CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125409Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Perjeta (pertuzumab)

Date: May 16, 2012
To: File for BLA 125409
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. Ringgold and labeling and secondary memorandum provided by Dr. Pilaro. I concur with Dr. Pilaro's conclusion that Perjeta may be approved and that no additional nonclinical studies are needed for the proposed indication.

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

/s/

JOHN K LEIGHTON
05/16/2012

MEMORANDUM

TO: The file
CC: Robert L. Justice, M.D., Director, Division of Oncology Products-1, Office of Hematology and Oncology (OHOP), Center for Drug Evaluation and Research (CDER)
John K. Leighton, Director, Division of Hematology and Oncology Toxicology (DHOT), OHOP, CDER
FROM: Anne M. Pilaro, Ph.D., Supervisory Toxicologist, DHOT, OHOP, CDER

STN BLA #: 125409/000
APPLICANT: Genentech, Inc.
PRODUCT: Perjeta™ (pertuzumab; recombinant, humanized monoclonal antibody 2C4)
SUBMISSION TYPE: original BLA application
DATE: May 11, 2012

SYNOPSIS:
Genentech Inc. has submitted an original licensing application for their recombinant, humanized monoclonal antibody, pertuzumab (Perjeta™) for the treatment of patients with locally advanced or metastatic breast cancer. Perjeta™ is indicated "…in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive metastatic breast cancer who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease."[1] Nonclinical studies investigating the pharmacology, pharmacokinetics and toxicology of pertuzumab in xenograft mouse models and cynomolgus monkeys were submitted with the biologics licensing application (BLA) in support of the safety of Perjeta™.

The human epidermal growth factor-2, also known as HER2, HER2/neu, or ErbB-2 is a member of the transmembrane family of ErbB epidermal growth factor receptors (EGFR). The ErbB family is composed of four cellular surface receptor tyrosine kinases including EGFR-1 (ErbB-1, HER1), HER2, HER3, and HER4, which function in cellular signaling, growth and survival in response to different growth factors. Upon ligand binding, the EGFR family members form either homodimers with the same receptor, or heterodimerize with other ErbB family members. Although HER2 has no known ligand, it can form heterodimers with any of the other three ErbB family members after binding of their respective ligand(s). Once dimerization occurs, autophosphorylation of the tyrosine residues within the cytoplasmic domain of the receptors, and subsequent transmembrane signal transduction ensue[2]. Signaling pathways activated by HER2 include mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), protein kinase C (PKC) and signal transducer and activator of transcription (STAT)

[1] from the current, draft labeling language for Perjeta™
[2] Olayioye, M.A. (2001). Update on HER2 as a target for cancer therapy: Intracellular signaling pathways of ErbB2/HER2 and family members. *Breast Cancer Res.*, **3**:385-389; Bublil, E.M. and Y. Yarden. (2007). The EGF receptor family: Spearheading a merger of signaling and therapeutics. *Curr. Opin. Cell Biol.* **19**: 124–134; Garrett, T. P., N.M. McKern, M. Lou, T.C.Elleman, T.E. Adams, G.O. Lovrecz, H.J. Zhu, F. Walker, M.J. Frenkel, P.A. Hoyne, R.N. Jorissen, E.C. Nice, A.W. Burgess, and C.W. Ward. (2002). Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor-α. *Cell* **110**: 763–773.

kinase. Once activated these kinases promote cellular proliferation and growth, and enhance cell survival by opposition of apoptosis pathways (*ibid.)*.

Overexpression of HER2 occurs in approximately 30-40% of breast cancers, and its amplification or overexpression, as detected by either fluorescent *in situ* hybridization or immunohistochemical staining of tumor tissue for HER2, respectively, is strongly associated with disease recurrence and a worse prognosis[3]. Therefore, development of therapeutic agents that target HER2 is desirable for treatment of patients with breast, ovarian, gastric or other cancers that overexpress this target. The first such anti-HER2 targeted therapy was the monoclonal antibody trastuzumab (Herceptin®), which was initially approved in 1998 for the treatment of women with metastatic breast cancers that overexpress HER2. The indication was later expanded to include earlier stages of HER2-positive breast cancer, and in October 2010 Herceptin® received approval for the treatment of HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinomas.

The present BLA presents data in support of the safety and efficacy of a second, anti-HER2 monoclonal antibody, pertuzumab (Perjeta™). In contrast to Herceptin®, whose mechanism of action in breast and gastric cancers is largely unknown, Perjeta™ acts by inhibiting dimerization of HER2 with itself, or with other ligand-bound ErbB receptors. Similar to the action of Herceptin®, pertuzumab also inhibits activation of the receptor tyrosine kinases, thereby blocking signal transduction through HER2. In a large scale, randomized, placebo controlled trial in 808 patients with metastatic breast cancer that was positive for HER2 overexpression, combination treatment with docetaxel, Herceptin® and Perjeta™ prolonged progression-free survival by approximately 6.1 months, as compared to the control cohort of patients treated with docetaxel, Herceptin® and the placebo for pertuzumab (hazard ratio 0.62; 95% confidence intervals 0.51, 0.75; $p \leq 0.0001$).

Reviewer comment: In the Highlights section of the label, Perjeta™ was defined by the Applicant as a "HER2/neu [redacted] (b) (4) for its pharmacologic class; however, to be consistent with the pharmacologic class of Herceptin® and other HER2-targeted therapies, the FDA has revised this language to label Perjeta™ as a "HER2/neu receptor antagonist" for its pharmacologic class.

The pivotal nonclinical data in support of the safety of Perjeta™ for the proposed indication were evaluated by the primary reviewer, Kimberly Ringgold, Ph.D., and are briefly summarized in the "Executive Summary" and "Integrated Summary and Safety Evaluation" sections of her review. Pharmacology, pharmacokinetic (PK) evaluations and toxicology studies supporting the BLA for Perjeta™ were conducted *in vitro* and *in vivo* in xenograft models using HER2-overexpressing human tumor cells including KPL-4 breast carcinoma and Calu-3 non-small cell lung carcinoma (NSCLC). *In vivo* safety was evaluated in several studies in non-human primates; however, the only studies pivotal for approval and reviewed for this submission were the single dose PK and

[3] Tan, M. and D. Yu. (2007). Molecular mechanisms of ErbB2-mediated breast cancer chemoresistance. *Adv. Exp. Med. Biol.*, **608**:119–29.

tolerability and the 6-month (Q weekly dosing x 26 weeks) repeat-dose toxicity studies in cynomolgus monkeys.

