sufficient to cause the new formulation, (b) (4) to fail the bioequivalence test. Nevertheless, these factors probably cannot account for the total difference between the two preparations. The initial drug product, (b) (4) is likely somewhat more bioavailable than the second drug product, (b) (4)

4) Evaluation of the Pharmacokinetics of PR0132365 (Trastuzumab-SMCC-DM1) Following a Single Intravenous Bolus Dose in Cynomolgus Monkeys with Preliminary Tolerability (Non-GLP)

Study no 04-0975-1459
File name 04-0975-1459- (b) (4) -final.pdf

Laboratory (b) (4)
Study Date November 2005
GLP No
Audited No
Drug Trastuzumab-SMCC-DM1, lot number 02077-001
Purity not specified

Methods

Species Naïve cynomolgus monkeys, Animal body weights ranged from approximately 3.5 to 5.5 kg and ages ranged from 2.5 to 6.0 years.

N 7 females and 7 males

Doses The following table shows the dose groups, doses, dose volumes and N. During preparation, dosing solutions were inadvertently switched during preparation in groups 2 and 3 (*). I will refer only to Control, LD, MD, and HD and not use the group numbers to avoid confusion.

Treatment Group	Dose Group	Nominal mg/kg	Nominal Dose ug/m2 ug/kg	Conc. (mg/mL) mg/mL	Dose Volume (mL/kg) mL/kg	Number of Females	Number of Males
1	Control	0	0	0	6	1	1
3*	LD	0.3	72.4	0.05	6	2	2
2*	MD	3	724	0.5	6	2	2
4	HD	30.0	7240	5.0	6	2	2

Formulation aqueous solution of 10 mM succinate, 100 mg/mL trehalose and 0.1% polysorbate 20, pH 5.0

Route IV

Clinical Obser. Twice daily

Body Weight Weekly

Hematology pre-dose and on Days 2, 4, 6, 8, 12, 15,19, 22, 25, 29, 43, 57 and 71.

Clinical Chem. pre-dose and on Days 2, 4, 6, 8, 12, 15,19, 22, 25, 29, 43, 57 and 71.

Toxicokinetics pre-dose, at 2 minutes and at 4 and 12 hours post-dose on Days 1, 2, 3, 4, 6, 8, 11, 15, 22, 29, 36, 43, 50, 57, 64, 71 and 76.

The investigators used WinNonLin to analyzed the data with different models for different drugs and different doses according to the following table. They did not provide a rationale for these choices nor did they specify a systematic elimination of other models.

Species	LD (0.3 mg/kg)	MD (3 mg/kg)	HD (30 mg/kg)
Total trastuzumab	Non-compartmental	3 compartment	2 compartment
Trastuzumab-SMCC-DM1	Non-compartmental	2 compartment	2 compartment
DM1	Non-compartmental	Non-compartmental	Non-compartmental

Antibody analysis Monkey antibodies to trastuzumab emtansine were measured using an electrochemiluminescent assay (ECLA). Serum samples taken from each animal pre-dose and at Day 76 post-dose were analyzed and compared to pooled sera from untreated cynomolgus monkeys.

Calibration

Results

Dose analysis The trastuzumab-SMCC-DM1 concentration was 5.1 mg/mL, pH 5.0, with a drug to antibody ratio of 3.4. The preparation was 98% monomer. Endotoxin was < 0.04EU/mg.

Clinical Obs. No toxicologically significant differences
Body Weight No toxicologically significant differences
Toxicokinetics

Table 2
Groups 2 and 4: Mean (±SD) Total Trastuzumab and Trastuzumab-MCC-DM1 PK Parameters following IV Administration of Trastuzumab-MCC-DM1

Parameter	Total Trastuzumab		Trastuzumab-MCC-DM1	
	Group 2 (3.0 mg/kg)	Group 4 (30 mg/kg)	Group 2 (3.0 mg/kg)	Group 4 (30 mg/kg)
AUC_{0-inf} (μg/mL • day)	302 ± 18.2	6940 ± 1760	180 ± 15.0	3110 ± 506
CL (mL/kg/day)	9.77 ± 0.584	4.66 ± 1.37	16.5 ± 1.53	10.1 ± 1.77
C_{max} (μg/mL)	73.5 ± 3.53	833 ± 36.5	74.2 ± 4.31	795 ± 76.0
V_1 (mL/kg)	40.2 ± 2.28	36.9 ± 1.64	39.8 ± 2.64	38.8 ± 3.71
V_{ss} (mL/kg)	90.6 ± 13.1	74.5 ± 3.06	60.7 ± 8.51	68.6 ± 1.32
MRT (days)	9.27 ± 1.18	16.9 ± 4.82	3.67 ± 0.199	6.96 ± 1.21
$t_{1/2, \alpha}$ (days)	0.506 ± 0.136	0.456 ± 0.0636	0.557 ± 0.175	0.498 ± 0.0379
$t_{1/2, \beta}$ (days)	4.45 ± 0.0899	12.2 ± 3.47	2.96 ± 0.093	5.30 ± 0.905
$t_{1/2, \gamma}$ (days)	15.9 ± 2.75	ND	ND	ND
$t_{1/2, effective}$ (days)	6.42 ± 0.820	11.7 ± 3.34	2.54 ± 0.138	4.82 ± 0.838

AUC_{0-inf} = area under the curve from zero to infinity; CL = clearance; C_{max} = maximum observed concentration; ND = not determined; MRT = mean residence time; $t_{1/2, \alpha}$ = half-life of the α phase; $t_{1/2, \beta}$ = half-life of the β phase; $t_{1/2, \gamma}$ = half-life of the γ phase; $t_{1/2, effective}$ = effective half-life; V_1 = volume of the central compartment; V_{ss} = volume of distribution at steady state.

Table 3
Mean (±SD) Total Trastuzumab and Trastuzumab-MCC-DM1 PK Parameters following IV Administration of Trastuzumab-MCC-DM1 (Group 3; 0.3 mg/kg)

Parameter	Total Trastuzumab	Trastuzumab-MCC-DM1
AUC_{0-inf} (μg/mL • day)	11.0 ± 3.71	7.53 ± 2.52
CL (mL/kg/day)	27.7 ± 11.0	40.4 ± 16.1
C_{max} (μg/mL)	8.10 ± 1.71	7.36 ± 1.43
V_1 (mL/kg)	35.2 ± 7.96	38.5 ± 7.56
V_{ss} (mL/kg)	66.7 ± 12.7	44.2 ± 10.8
MRT (days)	2.60 ± 0.843	1.14 ± 0.165
$t_{1/2,\ terminal}$ (days)	4.88 ± 2.03	0.916 ± 0.174

AUC_{0-inf} = area under the curve from zero to infinity; CL = clearance; C_{max} = maximum observed concentration; MRT = mean residence time; V_1 = volume of the central compartment; V_{ss} = volume of distribution at steady state; $t_{1/2,\ \lambda z}$ = terminal half-life.

6 General Toxicology

6.1 Single-Dose Toxicity

The submission includes reports of single dose toxicity studies done in rats given trastuzumab emtansine or DM1 which are not formally reviewed in this section. Nevertheless, Section 11 Integrated Summary and Safety Evaluation below includes summaries of these studies.

1) Single Dose Intravenous Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) in Cynomolgus Monkeys with a 3-Week Recovery Period

Study no	Sponsor reference number 04-0976-1459, (b) (4) Study number 6281-603
File name	04-0976-1459- (b) (4) -final.pdf
Laboratory	(b) (4)
Study Date	March 2005
GLP	Yes
Audited	Yes
Drug	Tmab-MCC-DM1 (PRO132365) FIL037D01 Purity 98% monomer by size exclusion chromatography Free maytansinoid 2.4% Drug to antibody ratio 3.4
Methods	
Species	Naïve male and female cynomolgus monkeys (Macaca *fascicularis*) Approximately 3 to 5 years old, weights ranged from 2.8 to 4.9 kg for the males and 2.5 to 3.5 kg for the females.
Dose Groups:	The following table from the study report shows the dose groups in this study

Group	Total No. of Animals M/F	Dose Level Tmab-MCC-DM1 (mg/kg)[a]	Dose Level Tmab-MCC-DM1 (μg/m2)[b]	Dose Concentration[c] (mg/mL)	Terminal Necropsy (Day 3) No. Euthanized M/F	Recovery Necropsy (Day 22) No. Euthanized M/F
1 (Vehicle)	6/6	0	0	0	3/3	3/3
2 (Low)	6/6	3	612	0.50	3/3	3/3
3 (Mid)	6/6	10	2040	1.67	3/3	3/3
4 (High)	6/6	30	6120	5.00	3/3	3/3

a Dose expressed as mg total Tmab-MCC-DM1/kg body weight.

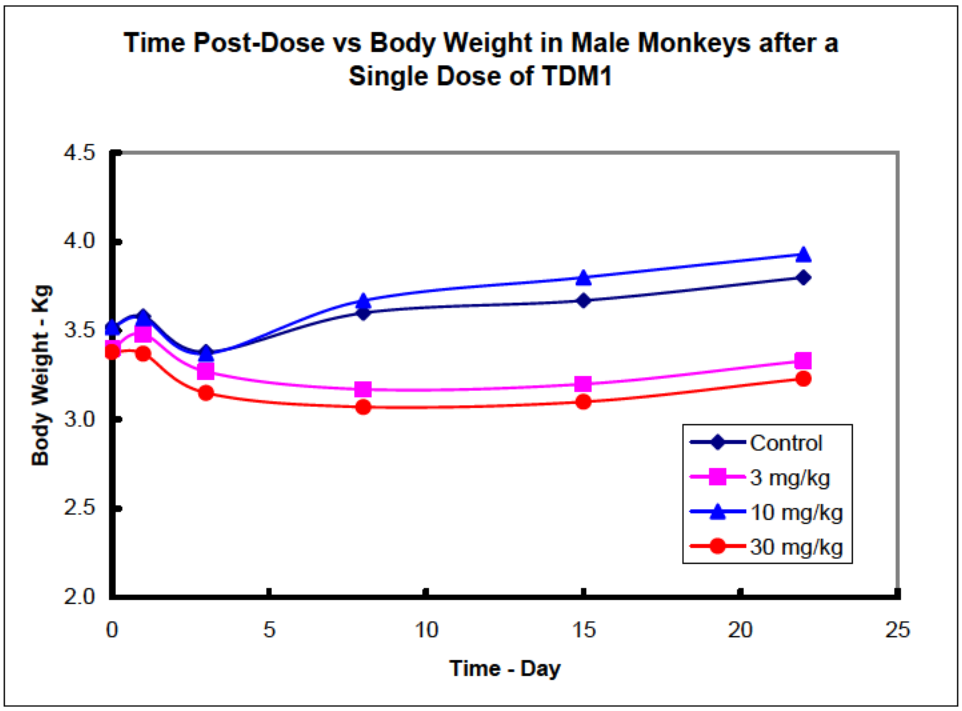
b Dose of Tmab-MCC-DM1 expressed as dose of DM1 (μg/m^2), calculated as follows: [Dose Level (mg/kg Tmab-MCC-DM1) x 12 x 0.017 (fraction of DM1 to trastuzumab in Tmab-MCC-DM1) x 1000]. The conversion factor of 12 is taken from U.S. Department of Health and Human Services Food and Drug Administration, CDER/CBER, Guidance for Industry and reviewers: Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, December 2002. The fraction of DM1 to trastuzumab in Tmab-MCC-DM1 is determined using the molecular weights (MW) of DM1 (MW 738) and trastuzumab (MW 145167), and the measured drug/Antibody ratio (DAR) 3.4, and is calculated as follows: (MW DM1 x DAR)/MW trastuzumab).

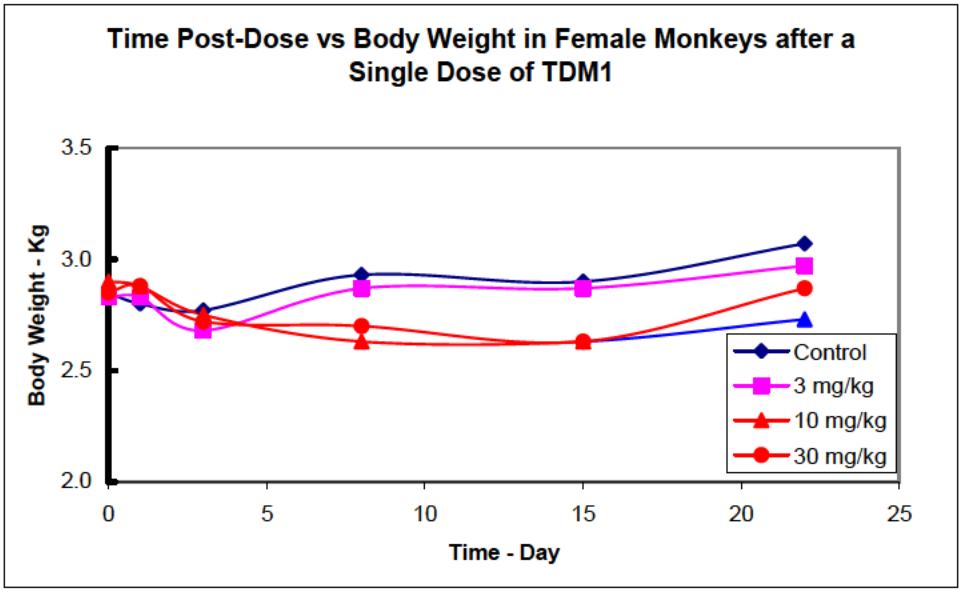
c The dose volume was 6.0 mL/kg for all groups.

Schedule	Single dose
Formulation	5 mg/mL TDM-1 in 10 mM succinate, 100 mg/mL trehalose, 0.1% polysorbate 20, pH 5.0.
Route	IV infusion, saphenous vein
Clinical signs	Twice daily
Body weights	Days 1, 3, 8, 15, and 22
Food cons.	Qualitative daily
Hematology	Days 3, 8, 15, and 22
Clinical chem.	Days 3, 8, 15, and 22
Urinalysis	Days 3 and 22
Necropsy	Day 3 and Day 22
Histopathology	Adequate battery
Toxicokinetics	Predose, Day 1, 5 minutes, Days 3, 4, 8, 15 and 22 ELISA, for trastuzumab and trastuzumab emtansine LC/MS/MS for TDM1 TK analysis only done on animals designated for necropsy on day 22 (N=3) The because of the sample size and sparse sampling, the data did not regress to a compartmental model, investigators at Genentech analyzed it using a non-compartmental model with WinNonLin.

Results

Mortality	All monkeys survived to scheduled necropsy
Clinical Signs	No toxicologically significant signs or symptoms
Body Weight	The following two tables show the changes in body weight with time post-dose.





All monkeys including the controls loose weight right after dosing probably because of the stress of the procedure. Except for the unusual result that the mid dose males gain weight like the controls, the data suggests that dosing prevents the monkeys from gaining weight normally. The maximum effect in males is about 15%; that in females is about 10%. The effect is small but probably toxicologically significant. The investigators say there was no effect on food consumption.

Antibody Analysis Negative

Hematology

Mild anemia in males and females with a compensatory increase in reticulocytes that are not resolved by day 22
Decrease in platelets in the HD males and females that did not reach statistical significance and showed signs of recovery
Increases in neutrophils on day three, < 30% in HD males not statistically significant, about 3X in females and statistically significant, recovered
Transient increases in monocytes in males and females (2X significant)
Other minor changes reached statistical significance but were not toxicologically significant

Clinical Chemistry

The following table shows the toxicologically significant changes in Clinical Chemistry parameters

Parameter	Day	Control	3 mg/kg	10 mg/kg	30 mg/kg	Recovery day 22
Male Albumin	3	4.6	0.0%	-4.3%	**-13.0%**	
Male Albumin	15	4.5	-2.2%	-4.4%	-20.0%	Remained low
Male AGR	3	1.3	7.7%	0.0%	**-15.4%**	
Male AGR	15	1.2	8.3%	0.0%	-25.0%	Partial recovery
Female Cholesterol	3	151	-16.6%	-15.9%	**-21.2%**	
Female Cholesterol	22	144	-13.2%	-10.4%	-22.2%	Remained low
Male Triglycerides	3	33	36.4%	42.4%	**57.6%**	Recovered
Male AST	3	48	35.4%	**81.3%**	**268.8%**	
Male AST	8	46	-10.9%	60.9%	**391.3%**	Recovered
Female AST	3	40	22.5%	97.5%	**215.0%**	
Female AST	8	51	-17.6%	33.3%	**360.8%**	Recovered
Male ALT	3	49	12.2%	38.8%	44.9%	Recovered
Female ALT	3	52	23.1%	19.2%	26.9%	Recovered
Male ALP	3	490	19.8%	**62.4%**	**64.9%**	Recovered
Female ALP	3	206	13.6%	85.4%	**131.6%**	Recovered
Male GGT	3	57	24.6%	68.4%	36.8%	Recovered

Organ Weights

On day 3 of the absolute weights of the brain and thyroids of females given 3 mg/kg were decreased relative to controls. The liver-to-body weight percentage of females given 30 mg/kg was increased as compared with that of females in the control group. These differences did not reach statistical significance and there was no dose response, but one of the female monkeys did show signs of microscopic inflammation in the brain.

Gross Pathology

Injection site damage

Histopathology

The following table shows the toxicologically significant microscopic toxicities in the monkeys necropsied on day 3 (referred to as terminal necropsy).

Terminal Necropsy N = 3 per sex	Male				Female			
	Control	3 mg/kg	10 mg/kg	30 mg/kg	Control	3 mg/kg	10 mg/kg	30 mg/kg
Brain								
Ependymal Inflamma ion						1		
Lung								
Hemorrhage						1		
Inflamma ion, Chronic			1	1				
Kidney								
Infiltrate, Lymphohistiocy ic, Bilateral			1	2	1			
Inflammation, Chronic, Unilateral				1				
Infiltrate, Lymphohistiocy ic, Unilateral		1						
Regeneration, Tubular, Unilateral								1
Infiltrate, Lymphoplasmacy ic, Bilateral			1					
Regeneration, Tubular, Medullary, Bilateral		1	1					
Mineralization, Papilla, Bilateral		1						
Mineralization, Medulla, Bilateral		1						
Liver								
Arrested mitoses in metaphase with hyperplasia, Kupffer cells			2	3		1	2	3
Hypertrophy, Kupffer Cell			3	3		1	2	3
Lipidosis, Tension		1						
Heart								
Infiltrate, Lymphohistiocy ic		2	2	1				
Spleen								
Arrested mitoses in metaphase			2	3			1	3
Fibrosis, Capsular		1	1					
Mesenteric lymph node								
Histocy ic Infiltra ion		2	2	2	2	3	3	3
Mammary Gland								
Increased (Arrested) Mitosis, Epidermis (Basal Layers)								3

The following table shows the toxicologically significant microscopic toxicities in the monkeys necropsied on day 3 (referred to as terminal necropsy).

Recoverey Necropsy, N = 3	Male				Female			
	Control	3 mg/kg	10 mg/kg	30 mg/kg	Control	3 mg/kg	10 mg/kg	30 mg/kg
Kidney								
Infiltrate, Lymphohistiocy ic, Bilateral			2				1	1
Infiltrate, Lymphohistiocy ic, Unilateral		1						1 1
Liver								
Infiltrate, Lymphohis iocytic	1	1	3	2	1		2	2
Heart								
Lymphohistocytic infiltrate	1			1		1		1

Toxicokinetics

The following table from the study report shows the toxicokinetic parameters determined by non-compartmental analysis for total trastuzumab and for trastuzumab emtansine.

Mean (±SD) Total Trastuzumab Non-Compartmental TK Parameter Estimates following IV Administration of Trastuzumab-MCC-DM1 in Cynomolgus Monkeys

Parameter	Group 2 (3 mg/kg)		Group 3 (10 mg/kg)		Group 4 (30 mg/kg)	
	Males	Females	Males	Females	Males	Females
AUC_{0-inf} (µg • day/mL)	301±25.0	325±33.4	1630±145	1430±231	5340±1070	5580±1140
AUC_{ALL} (µg • day/mL)	292±22.5	313±28.8	1360±88.9	1250±155	4020±378	4370±842
C_0 (µg/mL)	88.0±9.55	81.9±3.42	280±13.7	253±23.1	743±71.2	697±59.9
CL (mL/day/kg)	10.1±0.841	9.39±0.915	6.06±0.524	6.98±1.05	5.67±1.29	5.43±1.24
V_0 (mL/kg)	34.7±4.00	37.1±1.51	35.2±1.74	39.1±3.53	39.8±3.98	42.3±3.81
V_{ss} (mL/kg)	52.9±2.70	53.3±3.00	67.2±4.60	67.7±3.85	78.5±7.25	72.6±13.3
MRT (days)	5.24±0.319	5.71±0.630	11.1±1.14	9.81±1.22	14.4±4.00	13.4±0.620
$t_{1/2, terminal}$ (days)	4.39±0.328	4.58±0.410	8.53±1.08	7.40±0.960	10.9±3.29	9.91±0.240

AUC_{0-inf} = area under the serum concentration–time curve from Time = 0 to infinity; AUC_{ALL} = area under the curve from the time of dosing to the time of the last observation; C_0 = concentration at Time = 0 following an IV dose; CL = clearance; MRT = mean residence time; $t_{1/2, terminal}$ ($t_{1/2, \lambda_z}$) = terminal half-life; V_0 = volume of the central compartment; V_{ss} = volume of distribution at steady state.

Note: n = 3 for summary statistics because of interim necropsies.

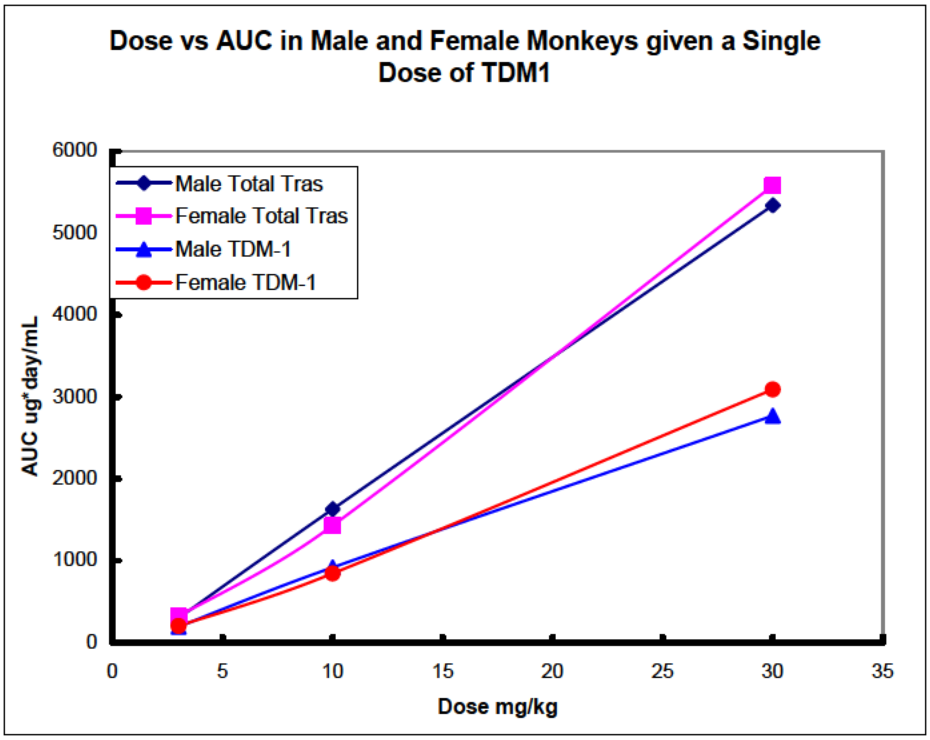
Mean (±SD) Trastuzumab-MCC-DM1 Non-Compartmental TK Parameter Estimates following IV Administration of Trastuzumab-MCC-DM1 in Cynomolgus Monkeys

Parameter	Group 2 (3 mg/kg)		Group 3 (10 mg/kg)		Group 4 (30 mg/kg)	
	Males	Females	Males	Females	Males	Females
AUC_{0-inf} (µg • day/mL)	194±19.0	203±19.2	918±68.6	844±121	2770±296	3090±693
AUC_{ALL} (µg • day/mL)	193±18.8	202±18.8	890±62.7	826±111	2640±226	2910±626
C_0 (µg/mL)	84.4±11.2	75.1±4.92	278±16.8	257±36.2	784±66.3	680±66.8
CL (mL/day/kg)	15.7±1.59	15.0±1.36	10.8±0.772	11.8±1.59	10.7±1.21	9.86±2.38
V_0 (mL/kg)	36.4±5.05	40.5±2.62	35.5±2.07	38.7±5.10	37.7±3.33	43.5±4.44
V_{ss} (mL/kg)	47.8±4.99	51.2±2.75	57.8±4.21	58.1±4.36	64.5±1.89	66.3±10.5
MRT (days)	3.03±0.0588	3.42±0.344	5.39±0.374	4.95±0.425	6.10±0.794	6.81±0.562
$t_{1/2, terminal}$ (days)	2.53±0.106	2.68±0.205	4.40±0.491	3.86±0.452	4.90±1.09	5.44±0.200

AUC_{0-inf} = area under the serum concentration–time curve from Time = 0 to infinity; AUC_{ALL} = area under the curve from the time of dosing to the time of the last observation; C_0 = concentration at Time = 0 following an IV dose; CL = clearance; MRT = mean residence time; $t_{1/2, terminal}$ ($t_{1/2, \lambda_z}$) = terminal half-life; V_0 = volume of the central compartment; V_{ss} = volume of distribution at steady state.