The *in vitro* and *in vivo* binding to HER2, pharmacodynamic and anti-tumor effects of pertuzumab were consistent with those observed with other HER2 receptor antagonists. As documented in Dr. Ringgold's review of the published literature provided with this BLA submission pertuzumab, but not trastuzumab, blocked Heregulin-induced activation of the PI3K cell survival pathway, as indicated by a lack of phosphorylation of Akt, a key enzyme in this pathway[4]. Both antibodies were capable of activating antibody-dependent cellular cytotoxicity with equal potency, which may contribute to the mechanism of both pertuzumab and trastuzumab's anti-tumor effects[5]. *In vitro* tissue cross-reactivity studies showed similar patterns of pertuzumab binding to human and cynomolgus monkey tissues, with haired skin, mammary gland, prostate, urinary bladder and stomach identified as the major target organs, consistent with the known distribution of HER2. *In vivo* effects in xenograft models included inhibition of tumor growth by either pertuzumab or trastuzumab administered as single agents, with greater activity (e.g. 100% inhibition of tumor growth of Calu-3 xenografts) when the mice were treated with the combination of pertuzumab and trastuzumab. Additionally, weekly or bi-weekly pertuzumab treatment of mice bearing xenografts of patient-derived mammary, ovarian, or non-small cell lung cancers showed anti-tumor activity against 1/6 primary mammary, 1/4 primary ovarian, and 4/18 primary NSCLC tumors. Lastly, weekly pertuzumab, but not trastuzumab treatment of Founder 2-134R tumor-bearing mice inhibited tumor growth in a dose-related fashion, with approximately 50% tumor growth inhibition reported at trough pertuzumab concentrations of 50 μg/ml and above. Importantly, although the Founder 2-134R tumor line is HER2-positive it does not respond to trastuzumab alone, suggesting that pertuzumab may have anti-tumor activity in patients whose breast cancers are resistant to Herceptin® treatment.

In the single dose toxicity and pharmacokinetic study of pertuzumab in cynomolgus monkeys (study #00-564-1821), there were no treatment-related effects or abnormal clinical signs reported, other than a mild reactivity at the injection site in the animals that were treated by subcutaneous injection. The pharmacokinetic profile of pertuzumab after intravenous injection of 15, 50, or 150 mg/kg was similar to other monoclonal antibodies of the IgG1κ isotype, with dose-related increases in C_{max} and AUC that were approximately linear, low clearance in the absence of significant expression of target (CL = 5 ml/kg/day), and a volume of distribution at steady state approximately equivalent to plasma volume (V_{ss} = 70 ml/kg). The elimination half-life of pertuzumab following a single dose was approximately 10 days.

Although it was never detected in the initial nonclinical toxicity studies with trastuzumab, cardiovascular toxicity has been a clinical safety concern with Herceptin®. There were

[4] Agus, D.B., R.W. Akita, W.D. Fox, G.D. Lewis, B. Higgins, P.I. Pisacane, J.A. Lofgren, C. Tindell, D.P. Evans, K. Malese, H.I. Scher, and M.X. Sliwkowski. (2002). Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell*, **2**:127-137.

[5] Scheuer, W., T. Friess, H. Burtscher, B. Bossenmaier, J. Endl, and M. Hasmann. (2009). Strongly enhanced antitumor activity of trastuzumab and pertuzumab combination treatment on HER2-positive human xenograft tumor models. *Cancer Res.*, **69**:9330-9336.

no remarkable treatment-related effects on electrocardiogram waveform patterns, heart rate, blood pressure or troponin levels in monkeys during the 6-month, repeat-dose toxicity study with pertuzumab, given weekly at doses of 15, 50, or 150 mg/kg/dose (study #00-458-1821). The most frequent toxicities observed in this study were liquid/non-formed feces (i.e. diarrhea) and elevations in blood urea nitrogen of 30 to 60% over the control group, which were reported for all pertuzumab-treated dose groups without a clear dose-relationship in either incidence or severity. Of note, diarrhea was the most commonly reported adverse reaction in the randomized clinical study, with a frequency of 67% overall and 8% for Grade 3-4 events. There were no other remarkable treatment-related changes in clinical observations, body weight and food consumption, clinical chemistry parameters including hematology and urinalysis, organ weights or serum testosterone levels in pertuzumab-treated monkeys. Evaluation of male fertility parameters (i.e. sperm counts, viability, motility) was not possible, as most of the male animals were not sexually mature. On microscopic evaluation, inflammation was noted in the lungs, pancreas and cecum in animals from the highest dose group of 150 mg/kg/dose, and was largely reversible by the end of the 8-week recovery phase. This dose level corresponds to an exposure (based on AUC) of approximately 9-fold greater than that achieved at the human recommended dose.

Reviewer Comment: Typically, comparative exposures for protein therapeutics between human and test species are not based on pharmacokinetic profiles, because the development of anti-drug antibody limits the reliability of the AUC values generated. In this study, however, there were no positive antibody titers against pertuzumab detected in any of the samples. Although the Applicant cannot exclude the possibility that pertuzumab present in the serum samples was interfering with the detection of anti-pertuzumab antibodies, it is apparent that exposure, as measured by AUC was not only maintained over the course of the 6-month study, but also showed accumulation. Therefore, AUC in this case is considered an appropriate basis on which to establish comparison between the cynomolgus monkey and human exposures.

As documented in Dr. Ringgold's review, an embryofetal developmental toxicity study conducted in cynomolgus monkeys showed that twice weekly treatment with pertuzumab during the period of organogenesis (Gestation Day 19 – GD50) resulted in fetal lethality/abortions occurring between GD25 and GD70 in 4/12, 6/12, and 10/12 dams treated on GD19 with loading doses of 30, 100, or 150 mg/kg pertuzumab followed by bi-weekly doses of 10, 33, or 100 mg/kg/dose pertuzumab, respectively (study #07-0925). After delivery by Caesarean section on GD100, oligohydramnios and discoloration of the amniotic fluid were noted in 2/8, 6/6 and 2/2 surviving offspring from these respective dose groups. Adverse effects on fetal growth and development were reported in the majority of surviving offspring (specifically, reduced fetal weight and decreased crown:rump and tail lengths at all dose levels; decreased head width, circumference, and hindfoot lengths as well as decreased lung and kidney weights at the mid- and high doses). Other visceral and skeletal anomalies present in offspring from the mid- and high dose groups included paw hyperextension/hyperflexion, microtia, small lungs, thin walls in the ventricular, fused caudal and sacral vertebra, and supernumerary lumber vertebra. Microscopic evidence of renal hypoplasia and impaired renal development (i.e. hypoplasia of the renal tubules, glomerulus, pelvis and

collecting tubules) was present in 100% of the surviving offspring delivered at GD100, with a dose-related incidence from slight to marked severity across the three dose groups. Both offspring delivered at GD100 from the dams treated with 150/100 mg/kg/dose pertuzumab had markedly severe renal findings. Exposure of offspring to pertuzumab was confirmed by toxicokinetic evaluation of fetal blood; levels of pertuzumab present at Caesarean delivery in offspring were approximately 30% of the maternal blood levels (based on C_{max} in the fetuses, and C_{last} in the dams at GD100), regardless of the dose of pertuzumab administered to the dams. The doses of pertuzumab tested in this study resulted in maternal exposures that were approximately 0.2 to 2-fold greater than the human exposure at the recommended dose, when scaled by AUC. Analysis of maternal and fetal sera for anti-pertuzumab antibodies was not conducted; however, all animals were negative for anti-pertuzumab antibody development in the 6-month, repeat-dose toxicity study so dose comparison based on AUC is likely appropriate for the reproductive toxicity study as well. A copy of the table from Dr. Ringgold's review, detailing the comparative dose information, is presented below.

<u>Animal to Human Exposure Multiples</u>

Dose (mg/kg)	AUC 0 - ∞ (µg·/mL)	Human Exposure AUC 0 - ∞ (µg·/mL)	Multiples of Human Exposure
10	644	2660	0.242
33.3	2030		0.763
100	5360		2.02

Reviewer comment: Given that pertuzumab will be labeled for use (b) (4) as a combination with Herceptin® (i.e., no monotherapy indications), and that Herceptin® has known human data with oligohydramnios and resulting deleterious effects on lung and renal development in exposed fetuses, the Division concluded that the pregnancy labeling for pertuzumab should more strongly convey these findings to inform patients and prescribing physicians of the risks of pertuzumab use in pregnant patients. The Division has revised the language in the appropriate sections of the label to follow the format proposed by the draft Physician's Pregnancy and Labor Labeling Rule (PLLR), and has requested assistance from the Maternal Health Team in drafting the language for Sections 5.3 (Warnings), 8.1 (Use in Specific Populations: Pregnancy) and 13.2 (Animal Toxicology Data).

There were no nonclinical genotoxicity or carcinogenicity studies performed with pertuzumab, as per the guidance provided in the International Conference on Harmonisation (ICH) guidance S6: *"Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals"* for protein therapeutic agents, and in ICH S9 *"Nonclinical Safety Evaluation for Anticancer Pharmaceuticals,"* which applies to new drugs used to treat advanced or metastatic cancers.

Recommendation: I concur with Dr. Ringgold's conclusions regarding the nonclinical findings for Perjeta™, her current recommendation that the licensing application be approved for marketing, and her recommendations regarding the language for the prescribing information. Dr. Ringgold will provide a separate labeling review to the file when the discussions with the Maternal Health Team and the Applicant are complete, and the language has been finalized. A copy of Dr. Ringgold's review, with supervisory sign-off, has been conveyed to the regulatory project manager for inclusion in the final action package, and has been uploaded into the DARRTS database.

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

/s/

ANNE M PILARO
05/15/2012

JOHN K LEIGHTON
05/16/2012

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	125409
Applicant's letter date:	12/6/2011
CDER stamp date:	12/8/2011
Product:	Pertuzumab
Indication:	HER2-positive metastatic or locally recurrent, unresectable breast cancer
Applicant:	Genentech, Inc
Review Division:	DHOT (for DHP)
Reviewer:	Kimberly Ringgold, PhD
Supervisor/Team Leader:	Anne M. Pilaro, PhD
Division Director:	John Leighton, PhD, DABT (DHOT) Robert Justice, MD (DOP1)
Project Manager:	Amy Tilley

TABLE OF CONTENTS

1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

The nonclinical studies submitted to this BLA provide sufficient information to support the use of pertuzumab for the treatment of patients with HER2-positive metastatic or locally recurrent, unresectable breast cancer.

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

See separate labeling review

1.2 Discussion of Nonclinical Findings

Pharmacology

Pertuzumab (rhuMab 2C4) is a recombinant humanized monoclonal antibody against the HER2/neu receptor (also referred to as ErbB2). The amino acid homology of human and monkey ErbB2 was 99%. Therefore, the cynomolgus monkey was selected as the appropriate model for nonclinical evaluation. Tumor growth was inhibited by pertuzumab at doses of 30 – 90 mg/kg in the Founder 2-134R tumor xenograft model, which is resistant to trastuzumab. Pertuzumab also exhibited anti-tumor activity in 1/6 of the mammary cancer models, 1/4 of the ovarian, and 4/18 of the NSCLC cancer models. Both pertuzumab and trastuzumab as single agents were significantly active against HER2 overexpressing (3^+) Calu-3 non-small cell lung cancer (NSCLC) xenografts [85% and 82% tumor growth inhibition (TGI), respectively]. The combination of pertuzumab and trastuzumab was greater in activity (100 % TGI) compared to either single agent effect.

Pharmacokinetics

The PK profile of pertuzumab was studied in the monkey; the nonclinical species used for the chronic toxicology and fetal toxicity studies. Pertuzumab was eliminated from plasma with a half-life of approximately 10 days. The plasma clearance and volume of distribution following intravenous administration were low (clearance = 5 mL/day/kg and volume of distribution, Vss = 70 ml/kg). Following subcutaneous administration, the peak plasma level (t_{max}) was reached within 2.28 days and rhuMab 2C4 was slowly eliminated from plasma with a half-life of approximately 10 days. The subcutaneous bioavailability was 81.5%.

Following intravenous administration of pertuzumab to monkeys for 26 weeks, pertuzumab exposures increased in a dose-proportional manner. Faster clearance was

observed in the 150 mg/kg dose group. Serum pertuzumab concentrations increased in a dose-proportional manner between all doses tested in pregnant monkeys and fetuses. Ratios of fetal to maternal pertuzumab levels were comparable (0.294, 0.399, and 0.338, respectively).

General Toxicity
The toxicological profile of pertuzumab suggests that pertuzumab appears to be well-tolerated in monkeys. Nonclinical findings show toxicities in the lung and gastrointestinal tract, which are expect given the distribution of the HER2/neu antigen.

Reproductive and Developmental Toxicity
Pertuzumab caused fetal lethality in pregnant monkeys treated with loading doses of ≥ 30 mg/kg followed by bi-weekly doses ≥ 10 mg/kg (approximately 0.2 to 2-fold greater than the exposure at the recommended human dose, by AUC). Fetal effects were also noted at doses ≥ 30/10 mg/kg. Malformations were observed at doses ≥ 100/33.3 mg/kg dose; the highest dose level tested was approximately 2.-fold higher than the recommended dose for patients. These malformations included paw hyperextension/hyperflexion, microtia, small lungs, thin walls in the ventricular regions of the heart, fused caudal and sacral vertebra, and supernumerary lumber vertebra. Thus, administration of pertuzumab during pregnancy may pose a risk to the human fetus.