Note: n = 3 for summary statistics due to interim necropsies.

Clearance decreases with increasing dose suggesting a non-first order process. The following graph shows that exposure for both total trastuzumab and trastuzumab emtansine increases linearly with dose ($r^2 > 0.99$), but the increase is not dose proportional. The difference in exposure between males and females is small, but is consistently greater in females.



The hydrolysis of DM1 was the rate limiting process in its elimination. Concentrations could be measured in the MD and HD groups, but were BLOQ after the five minute data point in the LD group. The investigators could not model DM1 elimination, but it followed the conversion of TDM1 to trastuzumab.

2) Single Dose Intravenous Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) with 5-7% Unconjugated Maytansinoid in Cynomolgus Monkeys with a 3-Week Recovery Period

Study no	Sponsor reference number 05-0848, [redacted] (b) (4) Study number 6281-644
File name	05-0848-[redacted] (b) (4) -final.pdf
Laboratory	[redacted] (b) (4)
Study Date	November 14, 2005
GLP	Yes
Audited	Yes
Drug	Tmab-MCC-DM1 (PRO132365) FIL037D01 Purity 98% monomer by size exclusion chromatography Free maytansinoid 5.3% Drug to antibody ratio 3.4
Methods	
Species	Naïve male and female cynomolgus monkeys (Macaca *fascicularis*) Approximately 2 to 9 years old, body weight 2.4 to 3.2 kg males, 2.4 to 3.1 kg females.

Dose Groups: The following table from the study report shows the dose groups in this study

Group	Total No. Animals Male (M)	Total No. Animals Female (F)	Dose Level Tmab-MCC-DM1 mg/kg[a] (μg/m^2)[b,c]	Dose Concentration[d] (mg/mL)	Terminal Necropsy (Day 3) No. euthanized M/F	Recovery Necropsy (Day 22) No. euthanized M/F
1 (Vehicle)	6	6	0 (0)	0	3/3	3/3
2 (Low)	6	6	3 (612)	0.50	3/3	3/3
3 (Mid)	6	6	10 (2040)	1.67	3/3	3/3
4 (High)	6	6	30 (6120)	5.00	3/3	3/3

a Dose expressed as mg total Tmab-MCC-DM1/kg body weight.
b Dose of Tmab-MCC-DM1 expressed as dose of DM1 (μg/m^2), calculated as follows: [Dose Level (mg/kg) Tmab-MCC-DM1 x 12 x 0.017 (fraction of DM1 to trastuzumab in Tmab-MCC-DM1[c]) x 1000]. The conversion factor of 12 is taken from U.S. Department of Health and Human Services Food and Drug Administration, CDER/CBER, Guidance for Industry and reviewers: Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, December 2002.
c The fraction of DM1 to trastuzumab in Tmab-MCC-DM1 was determined using the molecular weights (MW) of DM1 (MW 738) and trastuzumab (MW 145167), and the measured drug/antibody ratio (DAR) 3.4, and was calculated as follows: (MW DM1 x DAR)/(MW trastuzumab).
d The dose volume was 6.0 mL/kg for all groups.

Schedule	Single dose
Formulation	5 mg/mL TDM-1 in 10 mM succinate, 100 mg/mL trehalose, 0.1% polysorbate 20, pH 5.0.
Route	IV infusion
Clinical signs	Twice daily
Body weights	Days 1, 2, 7, 14, and 21.
Food cons.	Qualitative daily
Hematology	Days 3, 8, 15, and 22
Clinical chem.	Days 3, 8, 15, and 22
Necropsy	Day 3 and Day 22
Histopathology	Adequate battery
Toxicokinetics	Predose, Day 1, 5 minutes, Days 3, 4, 8, 15 and 22

Results

While the study report states the purpose of this study it does not clearly describe how that purpose was accomplished. Neither the non-clinical overview nor the non-clinical summary describe how this study was designed and accomplished. Only on reading the certificates of analysis and stability analysis described in the appendix does one discover the source of the drug used in this experiment. The study report lists the drug lot as FIL037D01 with a purity of 98% so it is unclear from the report how the drug used was 5 to 7% unconjugated. This is the same drug lot used in the multidose study 04-0977-1459 ([redacted] (b) (4) 6281-602) where dosing started on March 15, 2005. The Applicant analyzed this lot for purity on February 25, 2005 and again on November 29, 2005 after 99 days storage at -70 ºC than a further 178 days storage at 2 to 8 ºC. They found the following (certificate of analysis page 231 this report, text copied from the certificate):

"Stability Statement: Aged Tox Material, Study Number 05-0848, initiated on 14Nov2005 characterization testing completed on 25Feb2005, Storage conditions: 99 days at -70 ºC, the 178 days at 2-8 ºC."

Test[1]	Test Result Feb 25	Test Result November 29[2]
Drug Antibody ratio Size Exclusion Chromatography (b) (4) Identity by ELISA Protein Concentration pH	(b) (4)	

"[1] Testing performed (b) (4)
[2] Stability of the material is confirmed for the time period listed with the exception of (b) (4) (b) (4). Potency analyses performed in a developmental laboratory for initial characterization ((b) (4) of reference) and at this stability time point (b) (4) of reference) are consistent.
[3] The initial characterization was performed prior to method validation."
[4] N/R not defined

Thus, Genentech stored the material of this lot between February and November, then commissioned a second single dose study in monkeys to qualify the stored material to qualify the drug product with an elevated concentration of (b) (4)

Genentech also did an additional stability study at over 12 months at considerably higher temperatures. The following table shows that lysis of the DM1 from the antibody reaches a plateau at about 6 months and is no more severe than in the previous experiment when the drug is stored under normal refrigeration. This study qualifies the DP to (b) (4) (b) (4)

Date: March 10, 2006

From: Carolyn Broughton, Quality Control Clinical Development

To: File, Tmab-MCC-DM1 Lot FIL037D01

Re: Stability of Tmab-MCC-DM1 Lot FIL037D01 Used in Toxicity Study 05-0848

The data summarized in the attached stability statement demonstrate that results of stability testing were consistent with the initial values with the exception of (b) (4) The data summarized in Table 1 demonstrate that an increase of (b) (4) was observed during the 12-month storage at both 2–8°C and 15°C, and (b) (4) appear to plateau (6–12 months at 2–8°C and 3–6 months at 15°C). Results were within the release specification of (b) (4) At 2–8°C there was a (b) (4) increase in % (b) (4) over the 6-month period between 6 and 12 months. Based upon these data, it is unlikely that the levels of (b) (4) (b) (4) would have changed appreciably in the drug product stored at 2–8°C over the 15-day period from the date of the stability statement to the date of the study initiation.

Stability of this material with respect to (b) (4) is expected to be unchanged for the time period listed.

Table 1
Stability Data for Trastuzumab-MCC-DM1 Drug Product
Lot FIL037D01

(b) (4)

Mortality	None
Clinical Signs	No toxicologically significant or drug related signs
Body Weight	On Day 7, the mean body weight of females given 30 mg/kg Tmab-MCC-DM1 was 0.2 kg lower than the Day 1 value; the value was 0.4 kg lower (statistically significant) than that of the control group.
Antibodies	None of the animals developed antibodies against TDM1

Hematology The following table shows that a single dose of TDM-1 caused mild transient anemia and increases in white cell parameters consistent with mild inflammation.

Parameter	Day	Control	3 mg/kg	10 mg/kg	30 mg/kg
Male RBC	22	5.44	-2.6%	2.8%	-4.0%
Female RBC	22	5.26	1.5%	**-4.8%**	**-7.4%**
Male Reticulocyte	3	91.1	-11.6%	-9.1%	-26.1%
Female Reticulocyte	3	83.3	-22.2%	19.3%	-39.1%
Male % Reticulocyte	3	1.6	0.0%	-6.3%	-18.8%
Female % Reticulocyte	3	1.6	-25.0%	18.8%	**-37.5%**
Male Platelets	3	473	-3.8%	**-27.5%**	**-18.6%**
Female Platelets	3	410	6.8%	7.8%	-9.5%
Male WBC	3	11.88	-4.2%	**39.3%**	28.5%
Female WBC	3	11.04	12.4%	**81.2%**	**50.8%**
Male Neutrophils	3	7.43	-7.3%	**49.1%**	62.3%
Female Neutrophils	3	6.59	15.3%	**116.4%**	**99.8%**
Male Monocytes	3	0.3	33.3%	**163.3%**	3.3%
Female Monocytes	3	0.28	53.6%	**307.1%**	64.3%
Male Eosinophils	3	0.09	-33.3%	**188.9%**	**88.9%**
Female Eosinophils	3	0.1	0.0%	220.0%	30.0%
Male Basophils	3	0.03	0.0%	**66.7%**	-33.3%
Female Basophils	3	0.03	0.0%	66.7%	**0.0%**
Male Leukocytes	3	0.06	-16.7%	66.7%	**-33.3%**
Female Leukocytes	3	0.04	50.0%	**300.0%**	50.0%

Clinical Chemistry

The following table shows that a single dose of TDM1 caused either mild reversible liver damage, or because of the absence of increases in alanine aminotransferase possibly generalized tissue damage. Mild microscopic damage in the liver (vide infra) suggests the former.

	Day	Control	3 mg/kg	10 mg/kg	30 mg/kg
Male AST	3	81	-23.5%	-8.6%	**65.4%**
Male AST	8	40	12.5%	42.5%	**387.5%**
Male AST	22	42	4.8%	0.0%	**57.1%**
Female AST	3	45	6.7%	**57.8%**	**171.1%**
Female AST	8	48	12.5%	27.1%	**170.8%**
Female AST	24	48	-18.8%	-10.4%	2.1%
Male ALT	3	41	29.3%	56.1%	73.2%
Male ALT	22				Normal
Female ALT	3	69	-8.7%	-1.4%	63.8%
Female ALT	22				Normal
Male ALP	3	527	-7.0%	**3.0%**	**33.2%**
Male ALP	22				Normal
Female ALP	45	226	69.0%	64.6%	**123.0%**
Female ALP	45				Normal

Organ weights No toxicologically significant findings

Gross Pathology No toxicologically significant findings

Histopathology The table below shows the toxicologically significant microscopic changes associated with dosing at terminal necropsy (Day 3)

	Male				Female			
Terminal Necropsy N = 3 per sex	Control	3 mg/kg	10 mg/kg	30 mg/kg	Control	3 mg/kg	10 mg/kg	30 mg/kg
Brain								
Arrested mitoses, choroid plexus				1				1
Lung								
Inflammation, Acute				1				
Infiltrate, Lymphocytes			1				1	
Infiltrate, Macrophages, Alveolus			1	1			2	
Inflammation, Chronic-Active								
Pigment, Parasitic	3	3	3	2	2	3	3	3
Kidney								
arrested in mitoses during metaphase, cortical tubule - epithelial cells and interstitial cells				2				3
Liver								
Arrested mitoses in metaphase with hyperplasia, Kupffer cells		3	3	3		1	3	2
Infiltrate, Lymphocytes/Macrophages		2	1	1	2	1	2	2
Mineralization							1	
Pigment							1	
Heart								
Arrested mitoses in metaphase, interstitial histiocytic cells				1				
Spleen								
Arrested mitoses in metaphase, histiocytic cells in red pulp				3				3
Mesenteric Lymph Node								
Edema				1				1
Skin								
Acute inflammatory exudate								
Arrested mitoses in metaphase, basal epithelium			1	3				3
Arrested mitoses in metaphase, histiocytes			1					1
Edema								
Infiltrate, Lymphocytes/Macrophages					1	1		
Uterus								
Arrested mitoses in metaphase, histiocytic cells in the endometrium								2

The table below shows the toxicologically significant microscopic changes associated with dosing at recovery necropsy (Day 22)

	Male				Female			
Recoverey Necropsy, N = 3	Control	3 mg/kg	10 mg/kg	30 mg/kg	Control	3 mg/kg	10 mg/kg	30 mg/kg
Sciatic Nerve								
Degeneration, Axon				1				1
Inflammation, vascular			1					
Brain								
Arrested mitoses, choroid plexus								2
Lung								
Infiltrate, Lymphocytes								1
Infiltrate, Macrophages, Alveolus		1		1		1		1
Inflammation, Chronic-Active		1		1				1
Spleen								
Arrested mitoses in metaphase, histiocytic cells in red pulp		1						

Toxicokinetics

The toxicokinetic studies determined the concentrations of total trastuzumab, DM1 and TDM1. The quality of the data is unusually good. The following tables from the study report show the parameter estimates for total trastuzumab and TDM1.

Mean (±SD) Total Trastuzumab Non-Compartmental TK Parameter Estimates following IV Administration of Trastuzumab-MCC-DM1 in Cynomolgus Monkeys

Parameter	Group 2 (3 mg/kg)		Group 3 (10 mg/kg)		Group 4 (30 mg/kg)	
	Males	Females	Males	Females	Males	Females
AUC_{inf} (day • μg/mL)	245±18.7	237±29.9	1300±185	1300±143	5970±288	5970±848
AUC_{all} (day • μg/mL)	236±17.0	230±27.8	1140±125	1180±103	4350±53.0	4390±409
C_0 (μg/mL)	72.3±6.10	75.3±8.41	258±17.8	269±27.1	703±29.4	724±65.1
CL (mL/day/kg)	12.1±0.765	12.6±1.44	7.75±1.09	7.51±0.771	4.76±0.252	4.81±0.617
V_0 (mL/kg)	41.2±3.72	39.5±3.73	38.5±2.26	36.3±3.24	40.5±1.83	39.4±3.67
V_{ss} (mL/kg)	68.6±3.05	65.6±6.16	71.7±2.07	63.8±2.07	75.6±2.63	73.7±4.03
MRT (days)	5.68±0.397	5.22±0.277	9.36±1.27	8.55±0.866	15.9±1.35	15.5±1.79
$t_{1/2,terminal}$ (days)	4.61±0.216	4.32±0.294	7.10±0.932	6.44±0.687	11.8±1.27	11.6±1.27

AUC_{inf} = area under the serum concentration–time curve extrapolated to infinity; AUC_{all} = area under the curve from the time of dosing to the time of the last observation; C_0 = concentration at time = 0 following an IV dose; CL = clearance; MRT = mean residence time; $t_{1/2,terminal}$ = terminal half-life; V_0 = volume of distribution of the central compartment; V_{ss} = volume of distribution at steady state.

Note: n = 3 for summary statistics because of interim necropsies.

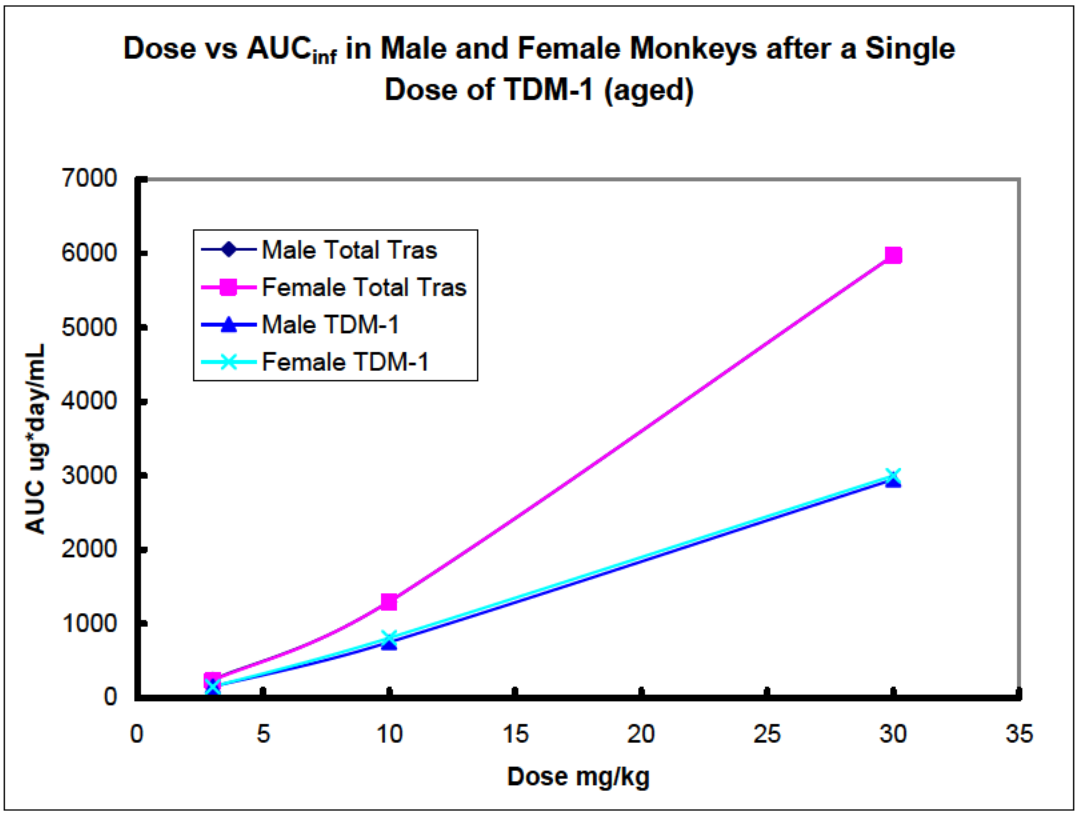
Mean (±SD) Trastuzumab-MCC-DM1 Non-Compartmental TK Parameter Estimates following IV Administration of Trastuzumab-MCC-DM1 in Cynomolgus Monkeys

Parameter	Group 2 (3 mg/kg)		Group 3 (10 mg/kg)		Group 4 (30 mg/kg)	
	Males	Females	Males	Females	Males	Females
AUC_{inf} (day • μg/mL)	156±11.0	154±21.6	752±91.6	810±67.7	2950±29.2	3000±253
AUC_{all} (day • μg/mL)	156±10.9	153±21.4	740±87.0	800±65.4	2790±47.0	2850±228
C_0 (μg/mL)	71.4±5.87	70.2±9.16	265±34.9	287±19.0	738±38.6	735±41.6
CL (mL/day/kg)	19.0±1.07	19.5±2.40	13.3±1.57	12.0±0.879	9.63±0.0321	9.49±0.734
V_0 (mL/kg)	41.6±3.66	42.5±4.76	37.8±4.39	34.0±1.89	38.5±1.78	38.7±2.44
V_{ss} (mL/kg)	57.8±3.43	56.0±4.49	61.2±4.35	52.3±3.12	63.6±1.77	61.5±2.64
MRT (days)	3.05±0.245	2.89±0.149	4.62±0.378	4.36±0.264	6.61±0.166	6.49±0.263
$t_{1/2,terminal}$ (days)	2.54±0.108	2.44±0.179	3.55±0.309	3.44±0.181	5.20±0.299	5.06±0.214

AUC_{inf} = area under the serum concentration–time curve extrapolated to infinity; AUC_{all} = area under the curve from the time of dosing to the time of the last observation; C_0 = concentration at time = 0 following an IV dose; CL = clearance; MRT = mean residence time; $t_{1/2,terminal}$ = terminal half-life; V_0 = volume of the central compartment; V_{ss} = volume of distribution at steady state.

Note: n = 3 for summary statistics because of interim necropsies.

Clearance decreases with increasing dose for both total trastuzumab and for TDM-1, with a concomitant increase in the terminal elimination half-life. Clearance is significantly less than hepatic blood flow. Volume suggests that antibody is confined to the plasma. The following graph shows that exposure increased linearly and that there was no difference between males and females.



While exposure increased linearly, the increase was considerably greater than dose proportional. The slope of the dose vs dose normalized AUC for total trastuzumab in males was 4.1 (r^2 = 0.97), for TDM-1 in males it was 1.6 (r^2 = 0.93).

6.2 Repeat-Dose Toxicity

1) Trastuzumab-SMCC-DM1 : Dose Range Finding Study in Cynomolgus Monkeys

Study no	Genentech No. 03-0674-1459; (b) (4).170.08
File name	03-0674-1459
Laboratory	(b) (4)
Study Date	March 15, 2005
GLP	No
Audited	No
Drug	Trastuzumab-MCC-DM1, Lot number 39458-39, Purity not specified Drug/Antibody Ratio = 2.44 Free Maytansinoid = 1.6% (done prior to method validation)
Methods	
Species	Naïve male and female cynomolgus monkeys (*Macaca fascicularis*)

Doses The following table shows the doses used in this study

Treatment Group	Dosing Days	Dose of DM1 ug/m^2	Dose DM1 mg/kg
1	1, 22	0	0.000
2	1, 22	4900	0.408
3	22	7400	0.617

Formulation: PBS
Route IV bolus in the Saphenous vein
N 2 per sex per dose group
Dose volume 14 to 26 mL

This non-GLP study was evidently the initial study in monkeys. The Applicant based the doses on ug/m^2 of DM1 and not on trastuzumab emtansine so it is difficult to relate this study to the other studies in the monkey. All animals survived to scheduled necropsy and there were no drug related clinical signs.

Monkeys in group 2 became anorexic during the first week but recovered to normal by day 10. Likewise food consumption decreased significantly in Group 3 monkeys but they too recovered. There was a concomitant decrease in body weight.

As in the other studies above, there was a transient decline in white cell parameters. Treatment was associated with a toxicologically significant increase in AST as much as four fold in group to and 5.5 fold in group 3. ALT and LDH also increased but less dramatically.

Plasma clearance of trastuzumab emtansine was only 7.5 mL/kg/day and volume of the central compartment approximated the plasma volume. The distribution half-life was approximately 0.5 days and the terminal elimination half-life was approximately 5.5 days in both dose groups.

In this initial study there were no drug related organ weight changes. Necropsy findings included yellow marrow in the femurs of one group 2 female; there was no microscopic correlate for this finding. There were diffuse petechiae in the lungs of one Group 2 female, this tissue was not examined microscopically. There were mild increases in the numbers of mitotic and apoptotic cells of the hepatic parenchyma. No abnormalities in bone marrow were noted microscopically in any monkey in any group. The study did not demonstrate any toxicities that were not seen in the subsequent GLP studies.

2) Multiple Dose Intravenous Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) Administered to Cynomolgus Monkeys Once Every 3 Weeks for 4 Doses, with a 3- or 6-week Recovery Period

Study no Genentech No. 04-0977-1459; (b) (4) 6281-602
File name 04-0977-1459- (b) (4) -final.pdf
Laboratory (b) (4)
Study Date March 15, 2005
GLP Yes
Audited Yes
Drug Trastuzumab-MCC-DM1, Lot number FIL037D01,
Purity 98.0% (monomer) by size exclusion chromatography

Drug/Antibody Ratio = 3.4
Free Maytansinoid = 2.4% (done prior to method validation)

Methods

Species: Naïve male and female cynomolgus monkeys (*Macaca fascicularis*)
The cynomolgus monkey expresses HER2, and the trastuzumab portion of PRO132365 has been shown to cross-react with cynomolgus HER2.
At initiation of treatment, the animals were approximately 3 to 5 years old, Body weights, from 2.4 to 4.4 kg for males and 2.4 to 3.5 kg for females.

Dose Groups: The following table from the study report shows the dose groups and number of animals in each group. The legend for the table explains the necropsy schedule.

Group	Number of Animals[a] Male	Number of Animals[a] Female	Dose Level Tmab-MCC-DM1[b] (mg/kg)	Dose Level Tmab-MCC-DM1[c] ($\mu g/m^2$)	Dose Concentration[b] (mg/mL)
1 (Vehicle)	7	7	0	0	0
2 (Low)	7	7	3	612	0.50
3 (Mid)	7	7	10	2040	1.67
4 (High)	7	7	30	6120	5.00

a Animals (7/sex/group) were administered 4 successive doses spaced 3-weeks apart; necropsies were done on Day 66 (terminal, 3/sex/group), Day 85 (first recovery, 2/sex/group), and Day 106 (second recovery, 2/sex/group).

b Dose expressed as mg total Tmab-MCC-DM1/kg body weight; the dose volume was 6 mL/kg.

c Dose expressed as dose of DM-1 ($\mu g/m^2$) estimated as follows: [Dose Level (mg/kg Tmab-MCC-DM1) x 12 x 0.017 (fraction of DM1 to trastuzumab in Tmab-MCC-DM1) x 1000]. The conversion factor of 12 is taken from U.S. Department of Health and Human Services Food and Drug Administration, CDER/CBER, Guidance for Industry and Reviewers: Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, December 2002. The fraction of DM1 to trastuzumab in Tmab-MCC-DM1 was determined using the molecular weights (MW) of DM1 (MW 738) and trastuzumab (MW 145167), and the measured drug/Antibody ratio (DAR) 3.4, and is calculated as follows: (MW DM1 x DAR)/MW trastuzumab).