Special Toxicity
Pertuzumab did not cause lysis of cynomolgus monkey or human erythrocytes and was compatible with cynomolgus monkey and human serum and plasma in in vitro test systems. Cell surface staining with pertuzumab in human tissues was noted in the haired skin, placenta, parathyroid gland, tonsil, mammary gland, ureter, and urinary bladder tissues. Cytoplasmic staining was noted in the salivary gland and prostate gland as well as in the stomach, haired skin, and thymic cyst of human tissues. In monkey tissues, cell surface staining was noted in the sweat and sebaceous gland, mammary gland, placenta, ureter, urinary bladder, and prostate gland. Cytoplasmic staining was noted in adenohypophysis and salivary gland.

2 Drug Information

2.1 Pertuzumab

2.1.1 CAS Registry Number:	380610-27-5
2.1.2 Generic Name:	Pertuzumab (formerly Omnitarg)
2.1.3 Code Name:	rhuMab 2C4
2.1.4 Chemical Name:	Immunoglobulin G1, anti-(human neu (receptor)) (human-mouse monoclonal 2C4 heavy chain), disulfide with human-mouse monoclonal 2C4 κ-

	chain, dimer
2.1.5 Molecular Weight	Approximately 148 kDa
2.1.6 Structure	recombinant, humanized, immunoglobulin (Ig)G1κmonoclonal antibody consisting of two heavy chains (449 residues) and two light chains (214 residues)
2.1.7 Pharmacologic class:	HER2/neu receptor antagonist

2.2 Relevant IND/s, NDA/s, and DMF/s

BB-IND 9900

2.3 Clinical Formulation

2.3.1 Drug Formulation

Composition of Pertuzumab Drug Product

Ingredients	Amount per Vial[a]	Concentration per Vial	Component Function	Pharmacopeia Specification
Pertuzumab	420 mg	30 mg/mL	Active Ingredient	-
L-Histidine	43.5 mg	3.1 mg/mL	(b) (4)	USP/Ph. Eur.
(b) (4)				USP/Ph. Eur.
Sucrose	(b) (4)			NF/Ph. Eur.
Polysorbate 20	(b) (4)			NF/Ph. Eur.
(b) (4)				USP/Ph. Eur.

NA = not applicable; NF = National Formulary.

[a] Amounts listed depict the extractable content.

2.3.2 Comments on Novel Excipients: none

2.3.3 Comments on Impurities/Degradants of Concern: none

2.4 Proposed Clinical Population and Dosing Regimen

Pertuzumab is intended for first line treatment of patients with HER2-positive metastatic or locally recurrent, unresectable breast cancer. Pertuzumab will be administered

intravenously (IV) at an initial dose of 840 mg over 60 minutes intravenous infusion, followed every 3 weeks thereafter by 420 mg over 30 – 60 minutes intravenous infusion

2.5 Regulatory Background

Genentech met with FDA on a number of occasions during which discussions on nonclinical, clinical, and chemistry, manufacturing and controls (CMC) aspects of the development of pertuzumab occurred, including a Pre-IND (3 May 2001) and other information request correspondences.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study No.
Primary Pharmacodynamics	
Cloning, Expression, and Characterization of Monkey ErbB2	01-249-1821
Characterization of the PK/PD Relationship of rhuMAb 2C4 in the Founder 2-134R Tumor Model	02-056-1281
In vivo activity of Pertuzumab in patient-derived mammary, ovarian, and non-small cell lung cancer (NSCLC) models	P60G, P60F, P60H
Pharmacodynamic Drug Interactions	
Evaluation of the anti-tumor effect of Omnitarg in combination with Herceptin in the Calu-3 NSCLC xenograft model in female Balb/c nude mice	1019398
Pharmacokinetics	
Absorption	
Single dose intravenous and subcutaneous pharmacokinetic study of Pertuzumab in cynomolgus monkeys	00-564-1821
General Toxicology	
Repeat-dose Toxicity	
Twenty Six-Week Intravenous Toxicity and Toxicokinetic Study with rhuMAb 2C4 in Cynomolgus Monkeys with an 8-Week Recovery Period	01-458-1821
Reproductive and Developmental Toxicity	
Embryo-Fetal Development Study of Pertuzumab Administered by Intravenous Injection to Pregnant Cynomolgus Monkeys	07-0925
Special Toxicity Studies	
Other Toxicity Studies	
Hemolytic Potential and Blood Compatibility of rhuMAb 2C4*	00-562-1821
Cross-Reactivity of rhuMAb 2C4 with Normal Human Tissues	01-014-1821
Cross-Reactivity of rhuMAb 2C4* with Normal Cynomolgous Monkey Tissues	01-015-1821

3.2 Studies Not Reviewed

Study Title	Study No.
Primary Pharmacodynamics	
Dose response of pertuzumab against BT474JB tumors	02-159A-1821
Dose response of pertuzumab in BALB/C Nude mice with CALU-3 tumor xenografts	0-163-1821
Dose response of pertuzumab in BALB/C Nude mice with MAXF449 tumor xenografts	0-164-1821
Dose response of pertuzumab in the NCLC xenografts NCI-H522	02-203-1821
Using Progynon-depot to evaluate the repeat-dose response of rhuMAb 2C4 versus MDA-MB-175 Tumors grown in gonadal fat pad	01-308C-1821
Pharmacodynamic Drug Interactions	
Anti-Tumoral Activity of Pertuzumab, Trastuzumab, and Bevacizumab as a Single Agent or in Combination in the KPL-4 Breast Cancer Xenograft (orthotopic, SCID beige mice)	RDR 1009892
Combination Study of Gemcitabine and Pertuzumab in the QG56 Xenograft Model (BALB/c nude)	RDR 1011230
Combination Study of pertuzumab and Gemcitabine in the CALU-3 NSCLC Xenograft Model	RDR 1011232
Combination Study of Pertuzumab and Paclitaxel in the CALU-3 NSCLC Xenograft Model	RDR 1015439
Combination study of rhuMAb 2C4 with Tarceva (RO0508231) or Irinotecan in the Calu-3 NSCLC Xenograft Model (Balb/c nude)	RDR 1011974
Evaluation of the Pharmacodynamic Effect of Gemcitabine and Pertuzumab Alone and in Combination in the IGROV-1 Human Ovarian Carcinoma Xenograft Model in Female SCID Beige Mice	RDR 1016330
Anti-Tumor Activity of Pertuzumab in Combination with Either Xeloda or Gemcitabine in Xenografts of Human Mammary, Colon, or Ovarian Cancers	P80K
Anti-Tumor Activity of Pertuzumab in Combination with Gemcitabine in a Patient-Derived Ovarian Cancer Passaged in Nude Mice	P80K (R)
Pharmacokinetics	
Absorption	
Pharmacokinetic Study of Pertuzumab Following Intravenous and Intraperitoneal Administration in CD-1 Mice	00-573-1821
A Single-Dose Pharmacokinetic Study of Pertuzumab Following Intravenous Administration in Male Rats	00-574-1821