Formulation:	5 mg/mL TDM-1 in 10 mM succinate, 100 mg/mL trehalose, 0.1% polysorbate 20, pH 5.0.
Route	IV bolus in the Saphenous vein
Schedule	Days 1, 22, 43 and 64
Dose volume	6 mL/kg
Clinical signs	twice daily
Physical Exam	Before treatment and during weeks 3 and 9
Body weights	Weekly
Food cons.	Daily qualitative
Ophthalmology	Before treatment and during weeks 3 and 9
ECG	Electrocardiograms and heart rates were recorded on each animal prior to treatment and during Weeks 2, 8, 12, and 15 under anesthesia. Parameters included heart rate, PR, QRS, QT, QTc (calculated), and RR intervals. QTc was calculated using Van de Water's equation.
Hematology	two separate days prior to initiation of treatment and on study days 3, 22 (predose), 24, 43 (predose), 45, 64 (predose), 66, 85, 94, and 106
Clinical chem.	Same as hematology

Necropsy	Vide supra, days 66, 85 and 106
Histopathology	Adequate battery
Toxicokinetics	Blood for toxicokinetic analysis was collected from all animals once prior to treatment and on Day 1 (approximately 5 minutes postdose and 6, 10, 24, 72, and 144 hours postdose), on Days 22 and 43 (predose and approximately 5 minutes postdose), on Day 64 (predose, approximately 5 minutes postdose, and approximately 10 hours postdose), and on Days 65, 67, 69, 71, 78, 85, 94, and 106 at one time point Analysis – ELISA range 0.3−17 ng/mL

Results

Dose analysis	Between 96.2 and 100% of target concentration
Mortality	All animals survived to scheduled necropsy
Clinical Signs	No toxicologically significant or drug related signs
Body Weight	No toxicologically significant changes
Physical Exam	No toxicologically significant changes
Food Consumption	No toxicologically significant changes
Ophthalmology	No toxicologically significant changes
ECG	No toxicologically significant changes
Coagulation	Small increases in APTT (<25%) and Fibrinogen (Female ~ 50%)

Clinical Chemistry

	Day	Control	3 mg/kg	10 mg/kg	30 mg/kg	Day of Maximum Effect	Recovery HD day 106
Male AST	3	43	62.8%	**81.4%**	**255.8%**		
Male AST	24	37	83.8%	**113.5%**	**405.4%**		
Male AST	45	48	106.3%	106.3%	**414.6%**		
Male AST	64	41	31.7%	17.1%	**107.3%**	45	Recovery day 106
Female AST	3	41	75.6%	139.0%	**385.4%**		
Female AST	24	37	48.6%	**162.2%**	**416.2%**		
Female AST	45	37	56.8%	191.9%	**618.9%**		
Female AST	64	34	17.6%	58.8%	**170.6%**	45	Recovery day 106
Male ALT	45	40	60.0%	72.5%	112.5%	85	Remained elevated
Female ALT	3	45	86.7%	113.3%	**275.6%**		
Female ALT	24	41	58.5%	92.7%	**168.3%**		
Female ALT	45	38	76.3%	152.6%	**205.3%**		
Female ALT	64	35	80.0%	80.0%	148.6%	45	Remained elevated
Male GGT	45	71	15.5%	-5.6%	-1.4%		
Female GGT	45	58	12.1%	0.0%	**67.2%**	45	Remained elevated
Male Albumin	3	4.5	-2.2%	-6.7%	**-8.9%**		
Male Albumin	24	4.3	-2.3%	-4.7%	**-11.6%**		
Male Albumin	45	4.4	-6.8%	-4.5%	**-13.6%**		
Male Albumiin	64	4.4	-2.3%	-6.8%	**-13.6%**	66	Signs of recovery
Female Albumin	24	4.3	0.0%	-4.7%	**-4.7%**		
Female Albumin	45	4.4	-4.5%	-9.1%	**-9.1%**		
Female Albumin	64	4.2	-2.4%	-4.8%	**-4.8%**	66	Signs of recovery
Male Globulin	24	3	6.7%	**16.7%**	**23.3%**		
Male Globulin	45	3.5	2.9%	**14.3%**	**25.7%**	45	Recovered
Male Globulin	64	3.2	9.4%	**15.6%**	**31.3%**		
Female Globulin	24	3.2	15.6%	**21.9%**	**21.9%**		
Female Globulin	45	3.8	10.5%	**15.8%**	**23.7%**	45	Recovered
Female Globulin	64	3.3	12.1%	**21.2%**	**27.3%**		
Male A/G Ratio	24	1.4	-8.4%	**-18.3%**	**-28.3%**		
Male A/G Ratio	45	1.3	-9.4%	-16.5%	**-31.3%**		
Male A/G Ratio	64	1.4	-10.6%	**-19.4%**	**-34.2%**	66	Partially recovered
Female A/G Ratio	24	1.3	-13.5%	**-21.8%**	**-21.8%**		
Female A/G Ratio	45	1.2	-13.6%	**-21.5%**	**-26.5%**		
Female A/G Ratio	64	1.3	-12.9%	**-21.4%**	**-25.2%**	85	Partially recovered
Male Triglycerides	24	22.0	63.6%	63.6%	**109.1%**		
Male Triglycerides	45	26.0	65.4%	**46.2%**	**73.1%**	45	Recovered
Female Triglycerides	24	39.0	-7.7%	**-12.8%**	**43.6%**	24	Recovered
Female Triglycerides	45	40.0	-12.5%	**-17.5%**	**30.0%**		

	Day	Control	3 mg/kg	10 mg/kg	30 mg/kg	Day of Maximum Effect	Recovery day 106
Male RBC	43	5.65	-6.0%	0.5%	**-5.8%**		
Male RBC	64	5.75	-0.9%	0.9%	**-7.1%**	66	Partial recovery
Female RBC	43	5.36	-0.6%	-4.7%	**-7.6%**		
Female RBC	64	5.36	0.2%	-2.1%	**-8.6%**	66	Partial recovery
Male Hbg	43	13.8	-5.8%	-2.2%	**-8.0%**		
Male Hbg	64	13.8	0.7%	-1.4%	**-8.0%**	66	Recovered
Female Hbg	43	13	-0.8%	-2.3%	**-8.5%**		
Female Hbg	64	12.7	0.0%	0.0%	**-9.4%**	66	Recovered
Male Hct	Dose related trend to slightly lower values					66	Recovered
Female Hct	43	41.9	-1.2%	-3.3%	**-8.6%**		
Female Hct	64	41.1	-1.2%	-1.2%	**-8.3%**	66	Recovered
Male Reticulocyte	43	62.9	1.0%	-3.8%	**37.7%**		
Male Reticulocyte	64	42.5	-8.5%	27.1%	**76.9%**	85	Partial recovery
Female Reticulocyte	43	59	-6.4%	-1.7%	41.9%		
Female Reticulocyte	64	48	-9.8%	-12.1%	**56.0%**	85	Recovered
Male % Reticulocyte	Dose and time related statistically significant increase					85	Recovered
Female % Reticulocyte	Dose and time related statistically significant increase					85	Recovered
Female Platelets	43	417	18.9%	-1.4%	7.4%		
Female Platelets	64	458	13.5%	-11.1%	**-7.2%**	66	Recovered
Male WBC	64	9.37	5.8%	23.4%	28.7%		Elevated NSS
Female WBC	64	13.89	-18.9%	-12.3%	28.6%		Elevated NSS
Female WBC	85	11.68	-26.5%	-9.2%	**49.5%**	85	Elevated NSS
Male Neutrophils	3	4.05	38.8%	**69.6%**	**84.4%**		
Male Neutrophils	24	3.79	-6.9%	49.6%	**103.7%**	24	rebound decrease
Female Neutrophils	3	3.72	79.8%	**95.7%**	**129.3%**		
Female Neutrophils	24	3.16	57.9%	112.7%	**190.2%**	24	Recovered
Female Neutrophils	45	4.62	13.6%	**22.1%**	**104.8%**		
Male Lymphocytes	22	4.65	24.1%	67.7%	**80.4%**		
Male Lymphocytes	43	4.88	26.6%	55.5%	**74.4%**	106	Elevated NSS
Female Lymphocytes	3	6.99	-35.1%	-41.1%	**-37.1%**		Control values were high throughout the study, these changes are probably not toxicologically significant
Female Lymphocytes	24	8.99	-33.1%	-32.9%	**-31.8%**		
Female Lymphocytes	43	8.06	-25.8%	-29.8%	**8.7%**		
Female Lymphocytes	45	9.81	-34.4%	-25.7%	**-31.3%**		
Female Lymphocytes	64	8.13	-32.2%	-26.0%	**13.5%**		Recovered
Male Monocytes	Small elevations (<2X) not consistantly dose dependant						
Female Monocytes	43	0.48	-6.2%	-18.8%	**139.6%**		
Female Monocytes	64	0.45	-8.9%	-11.1%	**166.7%**	85	Recovered
Male Basophils	Small elevations (<2X) dose dependant					64	Remained elevated
Female Basophils	Small elevations dose dependant					85	Remained elevated
Male Leukocytes	22	0.04	50.0%	50.0%	**125.0%**		
Male Leukocytes	43	0.04	50.0%	75.0%	**75.0%**		
Male Leukocytes	64	0.05	20.0%	40.0%	**120.0%**	64	Remained elevated
Female Leukocytes	43	0.09	-44.4%	-33.3%	**66.7%**		
Female Leukocytes	64	0.1	-50.0%	-40.0%	**80.0%**	64	Remained elevated

White cell percentages were mostly unchanged except for Leucocytes which were slightly elevated; occasionally this reached significance, but there was not a clear dose dependant pattern.

Organ weights

	Control	3 mg/kg	10 mg/kg	30 mg/kg	First Recovery HD	Second Recovery HD
Male Body Weight	2833	4.7%	-7.1%	-2.3%	Normal	Normal
Male Spleen	3.896	0.0%	-5.3%	56.6%	Increased	Remained elevated
Male Thymus	2.964	-4.2%	-31.2%	-44.1%	Remained decreased	Remained decreased
Male Salivary Gland	2.57	-7.7%	-24.6%	-22.7%	Remained decreased	Normal
Epididymis	1.021	7.5%	-2.2%	-25.1%	Signs of recovery	Normal
Prostate	0.624	14.3%	-1.4%	-34.8%	Signs of recovery	Normal
Testes	2.615	25.2%	-45.5%	-43.3%	Signs of recovery	Increased 2X+ over controls
Seminal Vesicles	1.074	47.0%	-6.1%	-43.0%	Remained decreased	Increased
Male Liver/Gall Bladder	50.059	10.6%	-3.1%	19.2%	Remained elevated	Remained elevated
Female Body Weight	2933	-9.1%	-12.5%	-9.1%	Normal	Normal
Female Spleen	4.757	-21.5%	1.3%	8.6%	Increased	Normal
Female Thymus	4.02	-42.4%	**-63.7%**	**-70.1%**	Remained decreased	Normal
Female Thyroid/Parathyroid	0.431	-26.2%	-17.2%	-36.2%	N = 1 decreased	Normal
Female Salivary Gland	2.029	-18.6%	-1.9%	-17.1%	Normal	Normal
Female Liver/Gall Bladder	57.663	-17.9%	-10.7%	-11.3%	Normal	Normal
Uterus	5.882	-12.2%	20.9%	-30.0%	Remained decreased	Remained decreased

The following table shows the weight of these organs as a percentage of body weight at first necropsy.

	Control	3 mg/kg	10 mg/kg	30 mg/kg
Male Body Weight	2833	4.7%	-7.1%	-2.3%
Male Spleen	0.14%	0.13%	0.14%	0.22%
Male Thymus	0.10%	0.10%	0.08%	0.06%
Male Salivary Gland	0.09%	0.08%	0.07%	0.07%
Epididymis	0.04%	0.04%	0.04%	0.03%
Prostate	0.02%	0.02%	0.02%	0.01%
Testes	0.09%	0.11%	0.05%	0.05%
Seminal Vesicles	0.04%	0.05%	0.04%	0.02%
Male Liver/Gall Bladder	1.77%	1.87%	1.84%	2.16%
Female Body Weight	2933	-9.1%	-12.5%	-9.1%
Female Spleen	0.16%	0.14%	0.19%	0.19%
Female Thymus	0.14%	0.09%	0.06%	0.05%
Female Thyroid/Parathyroid	0.01%	0.01%	0.01%	0.01%
Female Salivary Gland	0.07%	0.06%	0.08%	0.06%
Female Liver/Gall Bladder	1.97%	1.78%	2.01%	1.92%
Uterus	0.20%	0.19%	0.28%	0.15%

The following table shows weight of these organs as a percentage of brain weight at first necropsy.

	Control	3 mg/kg	10 mg/kg	30 mg/kg
Male Brain Weight	62.6	3.5%	4.8%	8.6%
Male Spleen	6.22%	6.01%	5.62%	8.97%
Male Thymus	4.73%	4.38%	3.11%	2.44%
Male Salivary Gland	4.11%	3.66%	2.95%	2.92%
Epididymis	1.63%	1.69%	1.52%	1.13%
Prostate	1.00%	1.10%	0.94%	0.60%
Testes	4.18%	5.05%	2.17%	2.18%
Seminal Vesicles	1.72%	2.44%	1.54%	0.90%
Male Liver/Gall Bladder	79.97%	85.45%	73.92%	87.75%
Female Brain Weight	64.3	-10.7%	-11.0%	-4.5%
Female Spleen	7.40%	6.51%	8.42%	8.41%
Female Thymus	6.25%	4.04%	2.55%	1.96%
Female Thyroid/Parathyroid	0.67%	0.55%	0.62%	0.45%
Female Salivary Gland	3.16%	2.88%	3.48%	2.74%
Female Liver/Gall Bladder	89.68%	82.51%	90.00%	83.27%
Uterus	9.15%	9.00%	12.44%	6.70%

Gross Pathology 3 of 14 animals had discolored livers, no other remarkable findings.

Histopathology

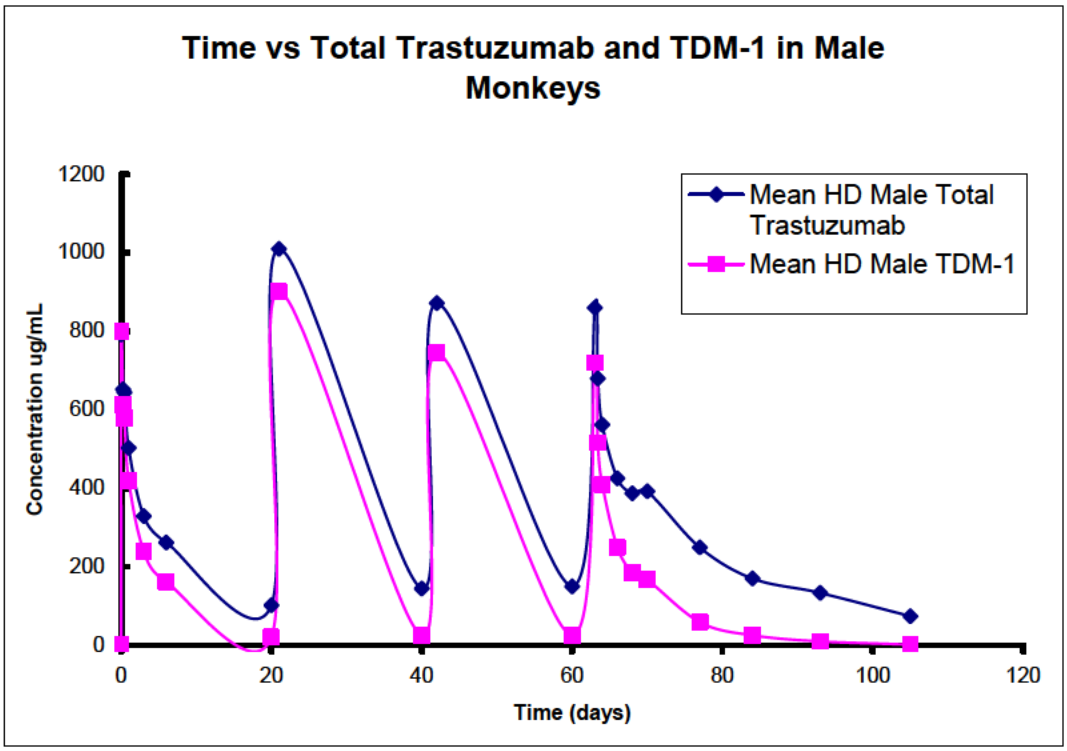
	Male				Female			
Terminal Necropsy N = 3 per sex	Control	3 mg/kg	10 mg/kg	30 mg/kg	Control	3 mg/kg	10 mg/kg	30 mg/kg
Spinal cord								
Axonal Degeneration, Dorsal Funiculus			3	3				3
Sciatic Nerve								
Axonal Degeneration			2	3			1	3
Hypertrophy/Hyperplasia, Schwan Cells				3				3
Infiltrate, Neutrophils				1				
Lung								
Infiltrate, Mononuclear Cell, Interstitium			1	2			1	
Kidney								
Infiltrate, Mononuclear Cell	3	3	2	2	2	3	1	2
Cast(s), Proteinaceous	1		1					
Mitoses/Arrested Metaphase, Tubular Epithelial Cells				3				3
Mineralization, Tubular		1			1		1	1
Degeneration, Glomerulus, Focal								1
Liver								
Vacuolation, Centrilobular		1	2	3				
Multinucleated Hepatocytes			1	3				2
Pigment Deposition, Hepatocytes			1					
Hypertrophy, Kupffer Cells		2	3	3		2	3	3
Mitoses/Arrested Metaphase, Kupffer Cells		2	3	3		2	3	3
Increased Sinusoidal Leukocytes		0	3	3			1	2
Vacuolation, Periportal					1			
Microgranuloma(s)					1			1
Angiectasis								1
Tongue								
Inflammation, Neutrophilic	1							
Infiltrate, Mononuclear Cell	1	1	2	3	2	1	2	2
Mitosis/Arrested Metaphase, Basal Epithelium		3	3	3		3	3	3
Spleen								
Increased Cellularity, Red Pulp		1		2			1	1
Lymphoid Depletion, Follicular Center				2				1
Hypertrophy, Reticuloendothelial Cells		1	2	3				3
Mitoses/Arrested Metaphase, Reticuloendothelial Cells		1	3	3			1	3
Pigment Deposition						1	1	
Thymus								
Lymphoid Depletion		1	2	3	1	2	3	3
Mesenteric Lymph node								
Lymphoid Depletion		1		2				
Esophagus								
Infiltrate, Mononuclear Cell		1	1	1				1
Skin								
Mitosis/Arrested Metaphase, Basal Epithelium		3	3	3		3	3	3

First Recovery Necropsy N = 2 per sex	Male				Female			
	Control	3 mg/kg	10 mg/kg	30 mg/kg	Control	3 mg/kg	10 mg/kg	30 mg/kg
Spinal cord								
Axonal Degeneration, Dorsal Funiculus			2	2			1	2
Sciatic Nerve								
Axonal Degeneration			1	2				2
Hypertrophy/Hyperplasia, Schwan Cells				2				2
Infiltrate, Neutrophils			1					
Infiltrate, Mononuclear Cell	1	1						
Lung								
Infiltrate, Mononuclear Cell, Interstitium		1	1	1				1
Alveolar Macrophages				1				
Liver								
Multinucleated Hepatocytes								1
Hypertrophy, Kupffer Cells								2
Mitoses/Arrested Metaphase, Kupffer Cells								2
Increased Sinusoidal Leukocytes				1				1
Microgranuloma(s)		1	1	2			2	
Angiectasis				1				
Infiltrate, Histiocytic	1							
F brosis, Focal				1				
Hyperplasia, Bile Ducts		1						
Infiltrate, Neutrophilic				1				
Tongue								
Infiltrate, Mononuclear Cell	2	1	1	2	1			
Mitosis/Arrested Metaphase, Basal Epithelium				2			1	2
Thymus								
Lymphoid depletion	1	1	1	2	2	2	2	2

Second Recovery Necropsy N = 2 per sex	Male				Female			
	Control	3 mg/kg	10 mg/kg	30 mg/kg	Control	3 mg/kg	10 mg/kg	30 mg/kg
Spinal Cord								
Axonal Degeneration, Dorsal Funiculus			1	2			1	2
Sciatic Nerve								
Axonal Degeneration			1	2			1	2
Hypertrophy/Hyperplasia, Schwan Cells				2				2
Liver								
Multinucleated Hepatocytes				1				
Microgranuloma(s)		2		2		1	2	2
Infiltrate, Neutrophilic		1		1		1		
Pigment Deposition				2				
Tongue								
Mitosis/Arrested Metaphase, Basal Epithelium				2				1
Infiltrate, Mixed Cell							1	

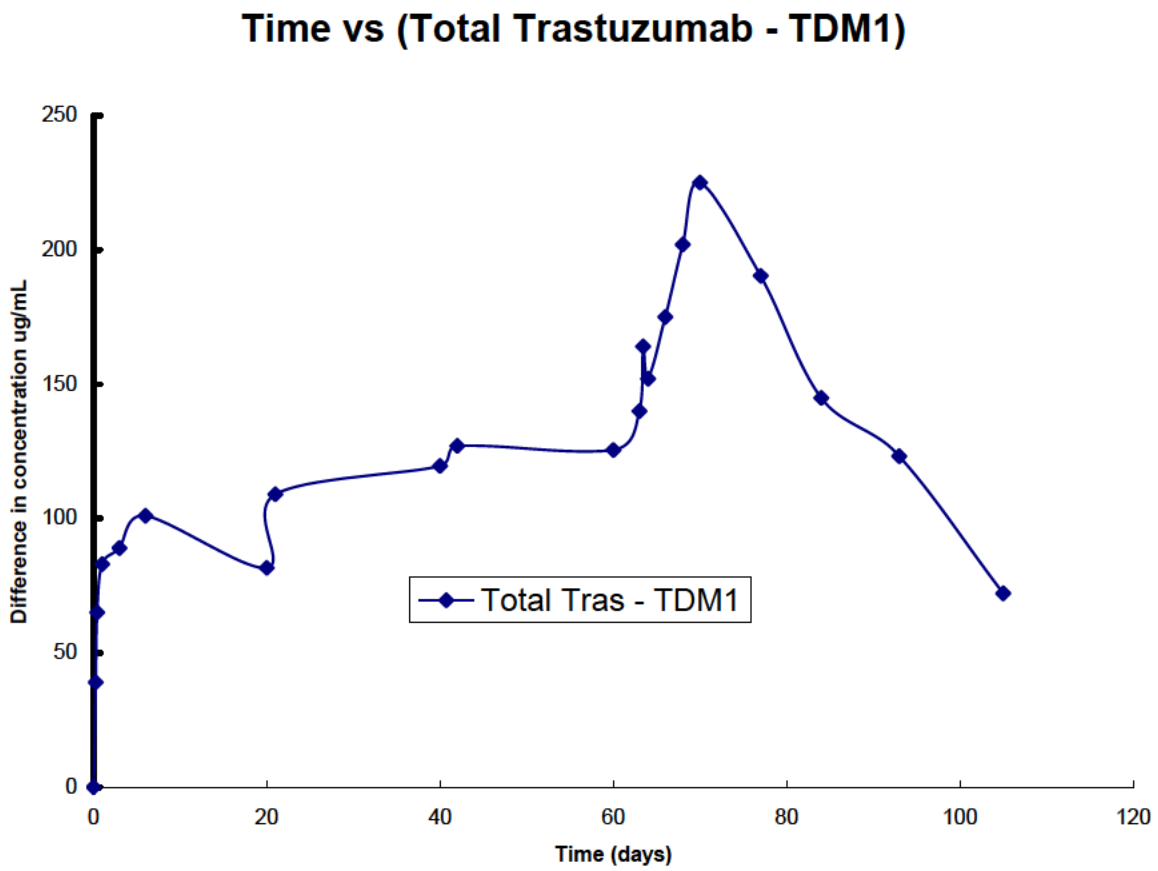
Toxicokinetics

The following graph shows the time course for the elimination of total trastuzumab and TDM-1 from high dose (30 mg/kg) male monkeys.



The curves for both total trastuzumab and trastuzumab emtansine show a rapid alpha phase, which most likely demonstrates binding of the antibodies to target and non-target receptor sites. After the last injection the curves show a slow terminal elimination half-life. The antibody does not appear to accumulate with subsequent doses; indeed the graph suggests that clearance may become slightly more efficient with time.

The curve shows that total trastuzumab is consistently greater than TDM-1. The following graph shows the difference between the concentration of total trastuzumab and intact trastuzumab emtansine.



If one looks at the first 60 days the difference increases slowly. This is reasonable as both moieties have long half-lives and one might expect an increasing amount of residual trastuzumab emtansine to hydrolyze. After the last injection on day 60, the difference between the two compounds increases rapidly over the first 10 days suggesting significant hydrolysis. Indeed if one looks at the last time point, the concentration of total trastuzumab is 74 μg/mL and that of trastuzumab emtansine is only 1.6 μg/mL, indicating almost complete hydrolysis of unbound residual trastuzumab emtansine at this point.

The Applicant fit the data to a two-compartment pharmacokinetic model using IV bolus input, first order elimination with macro constants in WinNonLin (Pharsight Corp. Mountain View CA). They did not say if they analyzed any other models but visual inspection suggests that this model is adequate. The curve does not indicate accumulation. The curves for the other dose groups and for females were similar to these. The following two tables from the study report show the results for total trastuzumab and trastuzumab emtansine.

Groups 2, 3, and 4: Mean (±SD) Total Trastuzumab TK Secondary Parameter Estimates following IV Administration of 3, 10, and 30 mg/kg of Trastuzumab-MCC-DM1 in Cynomolgus Monkeys

	Group 2 (3 mg/kg)		Group 3 (10 mg/kg)		Group 4 (30 mg/kg)	
Parameter	Males	Females	Males	Females	Males	Females
AUC_{0-inf} (μg • day/mL)	316±42.7	338±41.4	1760±351	1990±269	6570±1920	7670±697
C_{max} (μg/mL)	79.9±7.83	78.9±3.79	264±27.6	263±23.6	800±72.2	784±57.5
CL (mL/day/kg)	9.59±1.18	8.85±1.01	5.81±1.27	4.97±0.571	4.95±2.00	3.88±0.336
V_1 (mL/kg)	37.7±3.62	37.5±1.73	37.6±3.84	37.4±3.07	37.0±3.24	37.9±2.91
V_{ss} (mL/kg)	70.8±12.3	70.2±6.01	75.5±8.61	82.2±16.5	69.7±4.80	76.5±6.57
MRT (days)	7.40±1.06	7.98±0.551	13.6±3.31	16.6 ±3.27	15.6±4.65	19.9±2.58
$t_{1/2,\alpha}$ (days)	1.11±0.612	1.02±0.742	0.658±0.158	0.628±0.330	0.519±0.117	0.715±0.192
$t_{1/2,\beta}$ (days)	6.98±1.48	7.13±1.78	10.2±2.33	12.6±3.08	11.3±3.30	14.6±1.85
$t_{1/2,\,effective}$ (days)	5.13±0.735	5.53±0.381	9.43±2.29	11.5±2.27	10.8±3.23	13.8±1.79

AUC_{0-inf} = area under the serum concentration–time curve from Time = 0 to infinity; CL = clearance; C_{max} = concentration at Time = 0 following an IV dose; MRT = mean residence time; $t_{1/2,\,effective}$ ($t_{1/2,\,eff.}$) = effective half-life; $t_{1/2,\alpha}$ = half-life of the alpha phase (ln(2)/α); $t_{1/2,\beta}$ = half-life of the beta phase (ln(2)/β); V_{ss} = volume of distribution at steady state; V_1 = volume of the central compartment.

The volume of the central compartment is low, about 4% of total body weight, suggesting as one would expect that the antibody is confined to the plasma. Clearance is slow and decreases with increasing dose, which is reflected in terminal elimination half-lives that increase with increasing dose. This suggests that the rate limiting process in elimination, possibly release from binding sites or antibody destruction or cleavage of DM1, is not first order. At the dose most relevant to human experience the terminal elimination half-life is about 7 days.

Groups 2, 3, and 4: Mean (±SD) Trastuzumab-MCC-DM1 TK Secondary Parameter Estimates following IV Administration of 3, 10, and 30 mg/kg of Trastuzumab-MCC-DM1 in Cynomolgus Monkeys

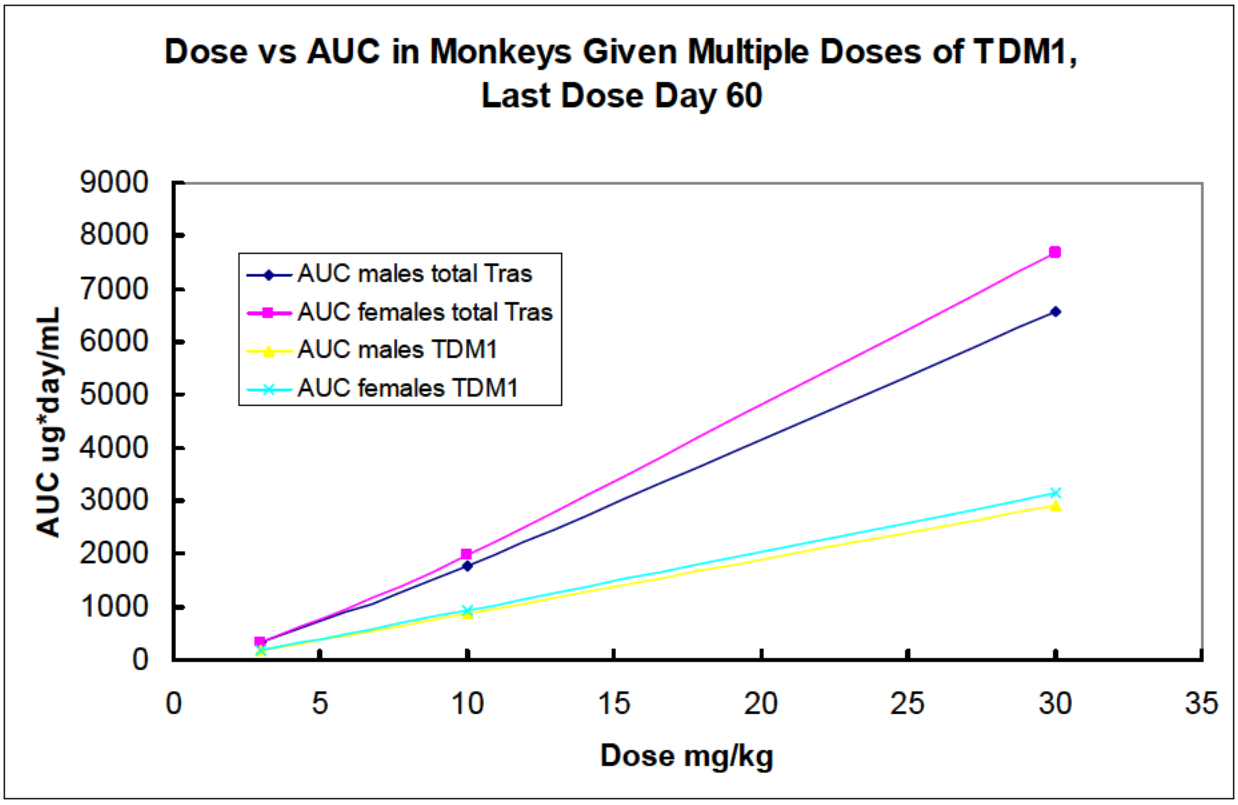
	Group 2 (3 mg/kg)		Group 3 (10 mg/kg)		Group 4 (30 mg/kg)	
Parameter	Males	Females	Males	Females	Males	Females
AUC_{0-inf} (μg • day/mL)	189±26.5	195±19.8	871±107	930±125	2900±552	3150±162
C_{max} (μg/mL)	75.7±6.51	78.0±3.16	266±31.8	265±20.7	787±76.9	776±39.9
CL (mL/day/kg)	16.1±2.15	15.3±1.52	11.5±1.34	10.7±1.31	10.5±2.33	9.39±0.561
V_1 (mL/kg)	39.8±3.45	38.0±1.40	37.5±4.41	37.1±2.71	37.7±3.59	38.2±2.22
V_{ss} (mL/kg)	56.5±10.0	58.4±3.74	61.6±6.55	63.7±9.43	68.1±4.71	70.0±4.74
MRT (days)	3.51±0.329	3.84±0.167	5.43±0.738	5.96±0.424	6.68±1.16	7.45±0.325
$t_{1/2,\alpha}$ (days)	0.856±0.564	0.397±0.168	0.515±0.112	0.494±0.129	0.412±0.0775	0.472±0.0705
$t_{1/2,\beta}$ (days)	3.13±0.414	2.94±0.161	4.20±0.465	4.61±0.421	5.04±0.862	5.65±0.241
$t_{1/2,\,effective}$ (days)	2.43±0.228	2.66±0.116	3.76±0.511	4.13±0.294	4.63±0.804	5.16±0.225

AUC_{0-inf} = area under the serum concentration–time curve from Time = 0 to infinity; CL = clearance; C_{max} = concentration at Time = 0 following an IV dose; MRT = mean residence time; $t_{1/2,\,effective}$ ($t_{1/2,\,eff.}$) = effective half-life; $t_{1/2,\alpha}$ = half-life of the alpha phase (ln(2)/α); $t_{1/2,\beta}$ = half-life of the beta phase (ln(2)/β); V_{ss} = volume of distribution at steady state; V_1 = volume of the central compartment.

Again here the volume parameters are low and suggest, as one would expect, the antibody is confined to the plasma space. The distribution half-life is remarkably consistent and shows relatively little variability except for low dose males, probably due to an outlier. This suggests that a consistent proportion of the drug binds to some site relatively quickly. The consistency of the volume and the distribution half-life suggest that titration of the binding sites (not necessarily just HER2 but all sites) is not saturated even at the high dose. The distribution half-life is less than the distribution half-life for total trastuzumab suggesting either more avid

binding or significant early cleavage of DM1; the latter seems most likely. Here again, the terminal elimination half-life and MRT increase with increasing dose and are about a factor of two different from their total trastuzumab counterparts. This again suggests that the rate limiting process in elimination is not first order and implies that the rate limiting process for elimination of trastuzumab emtansine is not destruction of the antibody, but more likely hydrolysis of the DM1.

The following graph shows that exposure for both total trastuzumab and trastuzumab emtansine increases linearly with dose (r^2 = 0.84 for total trastuzumab in males, similar for the others), but the increase in dose is greater than dose proportional (slope of dose vs dose normalized AUC = 3.7 for total trastuzumab in males, similar for the others). AUC is consistently higher in females for both total trastuzumab and trastuzumab emtansine.



Once hydrolyzed from the ADC, DM1 was cleared so rapidly that it could not be analyzed with accuracy sufficient to allow the investigators to fit the data to a model. In the kinetics of DM1 elimination, the hydrolysis step was rate limiting. The investigators used different methods to estimate exposures for the three different dose groups because so many of the data points in the low dose group were below the limit of quantitation. The tables below from the study report show these estimates.

Group 2: Mean (±SD) DM1 Plasma Exposure Parameters following IV Administration of Trastuzumab-MCC-DM1 in Cynomolgus Monkeys

Parameter	Group 2 (3 mg/kg)	
	Males	Females
AUC_{ALL} (day • ng/mL); Dose 1	1.66±0.557	1.76±0.581
AUC_{ALL} (day • ng/mL); Dose 4	3.25±2.26	3.17±1.46
C_{max} (ng/mL); Dose 1	5.95±0.770	5.85±1.13
C_{max} (ng/mL); Dose 2	6.98±1.03	6.72±1.23
C_{max} (ng/mL); Dose 3	8.04±0.574	7.09±1.01
C_{max} (ng/mL); Dose 4	7.02±0.957	7.53±1.46

AUC_{ALL} = area under the curve from the time of dosing to the time of the last observation; C_{max} = maximum observable concentration

Groups 3 and 4: Mean (±SD) DM1 Plasma Exposure Parameters following IV Administration of Trastuzumab-MCC-DM1 in Cynomolgus Monkeys

Parameter	Group 3 (10 mg/kg)				Group 4 (30 mg/kg)			
	Terminal Males	Recovery Males	Terminal Females	Recovery Females	Terminal Males	Recovery Males	Terminal Females	Recovery Females
AUC_{0-1} (day • ng/mL)	7.65±1.06	NA	6.92±0.477	NA	30.4±4.22	NA	20.7±0.226	NA
AUC_{63-64} (day • ng/mL)	9.53±2.68	NA	8.58±0.262	NA	40.5±9.80	NA	25.1±3.98	NA
AUC_{0-6} (day • ng/mL)	NA	19.8±4.50	NA	19.8±4.60	NA	53.7±14.2	NA	65.1±9.66
AUC_{63-69} (day • ng/mL)	NA	23.7±4.85	NA	22.5±4.55	NA	69.4±21.3	NA	92.8±6.80
C_{max} (ng/mL); Dose 1	21.9±1.90	20.8±2.41	19.6±1.00	22.5±2.99	74.6±9.27	61.8±0.755	58.6±2.12	66.0±4.78
C_{max} (ng/mL); Dose 2	23.6±10.1	24.8±5.49	24.2±3.39	27.9±5.52	81.8±9.82	64.5±8.85	65.1±10.5	70.1±6.24
C_{max} (ng/mL); Dose 3	25.1±3.99	26.3±5.06	23.6±3.88	25.9±4.23	86.1±11.1	69.0±8.91	66.1±4.70	67.0±24.4
C_{max} (ng/mL); Dose 4	23.6±2.15	23.4±2.99	23.4±1.10	24.4±2.30	76.8±7.55	75.9±11.3	59.7±4.77	76.1±7.85

AUC_{0-1} = partial area under the curve from time zero to 1 day post-dose; AUC_{63-64} = partial area under the curve from 63 days to 64 days post-dose; AUC_{0-6} = partial area under the curve from time zero to 6 days post-dose; AUC_{63-69} = partial area under the curve from 63 days to 69 days post-dose; C_{max} = maximum observable concentration; NA = not applicable.

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3) An Intravenous Chronic Toxicity Study of Trastuzumab-MCC-DM1 (PRO132365) Administered to Cynomolgus Monkeys Once Every 3 Weeks for 8 Doses, with a 6-Week Recovery Phase

Study no.:	07-0653 ([redacted] (b) (4) 6281-832)
Study report location:	EDR 4.2.3.2
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	November 5, 2007
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Trastuzumab-MCC-DM1/ 07p12-01-001/ 97% Antibody Drug Conjugate Vehicle/ 55122-75

Key Study Findings

- No early deaths occurred during the study.
- The liver was a target organ as evidenced by increases in ALT and AST and histopathological findings in the liver.
- Axon degeneration was noted in some treated animals during recovery (sciatic nerve and at injection site).
- Prostate and seminal vesicles had findings of mitotic abnormalities/apoptosis.
- There was evidence of mild inflammation.
- Spleen, thymus, lacrimal gland, and thyroid were targets.
- Toxicokinetic parameters were similar between males and females.
- Dose accumulation of T-DM1 and trastuzumab (but not DM1) at doses ≥ 3 mg/kg.

Methods

Doses:	0, 1, 3, 10 mg/kg
Frequency of dosing:	Every 3 weeks
Route of administration:	Intravenous injection
Dose volume:	2.5 mL/kg
Formulation/Vehicle:	Lyophililized T-DM1 reconstituted in sterile water and diluted in Antibody Drug Conjugate—Vehicle (control article)
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	6/sex/group
Age:	2-4 years
Weight:	2.3-3.8 kg (males), 2.1-3.0 kg (females)
Satellite groups:	
Unique study design:	
Deviation from study protocol:	Though an assessment of male fertility was initially planned, a decision was made to forgo this aspect of the study. There were no deviations from the study protocol that would significantly impact its interpretation.

Observations and Results

Mortality: checked twice daily
No early deaths occurred during the study.

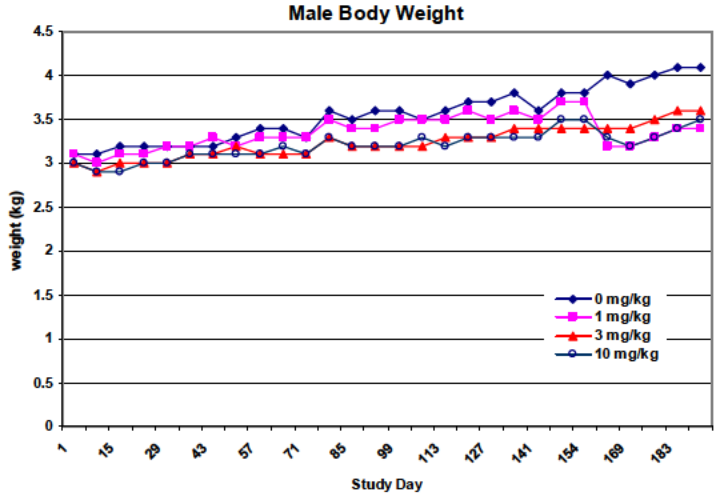
Clinical Signs: checked twice daily; cageside observations once daily or detailed observations once weekly (4 hrs. post-dosing on dosing days)

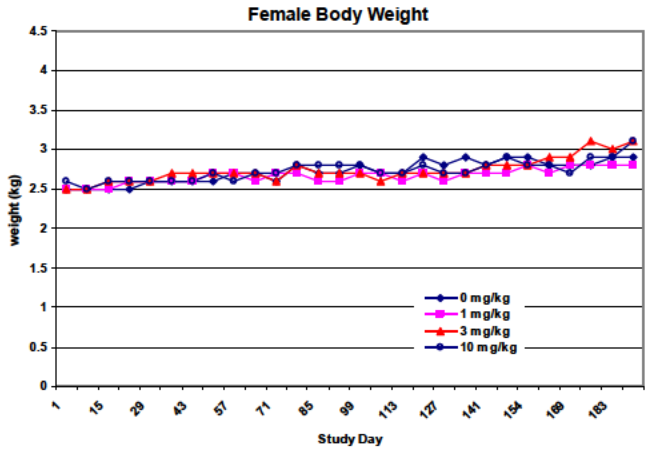
Observations Dose (mg/kg)	0		1		3		10	
Sex	M	F	M	F	M	F	M	F
N Main (Rec)	6 (3)	6 (3)	6 (3)	6 (3)	6 (3)	6 (3)	6 (3)	6 (3)
Vomitus, containing food			1		1	1	2	1
No food consumption					1	4	1	1
Low food consumption	6	5	6 (2)	5 (2)	6 (2)	6 (2)	5 (2)	6 (2)

Thin appearance				1		2		1
Liquid feces	3	4	1	2 (1)	2 (1)	5 (1)	3	4 (1)
Mucoid feces		1	1	1		1	(1)	1
Nonformed feces	5	5	4	5 (1)	6 (1)	6 (1)	5 (1)	6 (1)

Body Weights: recorded pre-dosing and once weekly thereafter

There were no significant differences in body weight between control and treated monkeys in any dose group during the dosing period. During the recovery period, there was a difference of up to 20% between control and treatment group males. While this difference did correlate with an observation of low food consumption in treatment group males of all doses during the recovery period, the difference appears mostly due to the inclusion of a heavier male in the control group recovery animals compared to the treatment group animals. Low food consumption was also observed in treatment group females of all dose levels during recovery, but there was no significant difference in body weight for females.





Feed Consumption: qualitative daily assessment only

Ophthalmoscopy: Study Days -21, 14, 149 (within a week of final dose), 184 (Recovery Day 30)

No significant observations.

Neurological Examinations: Study Days -15, 8, 154 (within a week of final dose), 189 (Recovery Day 35)
No significant observations.

ECG: Study Days -20, -8, 9, 79, 153 (within a week of final dose), 188 (Recovery Day 34)
There were no significant changes in breathing rate, body temperature, or oxygen saturation in treated animals over the course of the study.

ECG and blood pressure measurements were taken in anesthetized animals on non-dosing days. There were no significant changes in blood pressure or ECG parameters (including QTc prolongation) at any dose during the course of the study, though measurements were taken at least one week after the last administered dose throughout the study so that more immediate effects of the drug on these parameters might not be thoroughly appreciated.

Hematology: Collected twice during pre-dosing phase, Study Days 4, 8 and predosing on Study Days 22, 43, 64, 85, 106, 127; prior to terminal necropsy on Study Days 151, 155; Study Days 169, and 190 (prior to recovery necropsy)

There were mild and transient decreases in RBC, hematocrit, and hemoglobin levels compared to baseline levels that occurred in all groups, including controls, at around 8 days after treatment that may have been due to the antibody conjugate vehicle. This decrease (~10%) was smaller in the high dose group animals. Similarly, there were increases (~50-80%) seen in reticuloctye numbers from on Days 4 and 8 in all dose groups compared to baseline levels. These trends were also observed at 4 and 8 days following the final administration, though the reticulocyte response was less robust. At the high dose of 10 mg/kg there were decreases of ~30% observed in platelets compared to baseline at 4 to 8 days post dosing. Occasional transient increases during the dosing period (4-8 days post dosing Cycles 1 and 8) of monocytes, eosinophils and LUCs at the high dose for both males and females, may indicate some inflammation related to the drug product.

Clinical Chemistry: Collected twice during pre-dosing phase, Study Days 4, 8 and predosing on Study Days 22, 43, 64, 85, 106, 127; prior to terminal necropsy on Study Days 151, 155; Study Days 169, and 190 (prior to recovery necropsy)

Mild increases in total protein and globulin were apparent in the high dose group males and females throughout the dosing study. These increases indicate a possible chronic inflammatory response. Increases in AST were present at all dose groups for both sexes and were higher in the week following dosing (Days 4, 8, and 151). In males, CK was also increased following dosing. These increases may indicate some hepatic or muscle/cardiac toxicity. Finally, there were mild increases in cholesterol in the high dose group males and females in the week following dosing.

% Change from Control

Sex		Males				Females			
Dose (mg/kg)		0	1	3	10	0	1	3	10
N Main (Rec)		6 (3)	6 (3)	6 (3)	6 (3)	6 (3)	6 (3)	6 (3)	6 (3)
Parameter	Study Day								
TP	4	6.8		↑12%	↑15%	7			↑14%
	8	7.6				7.6			
	22	7.7			↑10%	7.8			
	85	7.4				7.4			
	R151	7.2			↑10%	7			↑10%
	R190	7.5				7.6			↑10%
GLOB	4	2.4		↑17%	↑29%	2.6			↑23%
	8	2.7			↑22%	2.8			↑21%
	22	2.8			↑25%	2.9			↑14%
	85	2.9			↑21%	2.9			↑17%
	R151	2.7			↑30%	2.8			↑21%
	R190	3.1			↑16%	3.3			
CHOL	4	142		↑14%	↑14%	139			↑27%
	8	149			↑16%	141			↑21%
	22	152	↓13%			152		↓11%	
	85	152	↓20%			139			
	R151	139				133			↑20%
	R190	142				161	↓22%	↓17%	↓27%
AST	4	39		↑79%	↑182%	44	↑27%	↑39%	↑156%
	8	44		↑34%	↑75%	52		↑12%	↑42%
	22	39			↑21%	43			
	85	40			↑33%	44			
	R151	69	↑42%	↑128%	↑216%	65		↑58%	↑155%
	R190	37			↑51%	38			
CK	4	220			↑126%	395	↑62%		
	8	368				595			
	22	198				457	↓39%	↓53%	↓53%
	85	234		↑76%	↑105%	413	↓39%	↓39%	↓56%
	R151	1164			↑267%	1142			↑127%
	R190	185	↑35%		↑101%	323	↓32%	↓47%	↑91%

Urinalysis

Urinalysis was performed only during the recovery period and not all recovery animals were included. While there were some differences in urinary volume between the dose groups, there was no significant difference in specific gravity. Urinary pH ranged from 6.8 to 8.5. No other parameters were recorded.