Seven-Week Intravenous Toxicity and Toxicokinetic Study with Pertuzumab in Cynomolgus Monkeys with a 4-Week Recovery Period	00-377-1821
A Repeated-Dose Toxicity Study of Pertuzumab Administered Subcutaneously to Cynomolgus Monkeys for 4 Weeks	00-604-1560
Single-Dose Pharmacokinetics of Pertuzumab and Bevacizumab as a Single Agent and in Combination in Rats	03-0659-1821
Pharmacokinetics of Pertuzumab (400 L Scale versus 2000 L Scale) following a Single IV Bolus in Rats	02-113-1821
[400 L Scale] versus Phase II Material Following a Single IV Bolus in Rats	03-0690-1821
Pharmacokinetics of Pertuzumab (New Cell Line, WCB 2450 [12K L Scale GMP] versus Phase II Material) following a Single IV Bolus in Rats	07-0688
Pharmacokinetics of Pertuzumab in Tumor- and Non–Tumor-Bearing Mice	02-056 A-1821
General Toxicology	
Repeat-dose Toxicity	
4-Week Intravenous Toxicity Study of Pertuzumab in Monkeys	99-520-1820
7-Week Intravenous Toxicity and Toxicokinetics Study of Pertuzumab in Monkeys	00-377-1821
4-Week Subcutaneous Toxicity and Toxicokinetic Study Of Pertuzumab in Monkeys	00-604-1560

3.3 Previous Reviews Referenced

Non-clinical reviews under BB IND 9900, Herceptin BLA review, and Herceptin label (BLA **# 103792)**. Pertuzumab will be approved for use only in combination with Herceptin.

4 Pharmacology

4.1 Primary Pharmacology

Study title: Cloning, Expression, and Characterization of Monkey ErbB2
Study no.: 01-249-1821
Study report location: eCTD 4.2.1.1

The objective of this study was to identify the appropriate species for nonclinical toxicology studies with pertuzumab. To do this, the full-length cDNA of the cynomolgus monkey erbB2 gene was cloned using standard techniques. Monkey ErbB2 was expressed in COS7 cells, and competitive binding analysis was compared with human ErbB2. The results show that the amino acid sequence homology between human and monkey ErbB2 extracellular domain is 99%. Therefore, the cynomolgus monkey was chosen as an appropriate model for assessing the safety of ErbB2-directed immunotherapeutics.

Table 1: Binding affinity of Pertuzumab for human and monkey ErbB2 (Excerpted from applicant's submission)

Species	Kd (nM) ± SE
Human	0.80±0.08
Cynomolgus monkey	0.53±0.07

Study title: Characterization of the PK/PD Relationship of rhuMAb 2C4 in the Founder 2-134R Tumor Model
Study no.: 02-056-1281
Study report location: eCTD 4.2.1.1

The anti-tumor activity of rhuMAb 2C4 was evaluated using the Founder 2-134R tumor xenograft model. Mice with established tumors were randomized by tumor volume and randomly assigned to the treatment groups shown below. Mice were treated weekly. Tumor growth was monitored bi-weekly and calculated using the following equation: $\text{Tumor volume } (mm^3) = 0.5(A) \times (B)^2$, where A=largest width, B=distance perpendicular to A.

Blood samples were collected via retro-orbital sampling to measure peak and trough rhuMab 2C4 levels. Samples were taken pre-treatment and 45 minutes post-treatment on Days 7, 14, 21, 28, 35, and 42. Terminal bleeds were performed on Groups 5 and 6 on Day 47. Terminal blood samples were collected from animals in Groups 1, 2, 3, and 4 on Days 28, 28, 35, and 32, respectively, because the animals had unacceptably large tumors and were euthanized according to protocol.

Group	No./Sex	Route	Dose (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/dose)
1 (E25)	10/F	IV	90	23.0	0.1
2 (rhuMAb 2C4)	10/F	IV	1	0.250	0.1
3 (rhuMAb 2C4)	10/F	IV	3	0.770	0.1
4 (rhuMAb 2C4)	10/F	IV	10	2.50	0.1
5 (rhuMAb 2C4)	10/F	IV	30	7.50	0.1
6 (rhuMAb 2C4)	10/F	IV	90	23.0	0.1

IV = Intravenous.

Treatment of tumor-bearing mice with rhuMab 2C4 inhibited the growth in the Founder 2-134 tumor xenograft model in a dose-related fashion. At serum trough concentrations of approximately 50 µg/mL (30 mg/kg dose or greater), tumor growth inhibition of approximately 50% was observed. These data are presented in Figures 1 and 2, below:

Figure 1. Dose-response curves for tumors treated with rhuMab 2C4
(Excerpted from applicant's submission)

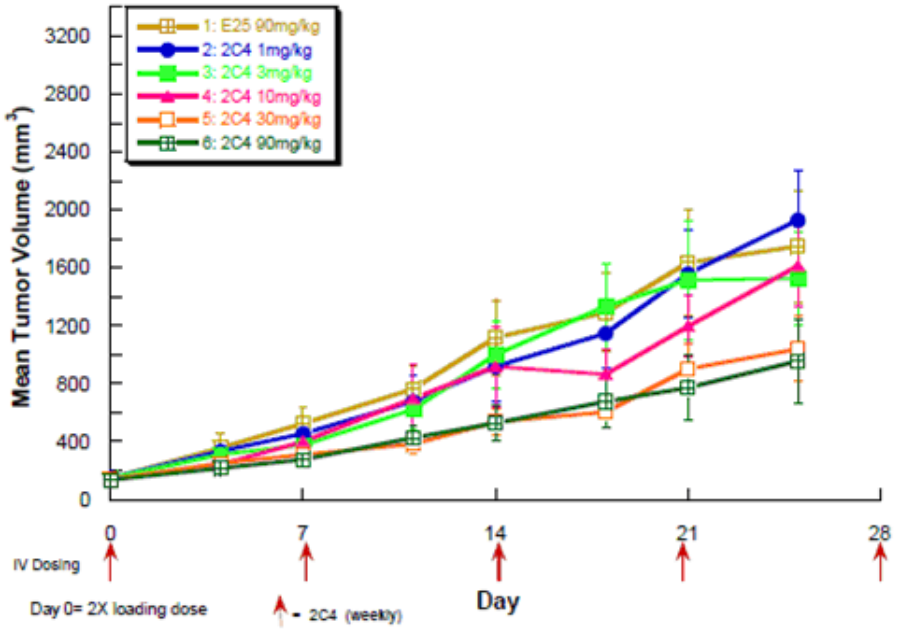
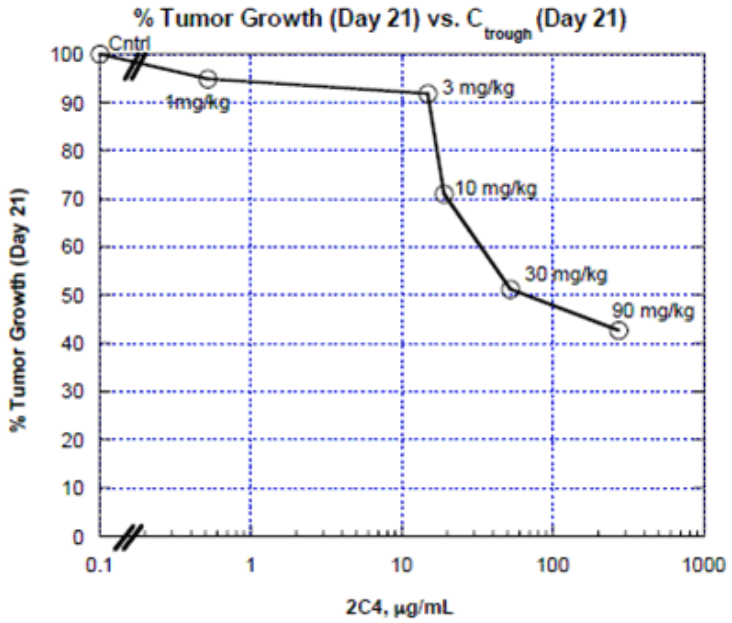


Figure 2. Founder 2-134 tumor growth inhibition vs. serum rhu MAb 2C4 trough levels
(Excerpted from applicant's submission)



Study title: In vivo activity of Pertuzumab in patient-derived mammary, ovarian, and non-small cell lung cancer (NSCLC) models
Study no.: P60G, P60F, P60H
Study report location: eCTD 4.2.1.1

These studies were conducted to identify Pertuzumab-responsiveness in xenograft tumor models established from patient-derived cancers. Tumors were surgically removed from patients and directly implanted and propagated in female (NMRI nu/nu) nude mice. Pertuzumab activity was screened against 6, 4, and 18 mammary, ovarian, and NSCLC cancer models, respectively.

Mammary cancer models (Study #P60G): rhuMAb 2C4 was investigated in 6 experiments with 6 human mammary cancer models. Pertuzumab was administered intraperitoneally at a dose of 100 mg/kg/dose (or 120 mg/kg loading dose followed by 60 mg/kg/dose in experiment #K404) once or twice per week from the start of the treatment up to the end of the experimental period. RhuMab 2C4 showed anti-tumor activity in 1 out of 6 of the human primary mammary tumor models tested, the MAXF 449 tumor model (data presented below).

Figure 3: Antitumor Activity of RhuMAb 2C4 (Twice Weekly Administration) on Human Mammary Cancer MAXF 449

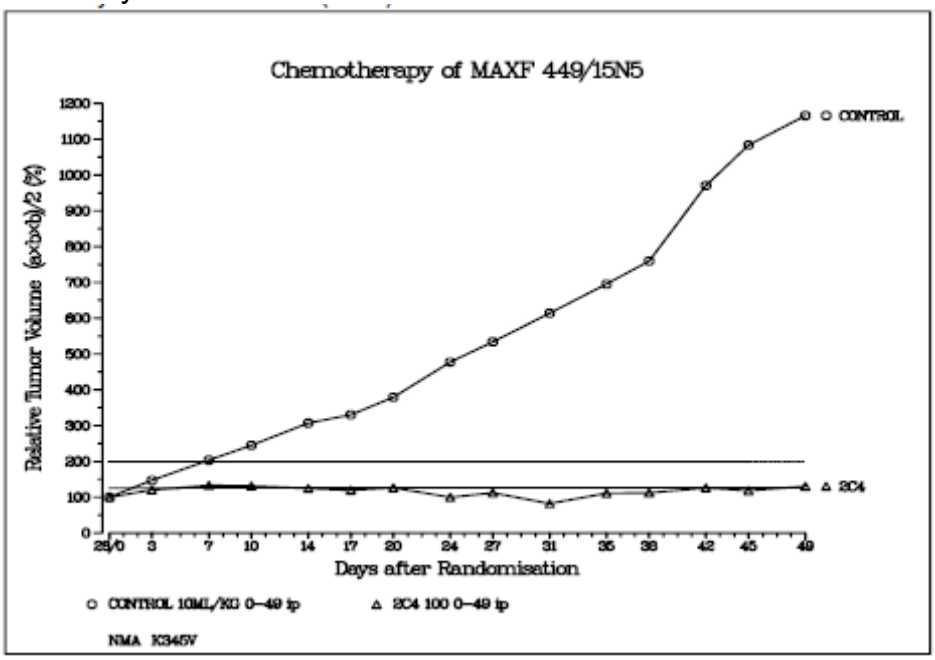
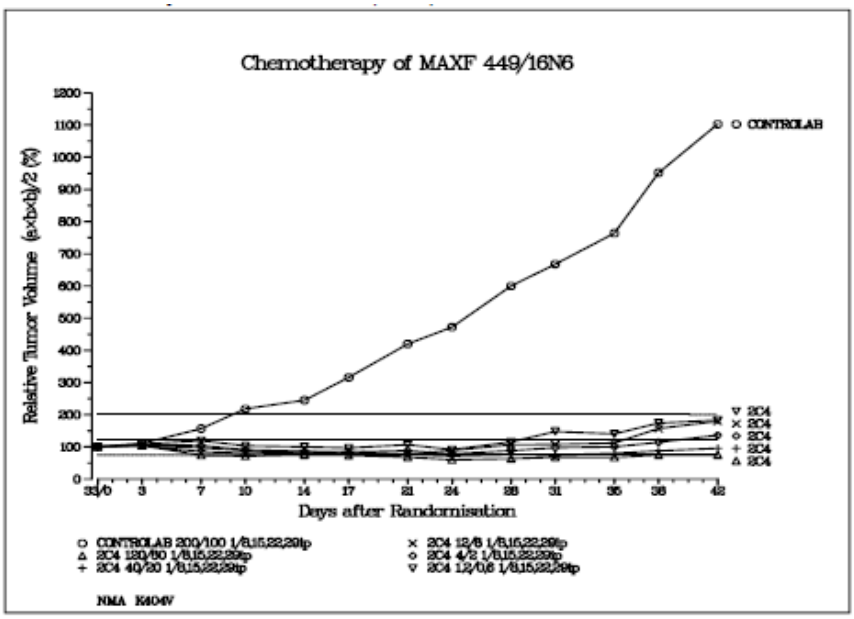