Gross Pathology

Macroscopic Observations

Sex		Males				Females			
Dose (mg/kg) N Main (Rec)		0 3 (3)	1 3 (3)	3 3 (3)	10 3 (3)	0 3 (3)	1 3 (3)	3 3 (3)	10 3 (3)
Liver	Discolored				1				1
Ovary	Cyst								1
IV Site (R)	Discolored Thickened	 1	1		1 (1)		1	1	2
Lung	Adhesion		(1)				1	(1)	(1)
Cecum	Discolored Raised Area							(1)	(1) (1)

Organ Weights

Table 1: Change in Absolute Organ Weights (% Change from Control)

Sex		Males			Females		
Dose (mg/kg) N Main (Rec)		1 3 (3)	3 3 (3)	10 3 (3)	1 3 (3)	3 3 (3)	10 3 (3)
Organ							
Body	Main Rec	↑12.5% ↓19.7%	↓13.5% ↓12%	↓2% ↓16.2%	↓5% ↓3%	↓11.4% ↑8%	↓6.3% ↑5%
Spleen	Main Rec	 ↓36%%	 ↓42%	↑52% ↓35%		 ↑54%	↑27% ↑196%
Liver	Main Rec			↑12%		 ↑20%	↑42% ↑17%
Thymus	Main Rec		↑22% ↓41%	↑20% ↑31%			↑45%
Mandibulary Sal. Gland	Main Rec	 ↓22%	↓32% ↓37%	↓21% ↓9%	↓29% ↑6%	↓26% ↑3%	↓36% ↓11%
Ovary	Main Rec						↑47%
Kidney	Main Rec						 ↑25%

Histopathology

Adequate Battery: yes
Peer Review: yes

Histological Findings

Microscopic Observations

Sex		Males				Females			
Dose (mg/kg) N Main (Rec)		0 3 (3)	1 3 (3)	3 3 (3)	10 3 (3)	0 3 (3)	1 3 (3)	3 3 (3)	10 3 (3)
Bone, Femur	Erosion/ulceration, articular cartilage, focal			1 (1)		1		1	1

Sex		Males				Females			
Dose (mg/kg)		0	1	3	10	0	1	3	10
N Main (Rec)		3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)
	Fibrosis, articular surface			1	(1)	1		1	1
	Hypertrophy, chondrocytes/ lacunae, art. surface			(1)		1			
Bone Marrow	Fat atrophy							1	
	Hypocellular				1				
Spinal Cord	Axonal Swelling							(1)	
	Mineralization							(1)	
Nerve, Sciatic	Axon Degeneration Min/Slight		(1)		(1)				
	Marked			(1)					
	Hemorrhage/edema, perineur		(1)	(1)		(1)			
	Hemorrhage, intraneur			(1)					
Pituitary	Cyst	1	(1)		(1)	(1)	1 (1)		
	Mineralization				1		(1)		
	Infiltrate, lymph			1					
Thyroid	Exfoliated cells, follicle		1 (1)	(2)	1	(2)	2 (1)	(1)	
	Mineralization			1					
Lung	Metaplasia, Sq. cell, bronchi		1 (2)	(1)			1 (1)	1 (2)	1
	Fibrosis, pleural/subpleural			(1)					
	Fibrosis, interstitial								(1)
	Inflamm, chronic								
Liver	Mitotic figs., Kupffer cells				1				2
	Hypertrophy, Kupffer/endo.				1				3 (1)
	Hepatocyte vacuolation				2			2	
	Atrophy, focal/multifocal				1				1 (1)
	Degen/necrosis, hepatocyte	(1)					1		1
	Fibrosis			1					
	Ito cell hyper-trophy/plasia				1			1	(1)
	Pigment, Kupffer cell								
Gallbladdder	Mitotic Figures, epith↑		1	1					(1)
Kidney	Glomerulopathy	1 (1)			(1)				(1)
	Tubule dilation		(1)	2	1		2 (1)	1 (2)	(2)
	Glomerulosclerosis/Perigl. Fibrosis			1	1	(1)			
	Tubule Atrophy				1		1		
	Fibrosis				1				
	Dilatation, blood vessels								1
Stomach, GI	Erosion/Ulceration		1					1	
	Amyloid							1	
Duodenum	Parasite								1
Colon	Inflamm., granulomatous				1		1		
	Hemorrhage/congestion			1 (1)	(1)			1	(1)
	Parasitic granuloma								(1)

Sex		Males				Females			
Dose (mg/kg) N Main (Rec)		0 3 (3)	1 3 (3)	3 3 (3)	10 3 (3)	0 3 (3)	1 3 (3)	3 3 (3)	10 3 (3)
Cecum	Pigment, submuc/tunica musc				(1) (1)			(1)	
	Parasite				(1)				
	Fibrosis								(1)
	Parasitic Granuloma								
Pancreas	Hemorrhage								(1)
Mandib. Sal. Gland	Mineralization, lumen/duct							1	
	Atrophy, diffuse						1		
Mand. LN	Mitotic figs ↑, germ. centers				1				
	Hyperplasia, lymph								(1)
	Granulopoiesis, extramed			(1)				(1)	(1)
Spleen	Mitotic figs ↑, mononuc. cells				2 1				3
	Fibrosis, capsule				1			(1)	(1)
	Pigment	2	1	(1)			1	2	(2)
	Hyperplasia, lymph			(1)					2
	Depletion, lymph.								
Mes. LN	Histiocytes, sinus							1	
	Fibrosis, capsule								(1)
	Pigment				(1)				
Thymus	Depletion, lymph		2 (1)	(1)			1		1
	Lymphocytolysis		(1)		1				
Muscle, Bi Fem	Atrophy				1		1		
Prostate	Mitosis/apoptosis epithel ↑				1				
Sem. Ves.	Mitosis/apoptosis epithel ↑				1				
	Exfoliated cells, lumen	(2)	1 (1)	2 (2)	2 (2)				
Epidid.	Exfoliated germ cells, lumen	1 (1)	2 (1)	(1)	(1) (1)				
	Cysts, intraepithelial								
Uterus	Dilation						1		
	Infiltrate, lymph/mac						1		1
Cervix	Metaplasia, sq. cell					(2)	1 (1)	(1)	(1)
Lacrimal Gl	Hypertrophy, epith. Cell			1	1 (1)				1
	↓Mucous cells								
	Min/Slight			1 (1)				1	2
	Mod				2 (1)				(2)
	Marked							1	
Heart	↑ Cyto. granularity, perinuc.						1	1	
	Inflamm. infiltrate, epicard.							1	
Brain	Infiltrate, lymph/mac				(1)		1	1	(1)
IV site, L	Infiltrate, lymph/mac	1		2		(1)		(1)	
	Fibrosis			2					1
	Hemorrhage, acute			2		1 (1)			
	Acanthosis		(1)	2	(1)	(1)		1 (1)	2 (2)
IV site, R	Mitotic figures/apop/necrosis ↑			(1)	1				2

Sex		Males				Females			
Dose (mg/kg)		0	1	3	10	0	1	3	10
N Main (Rec)		3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)
	Axonal degen (mod.)	2	2	(1)	1 (2)		2 (1)	2 (1)	3 (2)
	Hemorrhage, acute	2		1	1 (1)	1	1	1	2
	Fibrosis	2	1	(1)	1	1 (1)	1		1 (1)
	Infiltrate lymph/mac		(1)	(1)	2 (1)	(2)	1	2 (1)	2
	Acanthosis/hyperkeratosis				1				
	Hypertrophy, endot, saph vein						1		
	Thrombus							(1)	1
	Disruption, saphenous vein wall				1 (1)				
	Edema				(1)				
	Hypertrophy, sebaceous gl								
	Vaculitis, neutrophilic								

All findings were recorded as minimal or slight with the noted exceptions in the lacrimal gland, sciatic nerve, and injection site.

Special Evaluation

Anti-therapeutic Antibodies: Anti-therapeutic antibodies were detected in 3 of 12 animals in the 1 mg/kg dose group, no animals in the 3 mg/kg dose group, and 1 of 12 animals in the 10 mg/kg dose group. The positive finding in the 10 mg/kg dose group was not seen until recovery. The presence of these antibody positive animals did not appear to affect the toxicokinetic results significantly.

In addition, there was a single animal in the 0 mg/kg dose group that had positive findings in the post-dosing samples.

Bone Marrow Micronucleus Assay: Bone marrow smears were prepared from all animals at the terminal sacrifice (Day 155) timepoint--7 days after the final administered dose—in order to evaluate T-DM1 for *in vivo* clastogenic activity. The assay was considered valid by the Applicant because the toxicokinetic analysis confirmed the exposure of the bone marrow to the test article during the 7-day period from the last dose to the bone marrow collection. No positive control was included in the study. Histopathological evidence showed minimal bone marrow toxicity making T-DM1 exposure to the bone marrow questionable. There was a small decrease in the ratio of polychromatic erythrocytes to normochromatic erythrocytes in the bone marrow of male and female animals treated with T-DM1, suggesting some bone marrow toxicity. Overall numbers of micronucleated cells were small, however, these numbers were increased in all treatment groups for both males and females compared to the vehicle only control. The results of this assay are inconclusive.

(Adapted from Applicant's submission)

		Male				Female			
Treatment	Dose	Animal Number	# MN PCE/ 2000 PCE	Ratio PCE:NCE	Avg. Ratio PCE:NCE	Animal Number	# MN PCE/ 2000 PCE	Ratio PCE:NCE	Avg. Ratio PCE:NCE
Control	VC 2.5 mL/kg/day	I04766	1	0.70	**0.75 ± 0.06**	I04790	0	0.71	**0.74 ± 0.01**
		I04767	0	0.87		I04791	1	0.75	
		I04768	0	0.68		I04792	1	0.75	
Test Article	1 mg/kg (222 µg/m^2)	I04772	2	0.65	**0.73 ± 0.10**	I04796	2	0.69	**0.71 ± 0.05**
		I04773	0	0.63		I04797	1	0.63	
		I04774	1	0.93		I04798	0	0.82	
	3 mg/kg (665 µg/m^2)	I04778	1	0.60	**0 64 ± 0.04**	I04802	1	0.67	**0.63 ± 0.05**
		I04779	1	0.60		I04803	3	0.68	
		I04780	0	0.73		I04804	1	0.53	
	10 mg/kg (2216 µg/m^2)	I04784	1	0.58	**0 58 ± 0.04**	I04808	0	0.83	**0.74 ± 0.06**
		I04785	0	0.64		I04809	2	0.77	
		I04786	4	0.51		I04810	1	0.62	

Toxicokinetics

Study Days 1 (pre-dosing, 5 min., 4, 8, 24, 72, 144 (6 days), and 336 (14 days) hrs. post-dose); 43 (pre-dose, 5 min. post-dose); 64 (pre-dosing, 5 min., 4, 8, 24, 72, 144 (6 days), and 336 (14 days) hrs. post-dose); 85 (pre-dose, 5 min. post-dose); 127 (pre-dose, 5 min. post-dose); 148 (pre-dosing, 5 min., 4, 8, 24, 72, and 144 hrs.(6 days) post-dose); 162, 169, 176, 183, and 190 (recovery period-times similar to 5 min. post-dose)

Increases in exposure were both dose-dependent and roughly dose-proportional. There was evidence at doses ≥ 3 mg/kg of some dose accumulation for the antibody or the intact drug product. There was not evidence for accumulation of DM1 suggesting a steady rate of dissociation from the antibody and exposure in the animal.

TK parameter	Cycle	1 mg/kg	3 mg/kg	10 mg/kg
T-DM1				
C_{max} (µg/mL)	1	21.9±2.03	71.7±5.4	190±37
	4	26.8±2.76	77.3±9.17	267±27.8
	8	28.3±5.85	90.3±9.79	257±41.9
$t_{1/2}$ (day)	1	1.93±0.139	2.54±0.251	4.71±0.527
	4	2.72±1.03	3.74±0.384	5.3±0.36
	8	1.82±0.561	3.03±.441	4.55±1.23
AUC (day*µg/mL)	1	33.4±3.44	149±20.3	646±83.6
	4	35.2±4.9	183±29.5	874±128
	8	39.8±8.62	212±36.7	973±145
CL (mL/day/kg)	1	30.3±3.38	20.4±2.77	15±2.12
	4	28.8±4.26	16.6±2.78	11±1.77
	8	26.4±6.84	14.5±2.79	9.94±1.61
V (mL/kg)	1	66.6±7.29	68.5±3.78	94.3±11.2
	4	66.7±8.98	72.7±8.38	75.8±8.3
	8	55.4±6.46	57.6±5.83	65.6±11
Trastusumab				
C_{max} (µg/mL)	1	21.3±1.68	68.2±4.89	187±32.2
	4	26.9±2.63	76.3±8.63	267±30.4

TK parameter	Cycle	1 mg/kg	3 mg/kg	10 mg/kg
	8	29.4±3.95	84.4±9.16	330±48.2
$t_{1/2}$ (day)	1	2.89±.365	3.83±.497	7.61±1.17
	4	4.57±1.73	6.6±1.07	10±1.64
	8	2.88±1.19	5.06±1.36	9.42±2.7
AUC (day*µg/mL)	1	41.3±4.77	193±28.5	903±111
	4	48.1±8.12	279±54.7	1670±269
	8	52.1±13.5	319±75.9	1870±370
CL (mL/day/kg)	1	24.4±3.08	15.6±2.44	9.62±1.49
	4	20.7±4.13	10.1±2.23	4.78±1.15
	8	20.4±6.4	9.4±2.63	4.45±1.5
V (mL/kg)	1	81.8±8.6	77.7±4.75	100±12.6
	4	87±15.6	83.7±11.2	66.3±7.35
	8	64.2±8.97	61.9±6.47	56.1±9.55
DM1				
C_{max} (ng/mL)	1	1.56±.221	3.64±.452	13±3.88
	4	1.64±.853	4.35±.0.93	13.3±2.76
	8	1.86±1.22	3.45±0.534	11.2±1.94
$t_{1/2}$ (day)	1	.524	.518±.208	2.05±.447
	4	.34	1.45±3.08	2.32±.0853
	8	0.201±0.0172	1.03±1.27	3.52±1.28
AUC (day*ng/mL)	1	.886	1.21±.254	7.57±1.78
	4	.647	2.45±4.11	7.37±1.8
	8	0.399±0.0426	1.92±1.91	12.1±4.73

7 Genetic Toxicology

1) Study title: $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl) maytansine (DM1): Salmonella—Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay

Dr. Whitney Helms reviewed this study

Study no.: 09-2654 (Genentech)
8218567 (b) (4)
Study report location: CBER EDR 4.2.3.3
Conducting laboratory and location: (b) (4)

Date of study initiation: October 30, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: DM1/ 4006 AO 90109/ 93.5%

Key Study Findings

DM1 was negative in the Ames assay.

Methods

Strains: *Salmonella typhimurium*
TA98, TA100, TA1535, and TA1537
Escherichia coli
WP2*uvr*A

Concentrations in definitive study: 50, 160, 500, 1600, and 5000 µg/plate±S9

Basis of concentration selection: Initial dose range study: 1.6, 5, 16, 50, 160, 500, 1600, and 5000 µg/plate±S9

Negative control: Vehicle (DMS0)

Positive control:

Control Article	Strain	S9?
Benzo[a]pyrene	TA98	+
2-aminoanthracene	TA100 TA1535 TA1537 WP2*uvr*A	+ + + +
2-nitrofluorene	TA98	-
Sodium azide	TA100 TA1535	- -
ICR-191	TA1537	-
4-nitroquinoline-N-oxide	WP2*uvr*A	-

Formulation/Vehicle: DM1 in DMSO

Incubation & sampling time: Strains were plated during late exponential or early stationary growth phase. Plates (in duplicate for dose range or triplicate for confirmatory test) were cultured for 52±4 hrs. at 37C.

Study Validity

Excerpted from Applicant:

Characteristic Number of Spontaneous Revertants

TA98	8	-	60
TA100	60	-	240
TA1535	4	-	45
TA1537	2	-	25
WP2*uvr*A	5	-	40

Revertants on vehicle control culture plates must be within the range above in order for the assay to be accepted. Three non-toxic doses (cytotoxic defined as doses causing a decrease in

the number of revertants and/or by thinning of the bacterial lawn compared to the vehicle control) are required.

Results

DM1 was negative in the Ames assay.

(Excerpted from Applicant—Results of Confirmatory Assay)

Without S9

	Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle
	TA98	DM1	5000	12.0	3.0	0.9
			1600	8.3	2.3	0.7
			500	9.7	2.1	0.8
			160	13.7	5.0	1.1
			50.0	7.7	3.2	0.6
		Dimethyl Sulfoxide		12.7	3.8	
	TA100	DM1	5000	80.3	1.5	1.1
			1600	70.3	12.0	1.0
			500	63.7	2.3	0.9
			160	69.7	4.2	1.0
			50.0	86.0	22.7	1.2
		Dimethyl Sulfoxide		70.3	8.5	
	TA1535	DM1	5000	12.3	3.2	0.9
			1600	12.0	4.4	0.9
			500	13.3	5.0	1.0
			160	10.7	1.5	0.8
			50.0	12.7	2.5	1.0
		Dimethyl Sulfoxide		13.3	5.5	
	TA1537	DM1	5000	2.7	2.9	0.5
			1600	3.3	1.2	0.7
			500	4.0	0.0	0.8
			160	3.7	1.2	0.7
			50.0	5.3	1.5	1.1
		Dimethyl Sulfoxide		5.0	0.0	
	WP2*uvr* A	DM1	5000	5.7	2.9	0.4
			1600	14.0	1.7	0.9
			500	10.7	2.1	0.7
			160	13.7	3.5	0.9
			50.0	10.3	1.5	0.7
		Dimethyl Sulfoxide		15.0	12.1	
Positive Controls	TA98	2NF	1.0	189.7	30.2	15.0
	TA100	SA	2.0	1084.0	32.7	15.4
	TA1535	SA	2.0	711.7	70.1	53.4
	TA1537	ICR	2.0	183.7	4.0	36.7
	WP2*uvr* A	4NQO	1.0	673.0	69.9	44.9

With S9

	Strain	Compound	Dose level (µg/plate)	Mean revertants per plate	SD	Ratio treated/ vehicle
	TA98	DM1	5000	22.0	8.9	1.1
			1600	25.0	4.6	1.2
			500	15.7	2.5	0.8
			160	19.7	4.2	1.0
			50.0	20.0	2.6	1.0
		Dimethyl Sulfoxide		20.3	8.1	
	TA100	DM1	5000	85.0	4.0	1.0
			1600	87.3	4.9	1.0
			500	78.7	6.1	0.9
			160	81.3	17.2	1.0
			50.0	76.7	5.7	0.9
		Dimethyl Sulfoxide		85.0	0.0	
	TA1535	DM1	5000	8.7	2.5	0.9
			1600	13.0	6.2	1.3
			500	11.0	2.6	1.1
			160	8.0	1.7	0.8
			50.0	8.3	2.1	0.8
		Dimethyl Sulfoxide		10.0	5.3	
	TA1537	DM1	5000	5.0	1.0	0.6
			1600	3.0	1.0	0.3
			500	6.0	2.0	0.7
			160	6.0	1.7	0.7
			50.0	5.7	2.5	0.7
		Dimethyl Sulfoxide		8.7	2.1	
	WP2*uvr*A	DM1	5000	15.3	3.5	0.9
			1600	13.0	3.0	0.8
			500	10.7	2.1	0.7
			160	16.3	5.9	1.0
			50.0	11.7	0.6	0.7
		Dimethyl Sulfoxide		16.3	1.2	
Positive Controls	TA98	BP	2.5	404.3	13.6	19.9
	TA100	2AA	2.5	1832.3	137.0	21.6
	TA1535	2AA	2.5	193.3	30.4	19.3
	TA1537	2AA	2.5	101.7	5.0	11.7
	WP2*uvr*A	2AA	25.0	410.0	51.0	25.1

2) N^2-deacetyl-$N^{2'}$-(3-mercapto-1-xopropyl) maytansine (DM1): *In Vivo* Rat Bone Marrow Micronucleus Assay

Study no	Sponsor – 09-2726; (b) (4) – 8220198
File name	09-2726- (b) (4) -final.pdf
Laboratory	(b) (4)
Study Date	May 2010
GLP	Yes
Audited	Yes
Drug	DM1, Lot number 4006A090408, Purity 95.5%
Method:	

Species Young adult male CRL: CD (SD) rats
10 weeks old, body weights ranged from 311 to 393 g.
N Five per dose group
Doses The following table shows the doses and the number of rats in each group at 24 and 48 hour harvest

Test compound	Dose mg/kg	Dose $\mu g/m^2$	N at 24 hours	N at 48 hours
Positive control	60	-	5	
Vehicle	0	0	5	5
DM1	0.01	60	5	
DM1	0.05	300	5	
DM1	0.1	600	5	5
DM1	0.2	1200	5	5

Route PO gavage
Schedule Single dose
Vehicle Aqueous 0.5% DMA, 1 mM EDTA.
Dose volume 4 mL/kg
Positive control Cyclophosphamide
Marrow Collection 24 and 48 hours post dosing
Analysis Incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes for each rat. The PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring at least 500 erythrocytes per animal.

The investigators did not do a dose range finding study. They state that they observed no gender difference in a similar previous study ((b) (4) Study No. 6481-663). The Applicant did not submit this study to the NDA.

Results

Mortality None
Clinical signs None
Analysis

The following table from the study report demonstrates that DM1 causes a significant increase in the percentage of micronucleated PCE and a significant decrease in the PCE:NCE ratio. There was a distinct and significant dose response. The increase in micronucleated PCEs was as high or higher than that caused by the positive control. The high dose caused significant bone marrow toxicity at the 48 hour time point. The positive control was also positive. The assay was well conducted and validated. Thus N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl) maytansine (DM1) is a clastogen.

Study No.: 8220198
Test Article: $N^{2'}$-deacetyl-$N^{2'}$-(3-mercapto-1-oxopropyl) maytansine (DM1)
Initiation of Dosing: 05 May 2010 Species/Strain: Rat/Sprague-Dawley

Treatment	Dose[a]	Harvest Time	% Micronucleated PCEs Mean ± SD Male	Ratio PCE:NCE Mean ± SD Male
Controls				
Vehicle	VC 4 mL/kg	24	0.03 ± 0.04	0.94 ± 0.10
		48	0.05 ± 0.04	0.79 ± 0.11
Positive	CP 60 mg/kg	24	1.13 ± 0.44*	0.80 ± 0.08**
Test Article	0.01 mg/kg	24	0.05 ± 0.04	0.87 ± 0.16
	0.05 mg/kg	24	0.62 ± 0.34*	0.78 ± 0.12**
	0.1 mg/kg	24	1.13 ± 0.40*	0.51 ± 0.08***
	0.1 mg/kg	48	1.76 ± 0.72*	0.25 ± 0.03***
	0.2 mg/kg	24	0.72 ± 0.69*	0.52 ± 0.07***
	0.2 mg/kg	48	-- ± --[b]	-- --[b]

* Significantly greater than the corresponding vehicle control, $p \leq 0.01$.
** Significantly less than the corresponding vehicle control, $p \leq 0.05$.
*** Significantly less than the corresponding vehicle control, $p \leq 0.01$.
VC= Trastuzumab Drug Conjugate Vehicle with 0.5% DMA, 1mM EDTA
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte
[a] Dose DM1 expressed as mg/kg
[b] Four of the five animals had a PCE to NCE ratio less than 20% of the vehicle control value; this group was not used for calculation of group means or for statistical evaluation

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Not required and not submitted.

9 Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development
Not required and not submitted

Embryonic Fetal Development
Dr Anne Pilaro reviewed the studies of the reproductive toxicology of trastuzumab (Herceptin). The FDA agreed with the Applicant that these studies would be adequate to support the approval of TDM-1 because it is likely to be more toxic than trastuzumab, because trastuzumab is known to cause reproductive toxicity clinically, and because of the mechanism of action of DM1 which causes it to be genotoxic and toxic to rapidly dividing cells. This is consistent with ICH S9. In a review of cancer drugs used during pregnancy, O. Mir et al. reported that three pregnant women of six treated with Herceptin developed anhydramnios.[25]

[25] O. Mir et al. Annals of Oncology 19: 607–613, 2008

The text that follows is Dr. Pilaro's review without modification, except for the font which was originally a serifed font.

MEMORANDUM

TO: The file
THROUGH: Patricia Keegan, M.D., Director, Division of Biologic Oncology Products, Office of Oncology Drug Products, CDER
FROM:Anne M. Pilaro, Ph.D., Acting Supervisory Toxicologist, Pharmacology/Toxicology Branch, Division of Biologic Oncology Products, OODP, CDER
STN BLA #: 103792/5175
SPONSOR: GENENTECH, INC.
PRODUCT: humanized, monoclonal antibody trastuzumab (Herceptin®), directed against the Her2/*neu* receptor, for the treatment of Stage III or IV breast cancer
AMENDMENT TYPE: efficacy supplement, **labeling conversion to PLR format**
DATE: January 10, 2008

SYNOPSIS:

As part of the efficacy supplement STN BLA #103792/5175, the sponsor is converting the labeling to Physician's Labeling Rule (PLR) format. Changes in the *WARNINGS and PRECAUTIONS* (Section 5.6, *Embryo-fetal Toxicity [Pregnancy Category D]*), *USE IN SPECIFIC POPULATIONS* (Section 8.1, *Pregnancy* [*Teratogenic Effects: Pregnancy Category D]* and Section 8.3, *Nursing Mothers*), and *NONCLINICAL TOXICOLOGY* (Section 13.1; *Carcinogenesis, Mutagenesis, Impairment of Fertility,* and 13.2, *Animal Toxicology and/or Pharmacology*) required verification of the supporting nonclinical data. The data were included in the original labeling; however, there was insufficient information available to support the conversion to PLR format. Specifically, the types of studies conducted, the doses of trastuzumab tested and resulting exposure margins compared to human dosing, and the fetal outcomes, as well as any data regarding evaluation of male fertility parameters were required to support the labeling in PLR format.

The sponsor was requested to provide the supporting data for these sections of the label, and responded by submitting .pdf copies of three nonclinical, reproductive and/or developmental toxicology studies with trastuzumab, conducted in cynomolgus monkeys. These studies were initially reviewed in the nonclinical review for the original BLA (STN #103792/0000), and will not be subject to a complete review here. However, evaluation of the study reports confirms that they do contain the supporting data required for the labeling in PLR format. The three studies are summarized in context of the sections of the PLR labeling that they support, below.

Section 5.6 - *WARNINGS and PRECAUTIONS, Pregnancy Category D* and Section 8.1, *Teratogenic Effects*

1) Study #95-039-1450. GN1450. Intravenous embryo-fetal development study in the cynomolgus monkey.

Twelve presumed pregnant, cynomolgus monkeys per dose group were treated by i/v injection with trastuzumab during the critical period of organogenesis (gestational day [GD] 20 through GD50). The doses tested in this study were vehicle control (GN1450 vehicle, composition not specified), 1, 5, and 25 mg/kg trastuzumab, administered GD20, GD21, GD22, GD23, GD27, GD30, GD34, GD37, GD41, GD44, GD47, and GD50. Dams were followed for clinical and

laboratory signs of toxicity, toxicokinetic profiles, and immunogenicity. On GD100, offspring were delivered by Caesarian section and evaluated for skeletal and soft tissue malformations, as well as serum and amniotic fluid trastuzumab and anti-trastuzumab antibody levels.

In general, trastuzumab treatment was well-tolerated by the pregnant animals, and no evidence of maternal toxicity was noted. Early mortalities were present in a total of 5 dams during the study period, and occurred in all dose groups, including the control. In animals #3037and #4210 (1 mg/kg/dose group), and #208 (25 mg/kg/dose group), the deaths occurred 2, 10, and 35 days, respectively following spontaneous abortion by these dams. Early deaths for animals #3115 (vehicle control) and #3257 (1 mg/kg/dose group) were not associated with spontaneous abortion. In all cases, there were no apparent clinical signs of toxicity prior to death. However, on necropsy histological evidence of bacterial infection was present in skin lesions and in the intestines, and was identified tentatively as β-hemolytic *Streptococcus*, *Staphylococcus sp.*, and/or *Pasteurella* infections. Given the incidence of early mortalities and presence of infection in animals from all dose groups including the control, these findings appear unrelated to trastuzumab treatment.

Spontaneous abortions occurred in 2/12 control (17%), 5/12 low-dose (42%), 3/12 (25%) monkeys treated with 5 mg/kg/dose trastuzumab, and 4/12 (33%) of dams in the 25 mg/kg/dose group. There were no statistically significant differences between the control and trastuzumab-treated dose groups in the incidence of spontaneous abortions (p = 0.8973, *Chi* square)(*The data did not vary with dose by ANOVA, p>0.3 WDM*) . Additionally, the incidence of abortion in this study was within the range of historical controls for this laboratory (88/492 or 18%; from a total of 43 embryofetal toxicity studies, with a range of 0-50% spontaneous abortion).

Live fetuses were recovered from 10 monkeys in the control group, 7 animals treated with 1 mg/kg/dose trastuzumab, 9 dams in the 5 mg/kg/dose group, and 8 dams in the 25 mg/kg/dose group. Mean values for fetal measurements (body, placental, and organ weights, crown:rump lengths) in all trastuzumab-treated dose groups were within normal limits for cynomolgus monkeys. There were no gross skeletal or soft tissue malformations in the offspring from trastuzumab-treated dams at any dose level.

Toxicokinetic evaluation showed that maternal levels of trastuzumab increased rapidly during the first week of dosing (GD20 through GD27), and approached steady-state by the end of this period. During the course of the study, mean values for trastuzumab trough levels showed dose-related, approximately linear increases, and were maintained for the duration of the dosing period in all animals except dam #6292 in the 1 mg/kg/dose group. This animal had pre-existing anti-drug antibody and no detectable serum trastuzumab levels present at any time point on study (see below). The mean values for trastuzumab trough levels are presented in Table 1 below, which was abstracted from the sponsor's final study report.

Table 1. Summary Table of Mean (± S.D.) Trastuzumab Maternal Trough Serum Levels and Elimination Half-Life in Pregnant Cynomolgus Monkeys Dosed from GD20 through GD50

Parameter	Group 1 Control (n = 0 - 12)	Group 2 1 mg/kg (n = 6 - 10) [a]	Group 3 5 mg/kg (n = 10 - 12)	Group 4 25 mg/kg (n = 8 - 10) [b]
Trough Concentrations (mcg/mL) [c]:				
Maximum	0.000 [d]	46.1 (9.22)	349 (87.0)	1637 (474)
Minimum	0.000	27.8 (5.14)	205 (35.9)	936 (268)
Average	0.000	35.6 (6.04)	259 (50.4)	1261 (341)
Half-life (days) [e]	– [f]	7.09 (0.86)	7.72 (1.07)	10.1 (1.91)

a Concentrations from group 2 animal no. 6292 (all 'lts') were excluded from this table due to antibody reaction.

b Day 48 and 55 concentrations from group 4 animal no. 5462 were excluded from this table due to apparent misdosing. Half-life could not be calculated for this animal due to early removal from the study.

c Troughs concentrations reported here were from samples collected during the dosing phase of the study over Days 27 through 48.

d All concentrations for group 1 were 'lts' (less than the lowest standard on the standard curve, ie. less than 0.078 mcg/mL) and were therefore assigned a value of zero by convention. No standard deviation was calculated for this reason. Concentrations from animals no. 2914 and 6070 were excluded from this table due to apparent misdosing.

e Half-life was calculated over Days 55 through 100 by linear regression on the natural log of concentrations vs. time.

f No half-life was calculated for group 1 since all concentrations were 'lts'.

Placental transfer of trastuzumab, with subsequent fetal exposure was also detected in this study. Table 2 below, from the sponsor's final study report shows that at time of Caesarean section on GD100, trastuzumab was present at detectable levels in serum from fetuses in all dose groups, and in amniotic fluid from offspring of dams treated with 5 or 25 mg/kg/dose. The concentration of trastuzumab in fetal samples increased with increasing dose of antibody, and ranged from 10% to approximately 19% of maternal serum levels present in the fetal serum, and approximately 3 to 4% of maternal serum levels present in the amniotic fluid of fetuses in the mid- and high-dose groups. These results are summarized in Table 2, below.

Table 2. Summary of Mean (± S.D.) Trastuzumab Maternal and Fetal Serum and Amniotic Fluid Concentrations, and Concentration Ratios from Pregnant Cynomolgus Monkeys at GD100

Parameter	Group 1 Control (n = 0 - 9)	Group 2 1 mg/kg (n = 2 - 6) [a]	Group 3 5 mg/kg (n = 9)	Group 4 25 mg/kg (n = 8) [b]
Concentrations (mcg/mL):				
Maternal Serum	0.000 [c]	0.325 (0.255)	3.70 (1.22)	59.9 (41.3)
Fetal Serum	0.000	0.049 (0.077)	0.679 (0.210)	14.6 (23.2)
Amniotic Fluid	0.000	0.000 [d]	0.155 (0.082)	1.50 (0.851)
Concentration Ratios:				
Fetal / Maternal Serum	– [e]	0.100 (0.137)	0.188 (0.044)	0.171 (0.176)
Amniotic Fluid / Maternal Serum	–	0.000	0.039 (0.019)	0.028 (0.009)
Amniotic Fluid / Fetal Serum	–	0.000	0.214 (0.113)	0.291 (0.214)

a Concentrations from group 2 animal no. 6292 (all 'lts') were excluded from this table due to antibody reaction.

b Day 100 samples could not be obtained from all animals in each group because some animals were previously removed from study due to spontaneous abortion or death.

c All concentrations for group 1 were 'lts' (less than the lowest standard on the standard curve, ie. less than 0.078 mcg/mL) and were therefore assigned a value of zero by convention. No standard deviation was calculated for this reason. Concentrations from animals no. 2914 and 6070 were excluded from this table due to apparent misdosing.

d No standard deviation was calculated since all concentrations were 'lts'.

e No ratios could be calculated since both concentrations were 'lts'.

There were no detectable anti-drug antibody responses in any of the trastuzumab-treated dams or fetuses delivered at GD100, with the exception of one pair. Monkey #6292 and its fetus #6292f had positive titers for anti-trastuzumab antibody in both the ELISA assay for total binding

antibody, and the BT-474 neutralization bioassay. Antibody titer values in the dam at GD20 and GD50 were 3.2 and 3.4 absorbance units (AU), respectively, and at GD100 were approximately equivalent in the dam and offspring, with titers of 2.7 and 2.4 AU, respectively. Since anti-product antibodies were initially present in the dam on GD20 prior to treatment with trastuzumab; these results likely represent a pre-existing, cross-reactivity and are not related to a response to trastuzumab.

Comment: The labeling reports oligohydramnios, or decreased amniotic fluid in pregnant women receiving Herceptin® treatment during the second and third trimester. This finding was not observed in either of the cynomolgus monkey developmental toxicology studies (see additional study summary, below). However, since human data are available, Herceptin® is labeled Pregnancy Category D.

Comment: The sponsor reports in the labeling that there are no maternal or fetal toxicities associated with Herceptin® treatment at up to 25-times the human exposure. On a direct mg/kg basis, the dose of trastuzumab tested in the animal studies was only 12.5-fold greater than the recommended weekly human dose of 2 mg/kg. However, in the present developmental toxicity study and in the fertility study, trastuzumab was administered twice weekly. Therefore, the language in the labeling claim regarding the exposure margin is supported by the nonclinical dosing.

In conclusion, the results of this study support the reported findings in Section 5.6, *Pregnancy Category D* and Section 8.1, *Teratogenic Effects* of the revised, PLR formatted label for trastuzumab (Herceptin®).

Section 8.2 – *USE IN SPECIAL POPULATIONS, Nursing Mothers* and Section 13.2, *NONCLINICAL TOXICOLOGY, Animal Toxicology and/or Pharmacology*

2) Study #95-238-1450. Late gestational toxicity, placental transfer, and secretion in milk study with GN1450 in the cynomolgus monkey.

Two groups (n = 8/group) of pregnant, cynomolgus monkeys were treated by i/v injection with trastuzumab during the latter part of gestation, from approximately GD120 through either GD150 (Group 1), or through post-partum day (PPD) 28 (Group 2). All animals received 25 mg/kg/dose of trastuzumab by i/v injection daily from GD120 through GD123, then once weekly for the remainder of the dosing periods. Dams were followed for clinical and laboratory signs of toxicity, toxicokinetic profiles, and immunogenicity. For Group 1 animals, pregnancies were terminated by Caesarean section on GD150, while Group 2 females were allowed to deliver naturally and nurse the offspring until PPD29, at which time the animals were returned to the colony.

Maternal blood was sampled once pre-study, and then weekly prior to dosing for measurement of serum trastuzumab levels. For evaluation of serum anti-trastuzumab antibody levels, maternal blood was sampled once pre-treatment for all animals. Amniotic fluid, maternal blood and fetal umbilical vein blood were obtained at GD150 at the time of Caesarean section for Group 1 animals. Maternal and infant blood, and maternal breast milk samples were obtained from animals in Group 2 on PPD28.

Trastuzumab treatment was well-tolerated by the pregnant animals, with no evidence of maternal toxicity. Body weights, body weight gains, clinical chemistry and urinalysis profiles for the treated dams were within normal limits. There were three unsuccessful deliveries/stillbirths

during the study. Animal #1791 in Group 2 went into labor on GD163, had difficulty delivering the fetus, and was euthanized for humane reasons. A second Group 2 monkey (animal #3376) did not deliver successfully; the fetus was removed by Caesarean section on GD176, however, the dam survived. One Group 1 monkey (animal #5383) had a stillbirth at GD131.

Live fetuses were removed by Caesarean section from 7/8 animals in Group 1 at GD150, and evaluated morphologically for evidence of toxicity. No trastuzumab-related changes in fetal body measurements, fetal body or organ weights, or placental weights were detected. There were no treatment-related external, visceral or skeletal anomalies and no fetal malformations observed in the offspring in this group. Visceral findings, including hemorrhage in the cardiac area of the stomach or at the stomach wall were present in 5/7 offspring, and likely represent a post-mortem artifact. Minor skeletal variations, including ossification of the vertebra(e), sternebra(e), and/or carpal and tarsal bones were observed in 6/7 offspring in this group. These findings are considered to be normal developmental variations, and not related to trastuzumab treatment.

In Group 2, live infants were successfully delivered to 6/8 dams. There was no observable effect of trastuzumab treatment on infant body weights, as compared to historical control data from the study laboratory. Monkey #9536 from Group 2 had no detectable lactation and therefore could not breast-feed its offspring; this infant was fostered to another lactating female from the stock colony. From PPD11 through PPD28, alopecia at the base of the tail and back of the head was reported for this infant. There were no other clinical signs of toxicity reported for the infants in this group.

Using an ELISA assay, no anti-trastuzumab antibodies were detected in the dams at any time point on study, or in the fetuses at GD150 or the infants at PPD28. However, the sponsor states in the final study report that the interpretation of the negative findings in this study may be limited, since the presence of detectable trastuzumab in the blood samples may interfere with detection of the anti-drug antibody.

Maternal exposures were comparable between both groups of pregnant dams treated with 25 mg/kg/dose trastuzumab. A slight (approximately 40-60%) accumulation in serum trastuzumab levels was observed between the initial value on GD127, and the final values on GD150 (Group 1) and GD169 (Group 2). Following delivery, trastuzumab levels were still present in serum from nursing dams up until PPD28, at approximately similar levels to those observed during gestation (data not shown). The mean values for each group (± S.D.) are presented in Table 3 below, which was abstracted from the final study report.

Table 3. Summary of Mean (± S.D.) Maternal Serum Trastuzumab Levels in Pregnant Cynomolgus Monkeys Following Dosing with 25 mg/kg/dose from GD120 through Delivery

Day of Gestation	Group 1 Cesarean Section (n = 7 - 8) [a]	Group 2 Live Birth (n = 1 - 8)
Predose	0.00 [b]	0.00
120	0.00	0.00
127	1035 (201)	1065 (206)
134	1019 (355)	1220 (265)
141	938 (250)	1439 (348)
148	1746 (1029)	1327 (261)
150 ± 1	1487 (839)	– [c]
155	– [c]	1513 (399)
162	– [c]	1589 (473)
163	– [c]	1572 [d]
169	– [c]	1409 (42.0)
176	– [c]	906 [d]

a Each group contained eight animals, but for some time points samples were available from less than all eight animals.

b For predose and Day 120 samples all concentrations were 'lts' (less than the lowest standard on the standard curve, ie. less than 0.078 mcg/mL); they were therefore assigned a value of zero by convention and no standard deviation was calculated.

c No sample scheduled to be drawn at this time point for this group.

d Only one animal sampled at this time point; therefore no standard deviation was calculated.

Placental transfer of trastuzumab, with subsequent fetal exposure was also detected in this study. Table 4 below, from the sponsor's final study report shows that at time of Caesarean section on GD150, trastuzumab was present at detectable levels in both serum and amniotic fluid from of dams treated with 25 mg/kg/dose. The levels of trastuzumab in fetal blood and amniotic fluid were approximately 33% and 25%, respectively, of those present in the maternal serum at GD150, and the amniotic fluid trastuzumab levels were approximately 82% of that present in fetal serum. These results are summarized in Table 4, below.

Transplacental transfer of trastuzumab from dams treated with 25 mg/kg/dose was also evident in infants delivered at term. Mean values for neonatal serum trastuzumab levels were approximately 19% of those present in the dams at time of delivery. Low levels of trastuzumab were also detectable in maternal milk in 5 of the 6 lactating dams, with a mean value of approximately 0.3% of the maternal serum levels and <1% of the neonatal serum levels. These results are presented in Table 4, from the sponsor's final study report.

Table 4. Mean Maternal, Fetal, and Neonatal Serum Levels, and Fetal Amniotic Fluid, and Maternal Milk Levels of Trastuzumab Following Dosing of Cynomolgus Monkeys from GD120 Through GD150 (Group 1) or PPD28 (Group 2)

Parameter	Group 1 Cesarean Section Day 150 ± 1 (n = 7) [a]	Group 2 Live Birth Day 28 Post-Partum (n = 4 - 5) [a]
Concentrations (mcg/mL):		
Maternal Serum [b]	1487 (839)	1544 (862)
Fetal Serum [c]	411 (74.8)	– [e]
Amniotic Fluid [c]	305 (191)	– [e]
Maternal Milk [d]	– [e]	2.73 (1.12)
Neonate Serum [d]	– [e]	376 (213)
Concentration Ratios:		
Fetal / Maternal Serum	0.325 (0.110)	– [e]
Amniotic Fluid / Maternal Serum	0.249 (0.196)	– [e]
Amniotic Fluid / Fetal Serum	0.818 (0.571)	– [e]
Maternal Milk / Maternal Serum	– [e]	0.00280 (0.00269)
Neonate Serum / Maternal Serum	– [e]	0.191 (0.0695)
Maternal Milk / Neonate Serum	– [e]	0.00898 (0.00295)
Neonate Serum / Maternal Milk	– [e]	122 (42.3)

a Each group contained eight animals, but for some time points samples were available from less than all eight animals. For Group 1 animal no. 5383 did not produce a viable fetus; for Group 2 animals no. 1791 and 3376 did not produce a live birth. For Group 2 maternal milk concentration for animal no. 9536 was excluded, and neonate serum concentrations from the neonates of animals no. 6039 and 9536 were excluded. See exclusion listing 3-1 for details.

b Maternal serum reported here was collected on Day 150 ± 1 of gestation for Group 1 and on Day 28 post-partum for Group 2.

c Fetal serum and Amniotic fluid were collected on Day 150 ± 1 of gestation for Group 1; no fetus sampling scheduled for Group 2.

d Maternal milk and neonate serum were collected on Day 28 post-partum for Group 2; no live births scheduled for Group 1 and therefore no maternal milk or neonate serum were collected.

e No sample(s) scheduled to be collected for this group.

In summary, weekly i/v injections of trastuzumab to pregnant cynomolgus monkeys during late gestation through parturition and/or lactation did not result in detectable maternal, fetal, or neonatal toxicity. Transplacental transfer of trastuzumab was observed, with the levels in fetal serum approximately equal to one-third the levels present in maternal blood. Low, but detectable levels of trastuzumab were also present in milk from lactating dams, although the amount transferred to the offspring through milk could not be determined.

In conclusion, the results of this study support the reported findings in Section 8.1, *Teratogenic Effects* and Section 8.2, *Nursing Mothers* of the revised, PLR formatted label for trastuzumab (Herceptin®).

Section 13.1 – NONCLINICAL TOXICOLOGY, Impairment of Fertility

3) Study #95-038-1450. GN1450. Intravenous menstrual cycle study (fertility evaluation) in the female cynomolgus monkey.

The effects of trastuzumab treatment on female sex hormone levels and menstrual cycle duration were evaluated in cynomolgus monkeys. Six animals per group were treated for 3 menstrual cycles with vehicle control (GN1450 vehicle, composition not specified), or 1, 5, or 25 mg/kg/dose trastuzumab i/v, starting on Days 1-4 of treatment cycle 1, then twice weekly for the remainder of the treatment period. Three observation cycles were monitored for each animal prior to initiating treatment, and animals were also monitored for one recovery cycle following completion of treatment. Serum samples for measurement of progesterone, 17-β estradiol, and luteinizing hormone were obtained on Days 1, 4, 7, 10, 11, 12, 13, 14, 15, 18, 21, 24, and 27 of cycles 3, 4, 5, 6 and 7. Samples for toxicokinetic evaluation of serum trastuzumab levels were obtained once pre-treatment, monthly during the treatment cycles, and once during the second week of the recovery period.

There were no effects of trastuzumab treatment on clinical signs of toxicity, body weight, food consumption, clinical chemistry or urinalysis profiles. Trastuzumab treatment had no remarkable effects on the length of menstrual cycles, or the levels of, or timing to peak levels of the three female sex hormones measured. Toxicokinetic evaluation confirmed that exposure to trastuzumab was maintained over the duration of the three treatment cycles, and declined between the Day 64 (treatment cycle 6) and Day 98 (recovery cycle 7 samples. Mean trastuzumab serum concentrations for animals in the 1, 5, and 25 mg/kg/dose groups were 38, 205, and 1137 mcg/ml, respectively on sampling Day 4 (of Cycle 4). A slight increase in mean serum levels to 61, 360, and 1985 mcg/ml was observed for the three respective dose groups on Day 64, which then declined to 28, 308, and 1106 mcg/ml for the 1, 5, and 25 mg/kg/dose groups, respectively, at the end of the recovery cycle on Day 98. There was no anti-trastuzumab antibody detected by ELISA in any of the treated monkeys at any time point on study.

In conclusion, the results of this study demonstrate no adverse effects of trastuzumab treatment on parameters associated with female fertility, including menstrual cycle duration and sex hormone levels, at weekly doses of up to 25-fold greater than the human recommended dose of 2 mg/kg/week. These data support the labeling claims made by the sponsor in Section 13.2 of the PLR-formatted label, “Impairment of Fertility.”

Comment: For PLR labeling, data should be included from evaluation of both male and female fertility parameters. The sponsor was requested to provide information regarding measurement of male fertility (*i.e.*, sperm counts, viability, motility, testicular and epididymal histopathology). In a follow-up electronic mail message on January 11, 2008, the sponsor confirmed that male fertility parameters following trastuzumab treatment have not been evaluated. The licensed indication for trastuzumab is advanced breast cancer which is a disease predominantly of women, although occasional cases are reported in men. The lack of data regarding the effects of trastuzumab on male fertility will be addressed in Section 13.2 of the label. Please see “Suggested Language for Labeling,” below.

SUGGESTED LANGUAGE FOR LABELING:

All labeling revisions to Sections 5.6, *Embryo-fetal Toxicity (Pregnancy Category D)*, 8.1, *Pregnancy* (*Teratogenic Effects: Pregnancy Category D)*, and 8.3, *Nursing Mothers* have been completed and accepted by the sponsor. The following language is suggested for incorporation into Section 13.2, *Impairment of Fertility*, regarding the effects of trastuzumab treatment on parameters associated with female fertility, and the lack of data to address the effects of trastuzumab on male fertility.

Current language in label:

(b) (4)

Suggested revisions to current language:

A fertility study conducted in female cynomolgus monkeys at doses up to 25 times the weekly recommended human dose of 2 mg/kg trastuzumab revealed no evidence of impaired fertility, as measured by menstrual cycle duration and female sex hormone levels. Studies to evaluate the effects of trastuzumab on male fertility have not been conducted.

Addendum:

The above suggested language was conveyed to Genentech on January 14, 2008 and accepted for incorporation into the PLR label on January 15, 2008. No further actions are required from pharmacology/toxicology to support the approval of this supplement.

[here ends Dr. Pilaro's review]

11 Integrated Summary and Safety Evaluation

Mechanism of Action

S.M. Kupchan *et al.* of the Department of Chemistry at the University of Virginia originally isolated the ansa macrolide, maytansine, in an alcoholic extract of ***Maytenus*** *ouatus* Loes, a plant native to Ethiopia and Kenya.[26] These researchers identified the structure of the compound and determined that it was cytotoxic *in vitro* and *in vivo*. Researcher from this group eventually demonstrated that this class of compounds inhibits the polymerization of tubulin.[27] They also determined the structure activity relationship of the class.[28] The maytansinoids are isolated from members of the higher plant families *Celastraceae*, *Rhamnaceae*, and

[26] S.M. Kupchan et al. Maytansine, a Novel Antileukemic Ansa Macrolide from *Maytenus ovatus. J. Am. Chem. Soc.* **94**(4):1354-1356.
[27] S. Remillard et al. Antimitotic Activity of the Potent Tumor Inhibitor Maytansine. *Science*, 19 September 1975, **189**:1002-1005.
[28] S.M. Kupchan *et al.* Structural requirements for antileukemic activity among the naturally occurring and semisynthetic maytansinoids. J Med Chem. 1978 Jan;21(1):31-7.

Euphorbiaceae, as well as some mosses. There are 19-membered macrocyclic lactams related to ansamycin antibiotics of microbial origin, such as rifamycin B and geldanamycin.[29]

In 1980, K.A. Krolik *et al*. demonstrated the practicability of coupling a cytotoxin to an antibody to affect the destruction of specific cell types.[30] R.V. Chari *et al*. appear to have done the initial synthesis of DM1 from Ansamitocin P-3, or maytansinol isobutyrate (the reprint that the Applicant supplied is somewhat illegible).[31] These investigators also bound this compound covalently to mouse HER2/neu murine antibodies and demonstrated the cytotoxicity of the antibody-drug combination *in vitro*.

Human epidermal growth factor receptor 2 (HER2), also known as Neu, Erb2, CD340 (cluster of differentiation 340) or P185, is a transmembrane protein and member of the epidermal growth factor family (EGFR/ErbB or HER 1 through 4). The HER family of proteins is so named because of their homology with the avian erythroblastoma viral gene product, *v-erbB,* an oncogene later found to code for EGFR (HER1). The name HER2 derives from the protein's similarity to human epidermal growth factor receptor, or HER1. The name neu is derived from the fact that the protein was isolated from a rodent glioblastoma cell line, a neural tumor. Gene cloning showed that HER2, Neu, and ErbB-2 are all encoded by the same gene. The protein has an intracellular domain of 580 amino acids that has an intracellular tyrosine kinase domain with a regulatory carboxyl-terminal segment. The lipophilic transmembrane domain is only 22 amino acids while the extracellular domain is 632 amino acids.[32]

The oncogene that encodes for HER2 was initially discovered and sequenced by L. Coussens et al.[33] in 1985 and further characterized by M. Tal *et al*. two years later.[34] The HER2 promoter is different from the promoter of the epidermal growth factor receptor gene, and the GC boxes which are typical of the promoter of the epidermal growth factor receptor gene are absent from the HER2 promoter. HER2 is encoded by *ERBB2*, a known proto-oncogene located at the long arm of human chromosome 17 (17q12).