Figure 4: Antitumor Activity of RhuMAb 2C4 (Weekly Administration with loading dose) on Human Mammary Cancer MAXF 449 (K404)



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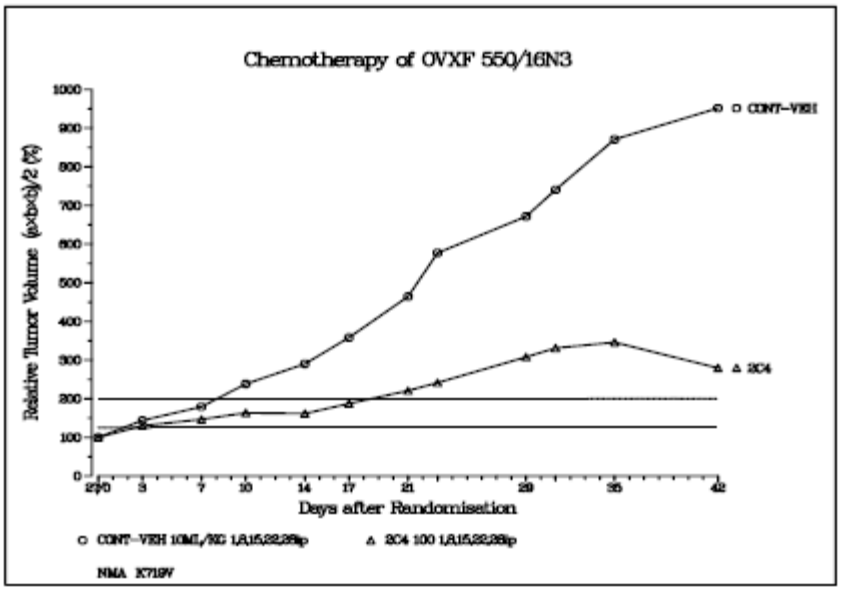
Ovarian cancer models (Study #AP60H): rhuMAb 2C4 was investigated in 4 different human ovarian cancer models and was administered intraperitoneally at a dose of 100 mg/kg/dose once weekly, from the start of the treatment up to the end of the experimental period. RhuMab 2C4 showed anti-tumor activity in 1 out of 4 of the models tested (OVXF 550); data are presented in Figure 5, below.

Figure 5: Antitumor Activity of RhuMAb 2C4 (Weekly Administration) on Human Ovarian Cancer OVXF 550 449 (K719)



NSCLC cancer models (Study #P60F): rhuMAb 2C4 was investigated in 18 human non-small cell lung cancer xenograft models, and was administered intraperitoneally at a dose of 100 mg/kg/dose once or twice per week from the start of the treatment up to the end of the experimental period. In the second series, in every experiment one additional group comprising three untreated animals was established for tumor sampling. Serum samples were taken from all other animals 24 hours after the last treatment. Treatment with rhuMab 2C4 showed anti-tumor activity in 4/18 of the models tested, and borderline anti-tumor activity in 2 of the models tested. Data are presented in Figures 6-9, below:

Figure 6: Antitumor Activity of RhuMAb 2C4 (Weekly Administration) on Human Non-Small Cell Lung Cancer LXFA 629 (H114)

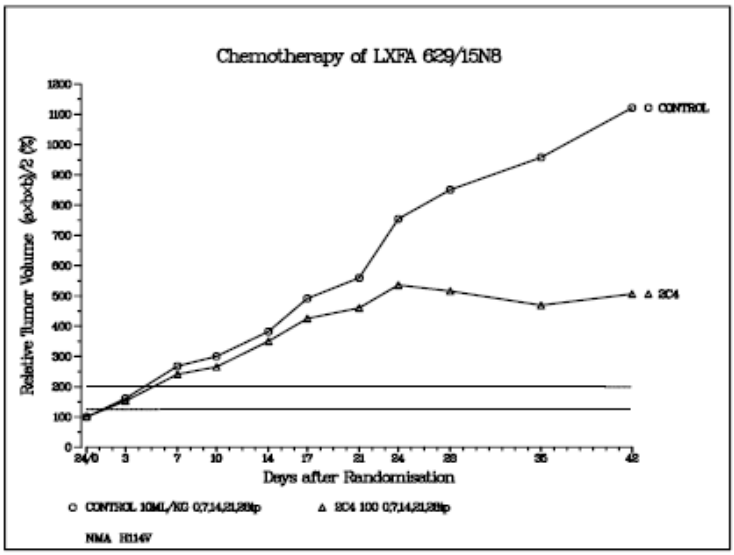


Figure 7: Antitumor Activity of RhuMAb 2C4 (Weekly Administration) on Human Non-Small Cell Lung Cancer LXFL 1072 (H124)

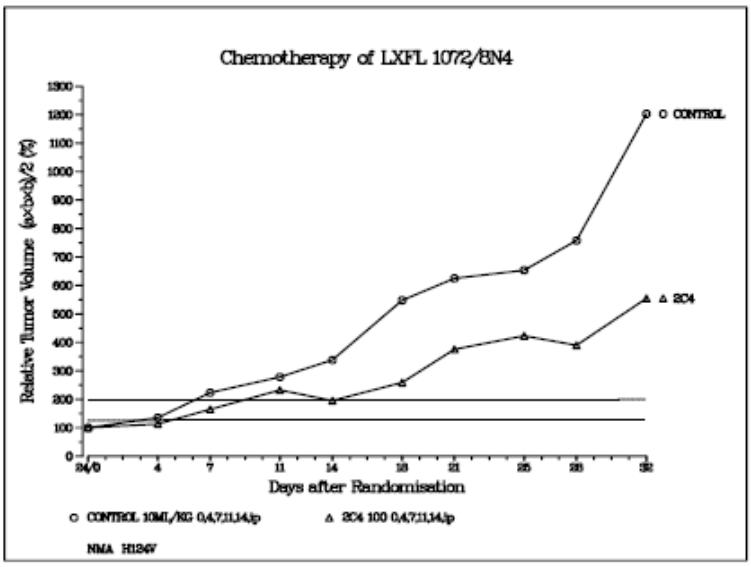


Figure 8: Antitumor Activity of RhuMAb 2C4 (Weekly Administration) on Human Non-Small Cell Lung Cancer LXFA 297 (K249)