Binding of a ligand to the extracellular region of HER1, 3 or HER 4 induces receptor dimerization or heterodimerization with any one of the other members of the family and subsequent activation of the cytoplasmic kinase activity of the molecules, which in turn leads to autophosphorylation and initiation of downstream signaling events.[35] HER1 and HER4 function as receptor tyrosine kinases (RTK) when dimerized or hetero-dimerized. The HER2 dimer is not an active tyrosine kinase but when it forms a heterodimer with any one of the other members its kinase activity manifests. There are at least 13 known polypeptide extra cellular ligands, which contain a conserved epidermal growth factor (EGF) domain. Nevertheless, HER2 has no known extracellular ligand and HER3 does not have a functional intracellular kinase domain.[36] HER2 is the only member of the family that can initiate mitotic activation

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[29] K.L. Rinehart Jr, L.S. Shield, Chemistry of the Ansamycin Antibiotics. Fortschr Chem Org Naturst. 1976;33:231-307.

[30] K. A. Krolick, K.A., Specific Killing of Normal or Neoplastic B Cells by Antibodies coupled to the A Chain of Ricin, *Proc. Natl. Acad. Sci.,* **77**:5419,1980.

[31] R. V. J. Chari et al. Immunoconjugates Containing Novel Maytansinoids: Promising Anticancer Drugs. Cancer Research, 52:127-131, January 1. 1992

[32] Y. Yarden. Biology of HER2 and Its Importance in Breast Cancer, Oncology 2001;61(suppl 2):1–13

[33] Lisa Coussens et al., Tyrosine Kinase Receptor with Extensive Homology to EGF Receptor Shares Chromosomal Location with neu Oncogene, Science, December 6, 1985, 230:1132-1139.

[34] M. Tal et al., Human HER2 (neu) promoter: evidence for multiple mechanisms for transcriptional initiation. Molecular and Cellular Biology, 1987, 7(7):2597-2601.

[35] Y. Yarden, M.X. Sliwkowski. Untangling the ErbB signaling network. *Nature Rev. Mol. Cell Biol.* **2**;127–137 (2001).

[36] L.N. Klapper *et al.* The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. *Proc. Natl Acad. Sci. USA* **96**, 4995–5000 (1999).

absent ligand binding when over-expressed. It is probably the preferred hetero-dimerization partner of the other HER receptors.[37] The following diagram by Klapper *et al.* (reproduced in Yarden 2001) shows the combinations of the HER receptors and the manifestations of their signals.[38]

Fig. 7. The HER signaling network. The layered structure of the HER signaling network is presented. Numbers refer to the four HER proteins. Note that HER3 carries an inactive catalytic domain. (Reproduced with permission from Klapper et al. 2000.)

Ami Citri and Yosef Yarden "regard ERBB2 as a non-autonomous amplifier of the network, rather than an additional growth-factor receptor."[39] The following figure from this elegant work provides a schematic representation of the complex network formed by the HER family of signal transducers.

[37] M.A. Olayioye. Update on HER-2 as a target for cancer therapy: Intracellular signaling pathways of ErbB2/HER-2 and family members. Breat Cancer Res. 2001:3(6);385-389.
[38] L.N. Klapper LN, Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors; in Klein G, Woude V (eds): Advances in Cancer Research. Academic Press, 2000, pp 25–79.
[39] A. Citri and Y. Yarden. EGF–ERBB signaling: towards the systems level. Nature Review, Molecular Cell Biology, July 2006, **7**;505-516.

As mentioned above, HER2 does not bind to any ligands, but it is the favored heterodimerization partner of the other ERBB receptors. Binding to a ligand is not necessary for this protein because its extracellular domain has a structure that resembles the ligand-bound form of HER1.[40] Domains I and II of HER2 interact strongly such that the dimerization arm is extended. This renders HER2 incapable of binding a ligand. Thus, HER2 is inherently configured for dimerization with ligand bound receptors of the family. The following figure from Citri and Yarden illustrates this configuration and its similarity to HER1.

[40] T.P.J Garrett et al., The Crystal Structure of a Truncated ErbB2 Ectodomain Reveals an Active Conformation, Poised to Interact with Other ErbB Receptors. Molecular Cell, Vol. 11, 495–505, February, 2003,

In 20% to 25% of breast cancers, the HER2 gene is amplified resulting in overexpression of the gene product. This amplification is associated with metastatic disease and decreased survival.[41,42,43] Trastuzumab is a humanized mouse anti–HER2 IgG1 antibody that binds to the juxta-membrane region of the HER2 extracellular domain.[44] The US Food and Drug Administration approved trastuzumab for the treatment of breast cancer in 1998. C.A. Hudis has reviewed the clinical experience with trastuzumab.[45] The binding of trastuzumab to HER2 prevents dimerization with other members of the HER family, thus preventing auto-phosphorylation and the attendant kinase activity. This inhibition of dimerization is probably accomplished through simple steric hindrance. The inhibition of auto-phosphorylation prevents the initiation of the downstream mitogenic signal.

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[41] S. Verma et al. N. Eng. J. Med. November 8, 2012. 367(19);1783-1791.
[42] M.D. Pegram et al., HER-2/neu as a predictive marker of response to breast cancer therapy. Breast Cancer Res Treat. 1998;52(1-3):65-77.
[43] D.J. Slamon et al., Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. 1987, Science 235:177-182.
[44] H-S Cho et al., Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Nature, Feb 13, 2003, 421:756-759.
[45] C.A. Hudis. Trastuzumab — Mechanism of Action and Use in Clinical Practice. N. Engl. J. Med., 2007, **357**:39-51.

Trastuzumab Emtansine (TDM1, or trastuzumab-MCC-DM1) is the trastuzumab antibody covalently bound to DM1. In TDM1, this antibody is linked to a hetero-bifunctional reagent, succinimidyl trans-4-[maleimidylmethyl] cyclohexane-1-carboxylate (SMCC). The other end of the SMCC linker molecule is covalently bound to DM1 by a labile thioether bond. The antibody binds to the linker predominantly at lysine residues with a net stoichiometry of DM1 to antibody of approximately 3.5. The resulting compound is referred to as an antibody drug conjugate or ADC. The covalent bonds adhering the DM1 to the linker and thence to the antibody do not involve π electron conjugation.

DM1 and other maytansinoids derivatives are as much as 100-fold more cytotoxic than vinca alkaloids and taxanes.[46] Trastuzumab emtansine binds to HER2 with an affinity similar to that of trastuzumab (*vide infra*). When trastuzumab emtansine binds to HER2, the damaged receptor protein is invaginated into the cell by endocytosis. Following internalization, the intracellular lysosomes degrade and release DM1-containing cytotoxic catabolites into the cell.[47, 48] The Applicant hypothesizes that the released DM1 then binds to tubulin and disrupts the microtubule network, resulting in inhibition of cell division. This inhibition eventually causes cell death via apoptosis. The Applicant further postulates that the combined actions of trastuzumab inhibiting the cancer cell mitogenic signals and DM1 cytotoxicity will affect a greater degree of clinical benefit that trastuzumab alone.

Pharmacology

The Applicant compared the binding of the trastuzumab emtansine to the extracellular domain of HER2 with that of trastuzumab and determined that they were approximately equivalent. TDM1 bound to the extracellular domain of HER2 with an EC_{50} of 170 pM while trastuzumab bound to the same site with an EC_{50} of 155 pM. Binding of trastuzumab emtansine to the high-affinity Fc gamma (Fcγ) cell surface receptor RIa (IC_{50} = 0.39 nM) was comparable with that observed for trastuzumab (IC_{50} = 0.038 nM). Likewise binding to the complement component C1q was comparable but of relatively low affinity. Binding of trastuzumab emtansine to low-affinity Fcγ receptors was somewhat more avid than that of trastuzumab.

Trastuzumab-MCC-DM1 induced increasing caspase-3 and -7 activation with increasing dose in SK-BR-3 and BT-474 cancer cell lines *in vitro*. The EC_{50} in SK-BR-3 cells was 0.028 μg/mL (~ 0.19 nM) and the EC_{50} in BT-474 cells was approximately 2 μg/mL (13 nM). Trastuzumab did not induce caspase-3 and -7 activation in any of these cell lines. These data suggest that, after internalization, DM-1 causes sufficient cell damage to initiate apoptosis in these cancer cell types *in vitro*.

The Applicant also evaluated the effect of trastuzumab emtansine on human tumor cell lines representing human ductal breast cancer, ovarian, gastric, or non-small cell lung cancer (NSCLC) and expressing varying amounts of HER2 *in vitro*. Cellular proliferation, cell viability, and activation of caspase-3 and -7 were assessed in cells cultured in the presence of increasing concentrations of trastuzumab emtansine, trastuzumab, free (unbound) DM1, or free MCC-DM1. Trastuzumab-MCC-DM1 caused cytotoxicity, decreased cellular proliferation, and activated effector caspase-3 and -7. These changes were not observed with trastuzumab, which inhibits cellular proliferation, but is not directly cytotoxic. Trastuzumab-MCC-DM1 toxicity

[46] Kovtun YV, Audette CA, Mayo MF, et al. Antibody-maytansinoid conjugates designed to bypass multidrug resistance. Cancer Res 2010, **70**(6):2528−37.

[47] Erickson HK, Park PU, Widdison WC, et al. Antibody-maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing. Cancer Res 2006, **66**(8):4426−33.

[48] Erickson HK, Widdison WC, Mayo MF, et al. Tumor delivery and in vivo processing of disulfide-linked and thioether-linked antibody-maytansinoid conjugates. Bioconjug Chem 2010, **21**(1):84−92.

increased with dose in cells expressing high levels of HER2. Trastuzumab-MCC-DM1 was cytotoxic in both trastuzumab-sensitive and resistant cell lines. In BT-474 cells, MCC-DM1 was 100 fold less toxic than trastuzumab emtansine and a factor of 4 fold less toxic than free DM1. In SKBR3 cells, MCC-DM1 was 10 fold less toxic than DM1. Using flow cytometry with HER2 positive breast cancer cells, experiments confirmed trastuzumab alone stopped cell division predominantly at G_0/G_1. DM1 binds β-tubulin and these experiments confirmed that cell cycle arrest with this compound was predominantly at G_2/M. Low dose trastuzumab emtansine caused cell cycle arrest predominantly at G_0/G_1, while at higher doses it stopped division at G_2/M thereby demonstrating that the trastuzumab emtansine has the mechanisms of both toxins.

In vitro binding experiments showed that the average equilibrium binding constant, K_D, for the binding of TDM1 to HER2 was 1.08 nM for T-DM1. The K_D for trastuzumab was very similar, 1.01 nM. HER2 ECD bound to T-DM1 lots with average k_a and k_d values of $2.07*10^5$ 1/Ms and $2.23*10^{-4}$ 1/s, respectively. The same protein bound to trastuzumab with average k_a $2.16*10^5$ 1/Ms and k_d of $2.21*10^{-4}$ 1/s. C_{max} is about 85 μg/mL in humans or about 537 nM, thus K_D is well in excess of the concentration of trastuzumab emtansine available *in vivo* in humans. All available HER2 sites should be titrated and should remain so until replaced by *de novo* synthesis.

Anti-tumor activity of trastuzumab emtansine was evaluated in three independent animal models of HER2-positive human cancers (not formally reviewed). Two of these are xenograft models using human tumor cell lines. The BT-474 EI model is a trastuzumab-resistant, estrogen-independent subline of the HER2-positive breast cancer cell line BT-474. The CaLu3 cell line is a HER2-positive NSCLC cell line that is sensitive to high doses of trastuzumab. The third model is a trastuzumab-resistant transgenic mouse model in which the human HER2 gene under transcriptional regulation of the murine mammary tumor virus promoter is overexpressed in mammary epithelium, causing spontaneous development of mammary tumors. These tumors overexpress the human HER2 receptor. The mammary tumor of one of these founder animals (founder NO. 5 (F05)) was propagated in subsequent generations of FVB mice or athymic nude mice by serial transplantation of tumor fragments. Trastuzumab-MCC-DM1 caused tumor growth inhibition in all three models in the dose range of 3 to 31 mg/kg, depending on the model. In the BT-474 EI breast cancer model, significant inhibition of tumor growth was seen at doses of 3, 10, and 15 mg/kg given once every 3 weeks for a total of three doses; 10% and 60% of the animals showed complete responses at 10 and 15 mg/kg, respectively. Trastuzumab alone showed minimal tumor growth inhibition and no complete or partial responses at 15 mg/kg. In the F05 model, complete responses with trastuzumab emtansine were observed in 13%, 38%, and 75% of animals when dosed once every 3 weeks for a total of three doses (similar to the proposed clinical schedule) at 10, 15, and 30 mg/kg, respectively. Eight of 9 animals with established CaLu3 tumors that received trastuzumab emtansine at 10 or 31 mg/kg once every 3 weeks for a total of three doses showed complete responses, compared with 2 of 9 and 0 of 9 animals that received trastuzumab or vehicle, respectively. No anti-tumor activity was observed in studies using trastuzumab emtansine against a HER2-negative tumor or a control ADC against F05 tumors.

In experiments designed to demonstrated diminution of cell growth in breast cancer cells, trastuzumab emtansine was effective at considerably lower concentrations than trastuzumab (e.g. IC_{50} 0.04 nM for trastuzumab emtansine and 1.68 nM in BR-474 cells) in cells that overexpress HER2. Nevertheless, while trastuzumab emtansine was effective in all cell lines overexpressing HER2, trastuzumab was not. This suggests that the mechanism for inhibition of cell growth involves factors other than simple overexpression of HER2. Again in this set of experiments, trastuzumab emtansine induced apoptosis as evidenced by caspase activation, while trastuzumab did not. When the individual components of trastuzumab

emtansine and its known *in vivo* metabolites were tested for cytotoxicity, only trastuzumab emtansine and unbound DM1 caused significant cytotoxicity.

In a second set of experiments to determine cell cycle arrest, trastuzumab emtansine or DM1 again caused accumulation at the G_2/M in trastuzumab sensitive and to a lesser degree in insensitive cells. Trastuzumab caused accumulation predominantly in G_0/G_1. Binding of both trastuzumab emtansine and trastuzumab to BT-474-M1 cells caused a rapid dose dependant but incomplete decrease in phosphorylation of AKT. The response of other cellular biomarkers (XIAP, pH3, and PARP) again suggested that both DM1 and trastuzumab emtansine induce apoptosis. The inhibition of AKT phosphorylation in cells not sensitive to trastuzumab is probably a feedback response after significant damage has already been done to the cell.

A further set of experiments demonstrated that the binding of trastuzumab or trastuzumab emtansine diminished the extent of shedding of the extracellular domain of HER2 from the cell surface *in vitro*. Maximal inhibition of HER2-ECD shedding was 42% with trastuzumab exposure and 43% with trastuzumab emtansine exposure. The IC_{50} values for the antibodies were 0.30 ± 0.05 µg/mL (about 2 nM) and 0.18 ± 0.04 µg/mL (about 1.2 nM), respectively. The evidence that this inhibition contributes to the mechanism of trastuzumab emtansine is incomplete and somewhat contradictory.

Safety Pharmacology

In a standard hERG assay *in vitro*, DM1 inhibited cardiac slow rectifier potassium current by 0.3 % at 2.6 µM, 1.0 % at 8.8 µM and 2.5 % at 29.5 µM. The IC_{20} and IC_{50} for the inhibitory effect of DM1 on hERG potassium current could not be determined because the hERG inhibition was so slight. The C_{max} for DM1 in humans after a dose of 3.6 mg/kg is about 5 ng/mL or about 7 nM. DM1 will not inhibit the slow rectifier current as physiologically relevant concentrations. In monkeys fitted with telemetric cardiac monitors, a single dose of 3, 10 or 30 mg/kg of TDM1 did not cause an increase in plasma troponin. Nor did this dose cause any toxicologically significant changes in the electrocardiogram of these animals. Dosing was associated with an increase in aortic systolic and diastolic pressure that while not statistically significant appeared to be dose dependent when viewed graphically. The effect persisted from about day three after dosing to day 21 the end of monitoring. Respiration was greater in HD animals than in controls, but again this did not reach statistical significance. There were no discernible differences in other cardiac parameters. These effects are probably toxicologically significant, but the dose at which they became apparent is 8 fold greater than the human dose on a mg/kg basis.
An antibody staining study investigating the binding of TDM1 to both human and monkey tissues obtained post-mortem demonstrated the distribution of the HER2 receptor. The ADC bound to both granular membrane and elements of the cytoplasm depending on the cell type. In epithelial cells binding was primarily at the membrane while in cells identified by the investigators as spindle cells which they presume to be Schwann cells staining was primarily in the cytoplasm. Glial and mononuclear cells were stained in both the membrane and membrane and the cytoplasm. Epithelial and glial cell staining was greater in intensity than spindle cell staining. Positive staining epithelial cells were found in the urinary bladder and ureter (urothelium), mammary gland (duct and gland epithelium), colon (mucosal epithelium), fallopian tubes (mucosal epithelium), eye (conjunctiva, ciliary body, cornea and retinal epithelium), pituitary (subset of cells in pars distalis and intermedia), thyroid (follicular epithelium), parathyroid (epithelium, chief cells), kidney (convoluted and collecting duct tubule epithelium), small intestine, (mucosal epithelium), exocrine pancreas (duct epithelium), lung (bronchiolar epithelium and Type 1 pneumocytes), ovary (follicular and surface epithelium), prostate (glandular epithelium), liver (hepatocytes and bile ducts), skin (epidermis, hair follicles, apocrine and sebaceous glands), testes (duct epithelium), thymus (epithelium), tonsil (mucosal

epithelium), cervix (mucosal epithelium) and uterus (mucosal and glandular epithelium). Ductal and apocrine gland epithelium stained with the greatest intensity and squamous epithelium with the least. Basal and basolateral cell surfaces stained more intensely than the apical cell surface. Cells identified as neural Schwann cells were stained in numerous tissues including connective tissue stroma of the adrenal gland, urinary bladder, colon, gastrointestinal tract, heart, cerebellum, cerebral cortex, kidney, liver, lung, ovary, pancreas, pituitary, lymph node, prostate, skin, spleen, skeletal muscle, testes, thymus, thyroid, tonsil, ureter, cervix and endometrium. Positive spindle cells were closely associated with peripheral nerve bundles and ganglia, located in the perineurium and epineurium. Positive staining glial cells were identified in the cerebellum, cerebral cortex, pituitary gland (pars nervosa) and spinal cord (white and gray matter). Binding in monkey tissues was similarly distributed by less intense.
In a study with blood from cynomolgus monkeys and a single human volunteer TDM1 did not cause hemolysis in either cynomolgus monkey or human blood and was compatible with cynomolgus monkey and human serum and plasma.

Pharmacokinetics and Toxicokinetics

Absorption

The elimination of trastuzumab emtansine from the plasma of mouse and rat is bi-exponential, with a terminal half-life of 0.9-6 days, volume of distribution of 40-50 mL/kg, and plasma clearance that decreases with increasing animal size (10-40 mL/day/kg)(not formally reviewed). The plasma clearance of trastuzumab emtansine was proportional to dose in rodents, but not proportional to dose in the cynomolgus monkey. It ranged from 41.6 mL/day/kg at 0.3 mg/kg to 10.1 mL/day/kg at 30 mg/kg. Clearance of trastuzumab emtansine was approximately 2-2.5 times faster and the half-life was about 50% less than that of total trastuzumab. The volume of distribution was similar for the two drugs and was about equal to plasma volume. The presence of a HER2-positive tumor xenograft (BT-474 EI) in mice did not appreciably change the PK characteristics of the ADC or total trastuzumab.

Distribution

In a plasma protein binding study, 92.5% of DM1 bound in human plasma, 91.5% bound in monkey plasma, and 97.1% bound in rat plasma. Binding did not vary with concentration over a range of 20 to 1000 ng/mL in any species.

Metabolism

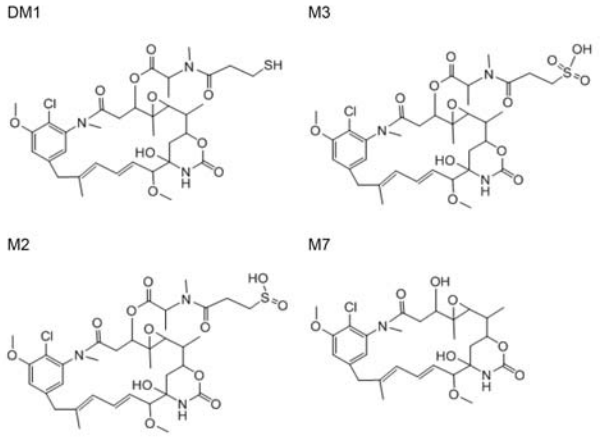
When human hepatocytes were treated with DM1, the cells maintained their viability during the assay at concentrations up to 1.0 μM for 48 hours. DM1 did not induce cytochromes P450 1A2, 2B6 or 3A4 or 5. The lack of induction potential indicates that DM1 at concentrations up to 1.0 μM will probably not interact with pregnane X receptor, constitutive androstane receptor, or the Ah receptor.

DM-1, in the presence of NADPH-generating system, inhibited cytochrome P450 1A2, cytochrome P450 2B6, cytochrome P450 2C8, cytochrome P450 2C9, cytochrome P450 2C19, cytochrome P450 2D6 and cytochrome P450 3A4 catalytic activities by less than 50 % at the highest concentration (678 nM; 500 ng/mL) examined. Pre-incubation of DM-1 for 30 min with human liver microsomes, in the presence of NADPH, resulted in inhibition of cytochrome P450 3A4 (midazolam 1'hydroxylase) activity with an IC_{50} value of 155 nM (114 ng/mL). These results suggest that DM-1 did not cause reversible inhibition at the concentrations examined, however, it may be a potential irreversible inhibitor of cytochrome P450 3A4.

Over the course of a 60 minute assay, human liver microsomes metabolize 73% of a DM1 dose in the presence of an NADPH regenerating system. In the absence of NADPH only 18% was metabolized. Studies with specific inhibitors suggested that this activity was associated with cytochromes P450 3A4 and 3A5. Studies with human recombinant cytochromes P450 corroborate the results with HLM, again showing that DM1 is metabolized by cytochromes P450 3A4 and 3A5 but also possibly to a lesser extent 1A2, 2C9 and 2E1. This study identified the following metabolites by mass spectroscopy. M3 and M2 should be more soluble than the parent compound. M7 may be less soluble and may be active.

Figure 1

Structure of DM1 and Proposed Structures of Its Metabolites



In a series of standard *in vitro* assays to determine the interaction of DM1 with MDR1 (p-glycoprotein), DM1 was a substrate for MDR1 membrane transport, but that it does not appear to inhibit the transport of other substrates.

Elimination

After rats were treated with a single dose of 10 mg/kg of TDM1 radiolabeled on the DM1 moiety, almost 100% of the radioactivity precipitated when the plasma was treated with acetone. In cannulated rats receiving this dose, 51% of the radiolabel was collected in the bile after seven days, less than 5% was recovered in the feces. Most of the radiolabel in the bile was associated with the acetonitrile extracted fraction, 97% or greater suggesting that the DM1 moiety has been cleaved from the antibody. Consistent with this finding, intact TDM1 was not present in the bile. HPLC analysis of the bile showed only low molecular weight peaks some of which migrated with times similar to DM1. In non-cannulated rats, the amount of radioactivity found in the feces, 50%, was comparable to that found in the bile of cannulated rats as one would expect with IV dosing. In these non-cannulated rats, only 8% of the total radioactivity was in the cumulative urine after 168 hours. The investigators did not measure the radioactivity in the carcass, but the mechanism of action of DM1 suggests that much of the radiolabel, about 40%, remains bound to various intracellular sites after this relatively short study.

Toxicology

Single Dose Studies

DM1 in Rats

The Applicant evaluated the toxicity of free DM1 in two single-dose non-GLP studies in rats (not formally reviewed). Rats that got an IV dose of 0.16 mg/kg showed transient elevations of liver enzymes and thrombocytopenia (Study 03-0140-1345). This dose of DM1 is roughly equivalent to the amount of DM1 given as a 10 mg/kg dose of ADC, which has no effect on clinical pathology parameters in rats. A dose range-finding study (Study 05-1157) identified a single IV dose of 0.2 mg/kg free DM1 as maximum tolerated dose; single IV doses of 0.4, 0.6, 0.8, and 1.0 mg/kg were lethal. Animals in this study showed dose-dependent elevation of liver enzymes and decreased white blood cells and platelets.

In a GLP study, rats received single doses of 0.07, 0.1 or 0.2 mg/kg of unconjugated DM1 and were necropsied on day 3 or 21 (05-1191) not formally reviewed). Severe damage due to extravasation at the tail vein injection site gave cause for two animals to be terminated early but all other animals survived to scheduled necropsy with no significant outward signs of toxicity. Pathology seen on day 3 included dose related thrombocytopenia, and decreased reticulocyte counts in all treated animals. Mid and high dose animals had higher absolute neutrophil and lower lymphocyte counts. High dose animals had elevated urea nitrogen and cholesterol and lower total protein. AST and ALT was elevated in mid and high dose males and high dose females. At necropsy on day three, high dose males but not females had hepatomegaly which correlated with microscopic damage in the liver. At the recovery necropsy, mean relative liver weights (relative-to-terminal body and brain weight percentages) were increased in high dose females, but not males and there was no microscopic correlation. Low dose animals showed microscopic signs of toxicity in the liver, kidney, bone marrow of the sternum and femur in both sexes, the prostate and seminal vesicle of males, and the skin or subcutis of females. Mid dose animals had microscopic toxicity in the liver, kidney, bone marrow of the sternum and femur, adrenal cortex, thymus, biceps femoris muscle, mandibular salivary gland, skin or subcutis, and intravenous injection site of one or more animals of both sexes; the thyroid, rectum, eye, prostate, and seminal vesicle of one or more males; and the tongue of one female and the duodenum of a second female. High dose animals had microscopic sings of toxicity in the liver, kidney, adrenal cortex, pituitary, thyroid, tongue, biceps femoris muscle, spleen, thymus, duodenum, rectum, mandibular salivary gland, skin or subcutis, eye, bone marrow of the sternum and femur, and intravenous injection site of one or more animals of both sexes; the jejunum, prostate, and seminal vesicle of one or more males; and the mammary gland of one female. Findings in the liver included liver included periportal to midzonal hepatocellular degeneration and necrosis, increased mitoses in biliary epithelial cells and hepatocytes, hypertrophy and vacuolation of Kupffer cells, increased mitoses in Kupffer cells and degeneration and necrosis of biliary epithelial cells. Findings in the kidneys included tubular degeneration and necrosis and increased mitoses in renal tubular epithelial cells. In the bone marrow of the femur and sternum there was hypocellularity and hemorrhage. Numerous organs showed signs of dose dependent increased mitoses. The high dose of DM1 in this study is approximately equivalent to a dose of 10 mg/kg of TDM1 on a molar basis.

In a single dose non-GLP study (05-0775-1459, not formally reviewed), investigators gave rats doses of 83, 165, or 248 µg/kg of MCC-DM1, that is the linker bound to DM1 without the antibody. These three doses represent the scenario in which 6% of the total DM1 dose of 10, 20, or 30 mg/kg ADC is present in the dosing solutions as free MCC-DM1. All animals appeared healthy and normal throughout the study. Serum chemistry and hematology parameters remained within normal limits for all animals at all time points. None of the animals showed signs of drug related microscopic or gross toxicities.

TDM1 in rats

The Applicant provided a single-dose GLP study with a 3-week recovery period in rats (Study 04-1214-1459, not formally reviewed) to assess the non-pharmacological toxicity of

trastuzumab emtansine. The doses were 6, 20, and 60 mg/kg IV. The animals did not well tolerate the highest dose; all of the rats in this group were dead or moribund by day 6 after dosing. These rats showed signs of loss of body weight and elevated liver enzymes and peripheral granulocytosis and thrombocytopenia. One mid dose male died on day 5, but other than this mortality animals that received 6 or 20 mg/kg of trastuzumab emtansine showed no clinical signs of toxicity except weight loss on day 3 in mid-dose animals secondary to anorexia. Analysis on day three showed that the rats in the mid and high dose groups had elevated absolute neutrophil count and lower platelet count. High dose rats had lower absolute reticulocyte and lymphocyte counts. The mid-dose animals showed signs of transient elevation of liver enzymes and mild thrombocytopenia. Low dose rats of both sexes showed microscopic damage in the liver and females showed damage in the kidneys on day 3. Mid dose rats of both sexes showed microscopic damage in the liver, kidney, and spleen. Mid dose males showed damage in the thymus and mammary gland. Females showed signs of microscopic damage in the pituitary and bone marrow of the. On day three, the high dose animals showed signs of microscopic damage in the liver, kidney, spleen, adrenal cortex, adrenal medulla, pituitary, thyroid, heart, tongue, thymus, duodenum, eye, bone marrow of the femur, bone marrow of the sternum, skin, mammary gland, and intravenous injection site of both sexes and in the testis and epididymis of males. In high dose males, degeneration of seminiferous tubules and hemorrhage in the testes were correlated with increased organ weights of the testes and epididymides. High dose females that survived to day six showed signs of hemorrhage and necrosis of the corpus luteum in the ovaries. The low and mid dose animals showed no signs of gross or microscopic toxicity after recovery at necropsy on day 22.

TDM1 in monkeys

The Applicant did a single-dose GLP study with a 3-week recovery period in cynomolgus monkeys (Study 04-0976-1459). Trastuzumab-MCC-DM1 was given IV at doses of 3, 10, and 30 mg/kg. None of the animals showed any clinical signs or symptoms after dosing. Animals in the mid and high dose groups had dose dependent increases in AST, ALT and ALP and thrombocytopenia. These toxicities resolved before the end of the study. None of the animals in any of the three dose groups showed drug related gross or microscopic changes at necropsy after a 3-week recovery period. In a sub-group killed humanely 3 days after dosing, there were increases in the number of mitotic cells in liver, spleen and mammary gland. The Applicant attributed these changes to direct DM1 toxicity; I consider this probably correct. There was also a dose dependent increase in the incidence of inflammation in the liver, heart and brain of some animals. By day 21 the monkeys demonstrated recovery but there were still signs of inflammatory infiltrates in the liver of some monkeys.

In order to qualify a higher percentage of free DM1 in the drug product, the Applicant stored the drug used in this study from March to November of 2005. The percentage of free maytansinoids increased from (b) (4) over this interval. They then conducted a virtually identical single dose study in monkeys using the stored drug. Again there was no mortality and no clinical signs or symptoms associated with dosing. The mild anemia seen at day 22 is worse in the first study. The mild decrease in reticulocytes on day 3 is worse in the second study. The mild thrombocytopenia is about the same in both studies and is worse in males than in females. The increases in white cell parameters were comparable in the studies in females but were somewhat more severe in males in the second study. The increases in AST, ALT and ALP were somewhat more severe in the first study. The incidences of microscopic toxicities, particularly inflammation and arrested mitosis were similar in both studies and found in the same organs. The stored preparation of the drug appears to be somewhat less hepatotoxic than the drug substance with less free maytansinoids. This result is probably due to the pharmacology of the ADC.

Multiple Dose Studies

In an exploratory non-GLP study (04-0669-1459 not formally reviewed), rats received weekly IV injections of vehicle or trastuzumab emtansine at 10.45, 26.19, or 52.37 mg/kg for 3 weeks. Weekly administration of 52.37 mg/kg for 3 weeks caused severe morbidity and mortality 1-3 days after the final dose. Clinical pathology findings in this group included thrombocytopenia and elevations in liver enzymes. This dose caused microscopic pathology including prominent extramedullary hematopoiesis in spleen and liver, a mildly hypocellular bone marrow, and increased number of mitotically arrested cells in the liver. Rats tolerated weekly doses of 10.45 and 26.19 mg/kg for 3 weeks without evidence of overt toxicity or changes in microscopic pathology 4 days following administration of the final dose.
In the initial multi-dose non-GLP range finding study in monkeys, investigators gave doses of TDM1 equivalent to 4900 μg/m^2 of DM1 on days 1 and 22 and 7400 μg/m^2 DM1 on day 22. The DM1:antibody ratio of the drug used in this study was only 2.4, so these doses are equivalent to about 30 and 50 mg/kg TDM1 respectively. The investigators did not specify the dose in terms of TDM1 so the numbers above are approximate and factor in the drug antibody ratio of 2.4. In the other monkey studies using a DM1:antibody ratio of 3.8 these doses are about the same as doses of 20 and 30 mg/kg TDM1 based on the DM1 total dose. Monkeys receiving TDM1 became anorexic and had a concomitant decrease in body weight. Dosing was associated with a transient decline in white cell parameters and a toxicologically significant increase in AST as much as four fold in group 2 and 5.5 fold in group 3. ALT and LDH also increased but less dramatically. There were mild increases in the numbers of mitotic and apoptotic cells of the hepatic parenchyma.

The Applicant did a repeat-dose GLP toxicity study in Cynomolgus monkeys (Study 04-0977-1459). The investigators gave IV doses of trastuzumab emtansine every 3 weeks for four doses followed by varying recovery periods. The doses evaluated were 0, 3, 10, and 30 mg/kg. None of these animals showed outward signs of toxicity. The 10 and 30 mg/kg doses caused reversible elevations of AST and ALT, but not ALP. Microscopic examination 3 days after the last dose showed an increased number of mitotic cells in liver, spleen, and skin, as well as lymphoid atrophy in spleen and thymus. These findings were more pronounced in the 30 mg/kg group than in the 10 mg/kg group and were not seen in the 3 mg/kg group. At the 21-day post dosing recovery necropsy, increased numbers of mitotic cells in the liver and thymic atrophy remained in the 30 mg/kg group. The 10 and 30 mg/kg dose levels were associated with microscopic evidence of axonal degeneration in the sciatic nerve and in the dorsal funiculus of the spinal cord. These changes were seen in animals from the two higher dose groups at both necropsy times and were more pronounced in animals that received 30 mg/kg. This finding suggests the possibility of an irreversible or slowly reversible neurotoxicity. Neurological examinations during life did not predict this toxicity. Animals that received 3 mg/kg ADC did not show any histopathological changes at any of the three necropsy time-points. Based on overall tolerability and reversibility of findings, the Applicant considered 10 mg/kg of trastuzumab emtansine given as four injections to Cynomolgus monkeys every 3 weeks to be the highest non-severely toxic dose.

The Applicant did a second multidose toxicology study in Cynomolgus monkeys (study 07-0653). The animals received doses of TDM1 of 0 1, 3, or 10 mg/kg once every three weeks for 8 doses. Half the monkeys were allowed to recover for six weeks after the last dose. There were no toxicologically significant outward signs of toxicity in any dose group. There were mild transient decreases in RBC, hematocrit, and hemoglobin levels compared to baseline in all groups, including controls, at around 8 days after treatment that may have been due to the antibody conjugate vehicle. This decrease (~10%) was smaller in the high dose group animals. Similarly, there were increases (~50-80%) in reticuloctyes from on Days 4 and 8 in all dose

groups compared to baseline levels. These trends were also observed at 4 and 8 days following the final administration, though the reticulocyte response was less robust. At the high dose of 10 mg/kg there were decreases of ~30% observed in platelets compared to baseline at 4 to 8 days post dosing. Occasional transient increases during the dosing period (4-8 days post dosing Cycles 1 and 8) of monocytes, eosinophils and LUCs at the high dose for both males and females, may indicate some inflammation related to the drug product. Mild increases in total protein and globulin were apparent in the high dose group males and females throughout the dosing study. Increases in AST were present at all dose groups for both sexes and were higher in the week following dosing (Days 4, 8, and 151). In males, CK was also increased following dosing. These increases may indicate some hepatic or muscle or myocardial toxicity. Finally, there were mild increases in cholesterol in the high dose group males and females in the week following dosing. Microscopically there were dose related increases in the incidence of mitotic figures in the gall bladder, liver, spleen, lymph nodes, and injection site. There were signs of inflammation in the lungs (fibrosis and metaplasia), liver (Kupffer cell and Ito cells with vacuolation). In the kidney there were signs of tubular dilation and atrophy. There were signs of atrophy in the liver and bone marrow. In the spinal cord and the sciatic nerve and in neurons at the injection site there was axonal swelling or degeneration in some animals.

Genotoxicity

DM1 did not cause reverse mutations in the Ames assay. Nevertheless, in an *in* vivo bone marrow micronucleus assay in rats DM1 did cause a significant increase in the percentage of micronucleated PCE and a significant decrease in the PCE:NCE ratio. There was a distinct and significant dose response. The increase in micronucleated PCEs was as high or higher than that caused by the positive control. The high dose caused significant bone marrow toxicity at the 48 hour time point. Thus N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl) maytansine (DM1) is a clastogen.

In the multi-dose toxicology study above (07-0653) the investigators incorporated an analysis of bone marrow for the presence of micronuclei into the protocol to evaluate the clastogenic potential of TDM1. Bone marrow smears were prepared from all animals at the terminal sacrifice (Day 155), seven days after the final dose. The Applicant considered the assay valid because the toxicokinetic analysis confirmed the exposure of the bone marrow to the TDM1 during the 7-day period from the last dose to the bone marrow collection. Nevertheless, the bone marrow showed only minimal damage microscopically. There was a small decrease in the ratio of polychromatic erythrocytes to normochromatic erythrocytes in the bone marrow of male and female animals treated with T-DM1, suggesting some bone marrow toxicity. Overall numbers of micronucleated cells were small, however, these numbers were increased in all treatment groups for both males and females compared to the vehicle only control. The delay of when the bone marrow was harvested from these monkeys following the previous administration of trastuzumab emtansine (i.e., 7 days) may have contributed to the negative result. In addition, there was no binding of trastuzumab emtansine to bone marrow from human or Cynomolgus monkey in a tissue cross-reactivity assay, which suggests bone marrow cells may not internalize trastuzumab emtansine. The relative exposure to free DM1 in the plasma was low in these animals. The results of this assay are inconclusive.

Reproductive toxicity

After consultation with the FDA, the Applicant did not conduct new reproductive toxicity studies with trastuzumab emtansine. The small molecule component, DM1, is a microtubule inhibitor which is genotoxic. In addition, DM1 caused toxicity to rapidly dividing cells in toxicity

studies. Therefore, based on recommendations in ICH S9 reproductive and developmental toxicology studies were not needed.

Zagouri F, *et al*. have provided a review and meta-analysis of the current experience in women who are pregnant or become pregnant while being treated with trastuzumab. [49] They examined 17 reports with 18 pregnancies and 19 newborns. In these studies 55.6 % (10 of 18) of the women receiving trastuzumab had metastatic disease. The mean duration of trastuzumab administration was 14.8 weeks. Occurrence of oligohydramnios or anhydramnios (O/A) was the most common (61.1 %) adverse event and two of the women showed signs of cardiac toxicity. 73.3 % of pregnancies exposed to trastuzumab during the second or third trimester were complicated with O/A. The mean gestational age at delivery was 33.8 weeks, and the mean weight of babies at delivery was 2,261 gr. In 52.6 % of cases (10 of 19), a healthy neonate was born. At the long-term evaluation, all children without problems at birth were healthy after a median follow-up of 9 months. Four of the nine children facing troubles at birth were dead within an interval ranging between birth and 5.25 months. All children exposed to trastuzumab *in utero* exclusively in the first trimester were healthy at birth. The authors concluded that trastuzumab should not be administered during pregnancy. Nevertheless, they also concluded that women who become pregnant during trastuzumab treatment should stop trastuzumab treatment and continue the pregnancy if that is their desire. Trastuzumab and by implication trastuzumab emtansine are reproductive toxins in humans.

Toxicokinetics

Species		Human[1]		Monkey[2]		Monkey[2]		Monkey[2]		Rat[3]	
		TDM1	Total Trastuzumab	TDM1	Total Trastuzumab	TDM1	Total Trastuzumab	TDM1	Total Trastuzumab	TDM1	Total Trastuzumab
Dose mg/kg		3.6	3.6	3	3	10	10	30	30	6	6
Number of doses				4	4	4	4	4	4	1	1
Schedule		q3w	q3w	q3w	q3w	q3w	q3w	q3w	q3w	single	single dose
C_{max}	μM	0.57	0.60	0.52	0.54	1.78	1.81	5.25	5.44	1.11	1.03
AUC	μM*day	3.19	4.15	1.29	2.25	6.05	12.9	20.3	48.9	2.90	5.32
Clerance	mL/day/kg	7.1	4.7	15.7	9.2	11.1	5.4	9.9	4.4	13.65	7.67
Hepatic Flow[4]	mL/day/kg	29829		62784		62784		62784		79488	
V_1	ml/kg	33.3	41.4	39.0	37.6	37.3	37.5	38.0	37.5	35.6	39.5
Vss	mL/kg			57.5	70.5	62.7	78.9	69.0	73.1	77.6	100.0
Plasma Volume[4]		42.9	42.9	44.8	44.8	44.8	44.8	44.8	44.8	31.2	31.2
MRT	Days			3.7	7.7	5.7	15.1	7.1	17.8	5.7	13.2
$t_{1/2}\alpha$	Days			0.63	1.07	0.50	0.64	0.44	0.62	0.285	0.322
$t_{1/2}\beta$	Days	4.2	6.9	2.5	7.5	4.4	11.4	5.3	13.0	4.3	9.7

[1] Trial TDM4370g/ BO21977 cycle 4
[2] Multiple Dose Intravenous Toxicity Study in Cynomolgus Monkeys, Number 04-0977-1459
[3] Single Dose Intravenous Toxicity Study In Rats Number 04-1214-1459
[4] Brian Davies and Tim Morris, Pharmaceutical Research, Vol. 10, No. 7, 1993

In the case of the monkey studies submitted to this application, the Applicant fit the data to a two-compartment pharmacokinetic model using IV bolus input. Neither trastuzumab nor trastuzumab emtansine appeared to accumulate significantly in these studies. The volume of the central compartment is low, about 4% of total body weight, and was about the same as the plasma space. Clearance is slow and decreases with increasing dose, which is reflected in terminal elimination half-lives that increase with increasing dose. This suggests that the rate limiting process in elimination, possibly release from binding sites or antibody destruction or cleavage of DM1, is not first order. At the dose most relevant to human experience the terminal

[49] F. Zaqouri et al., Trastuzumab administration during pregnancy: a systematic review and meta-analysis. Breast Cancer Res Treat, 2012, Dec. 15 [Epub ahead of print].

elimination half-life is about 7 days. At the lowest dose tested, 3.0 mg/kg, monkeys have C_{max} values similar to humans but the AUC is somewhat lower. The monkeys clear trastuzumab emtansine somewhat more quickly than humans. Trastuzumab emtansine half-lives are shorter than trastuzumab half-lives and clearance is quicker. The distribution half-lives are relatively small suggesting that TDM1 binds to available binding sites quickly. The consistency of the volume and the distribution half-life suggest that titration of the binding sites (not necessarily just HER2 but all sites) is not saturated even at the high dose. The distribution half-life is less than the distribution half-life for total trastuzumab suggesting either more avid binding or significant early cleavage of DM1; the latter seems most likely. Exposure for both total trastuzumab and trastuzumab emtansine increases linearly with dose, but the increase in dose is greater than dose proportional. AUC is consistently higher in female monkeys for both total trastuzumab and trastuzumab emtansine, but the differences are small and did not manifest in increased toxicity.

Once hydrolyzed from the ADC, DM1 was cleared so rapidly that it could not be analyzed with accuracy sufficient to allow the investigators to fit the data to a model. In the kinetics of DM1 elimination, the hydrolysis step was rate limiting.

Correlation with Clinical Toxicity

The following table correlates other toxicities seen at an incidence of greater than 10% in clinical trials.

Human Toxicity	Human Incidence (%, all grades)	Monkey Studies
Diminished LVEF		Predicted by in vitro models for HER2
Fatigue	36.3	Not noted
Nausea	39.8	Dose dependant vomiting
Pyrexia	18.6	Not seen
Asthenia	17.8	Not noted
Musculoskeletal pain	36.1	Not observable
Arthralgia	19.2	Not observable
Myalgia	14.1	Not observable
Thrombocytopenia	31.2	Dose dependant but transient
Headache	28.2	Not observable
Peripheral Neuropathy	21.2	Axonal degeneration, Schwann Cell hyperplasia and hypertrophy
Increased transaminases	28.8	Increased transaminases, Kupffer cell hypertrophy, centrilobular vacuolization, multinucleated hepatocytes
Constipation	26.5	Not seen
Anemia	14.3	Mild (<10%)
Diarrhea	24.1	Seen in all groups including controls
Abdominal Pain	18.6	No observable
Stomatitis	14.1	Inflammation of the tongue
Epistaxis	22.5	Not seen
Dyspnea	12.0	Mononuclear cell infiltrates
Pneumonitis	1.2	Mononuclear cell infiltrates
Hypokalemia	10.2	Not seen

The studies in monkeys well predicted most of the toxicities seen humans that could be objectively observed. These studies could not predict those toxicities that are patient reported

(not observable in the table above). Pyrexia was probably absent from the monkey studies because of the weaker interaction monkey tissues have with the human antibody. The peripheral neuropathy seen in humans correlates with axonal degeneration. Unlike neuropathy seen with other cancer drugs this toxicity may be only very slowly reversible because of the damage to the Schwann cells.

The damage in the liver is probably due to direct toxicity from DM1. The concentrations of DM1 in the serum are very low and metabolism studies suggest that this compound is cleared rapidly by the liver. The presence of centrilobular vacuolization suggest significant direct toxicity to the hepatocytes. Serious hepatobiliary disorders, including at least two fatal cases of severe drug-induced liver injury and one fatal case of metabolic encephalopathy, have been reported in clinical trials with trastuzumab emtansine (n=884 treated patients). In clinical trials, cases of nodular regenerative hyperplasia (NRH) of the liver have been identified from liver biopsies (3 cases out of 884 treated patients). NRH is a rare liver condition characterized by widespread benign transformation of hepatic parenchyma into small regenerative nodules; NRH may lead to non-cirrhotic portal hypertension. The diagnosis of NRH can be confirmed only by histopathology.

Since there was an unusual incidence of microscopic pathology in the tongue of monkeys exposed to TDM-1 for 12 weeks (30 mg/kg = HD), I asked Dr. Blumenthal, the clinical reviewer for this BLA, if similar toxicities occurred clinically. He did a search of the clinical database and reported the following:

Preferred terms	TDM-1 Arm	Lapatinib-capecitabine Arm
Tongue discoloration	1	0
Tongue hemorrhage	1	0
Tongue ulceration	1	0
High Level Group Term		
Tongue Conditions	4	0
Oral soft tissue conditions	35 (7%)	88 (18%)

The toxicity seen in the monkey possibly has a clinical correlate, but cannot be distinguished from stomatitus in this clinical study. HER2 is almost certainly involved in the production of new epithelium in the tongue.

LP Grazette *et al.* have shown that Inhibition of HER2 causes mitochondrial dysfunction in cardiomyocytes.[50] HER2 inhibition activates the mitochondrial apoptosis pathway through modulation of Bcl-xL and Bcl-xS, causing impairment of mitochondrial function and integrity and disruption of cellular energetics. This leads to decreased cardiac contractility and congestive failure. The damage may be irreversible or only very slowly reversible. This mitochondrial toxicity probably manifests in any tissue that expresses HER2, but cardiac toxicity is sentinel because this tissue has the highest concentration of and greatest dependence upon mitochondria. While the monkey studies demonstrated no obvious cardiac toxicity associated with trastuzumab emtansine given every three weeks for 4 doses, the studies did not confirm the adequacy of this model species to predict this toxicity. If the expression of HER2 is higher on the human heart than on the simian heart the species could under-predict the potential for cardiac toxicity. In this case, a large dose of DM1 could be delivered to cardiac tissue exacerbating the known cardiac toxicity of trastuzumab. In ongoing and complete clinical trials, the Applicant is monitoring patients for cardiac toxicity and protocols exclude patients with preexisting cardiac insufficiency. This toxicity correlates with the incidence of diminished left ventricular ejection fraction seen clinically.

[50] LP Grazette *et al*. J Am College Cardiology, **44**(11); 2231-2238

The pneumonitis seen clinically was predicted by cellular infiltrates in the monkeys indicative of inflammation.

W. David McGuinn, Jr., Ph.D., D.A.B.T.
January 2, 2013
Revised January 14, 2013

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

WILLIAM D MCGUINN
01/15/2013

TODD R PALMBY
01/15/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125427 **Applicant: Genentech** **Stamp Date: 8/27/2012**

Drug Name: TDM-1 **BLA Type: 351(a)**

On **initial** overview of the NDA/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?	☺		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	☺		
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).	☺		
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?	☺		
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?	☺		

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
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.)	☺		
11	Has the applicant addressed any abuse potential issues in the submission?	NR		
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	NR		

NR = Not relevant to this application

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.

W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T. September 18, 2012
Reviewing Pharmacologist Date

Todd R. Palmby September 19, 2012
Team Leader/Supervisor Date

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

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/s/

WILLIAM D MCGUINN
09/19/2012

TODD R PALMBY
09/19/2012