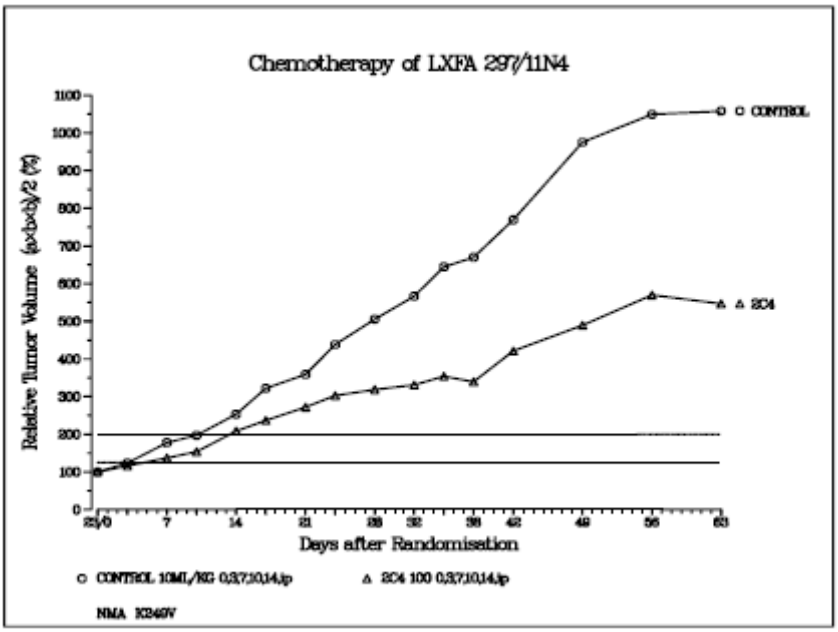


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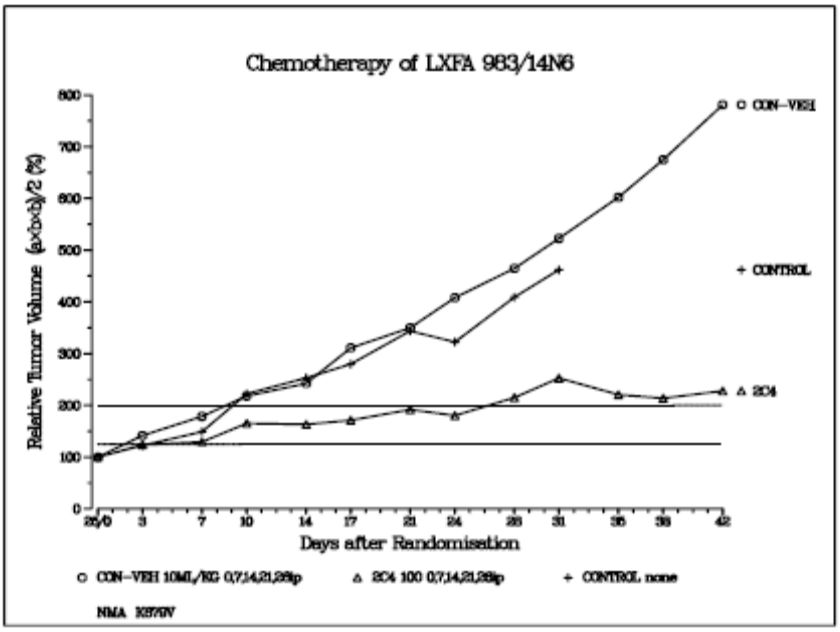
Figure 9. Antitumor Activity of rhuMAb 2C4 (Weekly Administration) on Human Non-Small Cell Lung Cancer LXFA 983 (K679)



4.2 Secondary Pharmacology

Studies not conducted.

4.3 Safety Pharmacology

Studies not conducted. Evaluations of cardiac (electrocardiogram measurements; ECG) and respiratory safety pharmacology parameters were included in the pivotal, repeat-dose toxicology studies and the findings are discussed with those studies, below.

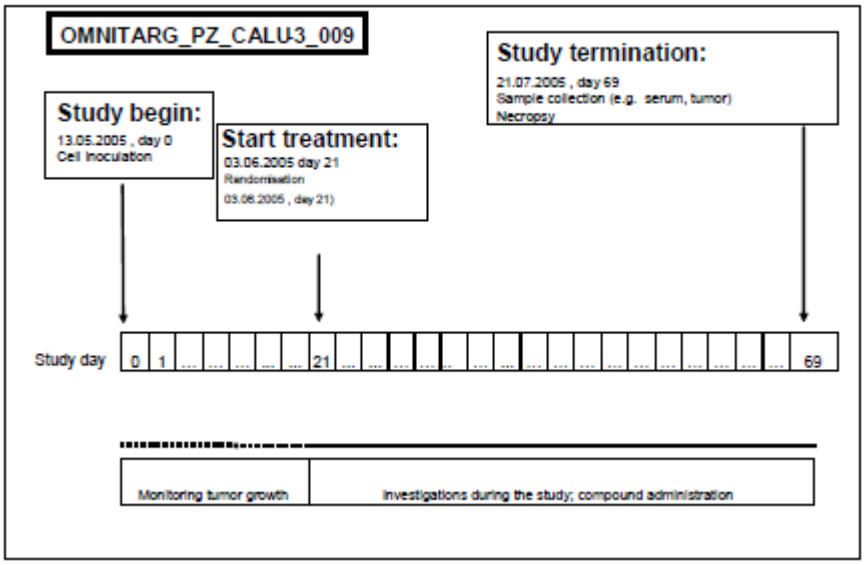
4.4 Pharmacodynamic Drug Interactions

Study title: Evaluation of the anti-tumor effect of Omnitarg in combination with Herceptin in the Calu-3 NSCLC xenograft model in female Balb/c nude mice
Study no.: 1019398
Study report location: eCTD 4.2.1.4

Objective: to evaluate the in vivo antitumor efficacy of combination treatment with pertuzumab and trastuzumab in Calu-3 NSCLC xenografts.

Study Design:

STUDY DESIGN AND TREATMENT SCHEDULE



Experimental schedules

Group	Compound	Dosage (mg/kg)	N° of mice	Route	No. of treatments	Cumulative Dose (mg/kg)
1	vehicle control	-	10	i.p. q7d	7	-
2	Omnitarg (Pertuzumab)	30/15	10	i.p. q7d	7	120
3	Herceptin (Trastuzumab)	30/15	10	i.p., q7d	7	120
4	Omnitarg (Pertuzumab) + Herceptin (Trastuzumab)	30/15 30/15	10	i.p., q7d/ i.p., q7d	7 7	120 120

Results:

Figure 10. Effect of treatment on tumor volume (day 96)

Compound	Dosage	TCR	CI	TGI %
Omnitarg	(30/15 mg/kg) q7d	0.23	0.08-0.53	85
Herceptin	(30/15 mg/kg) q7d	0.27	0.06-0.57	82
Omnitarg / Herceptin	(30/15 mg/kg) q7d (30/15 mg/kg) q7d	0.05	0.02-0.11	> 100

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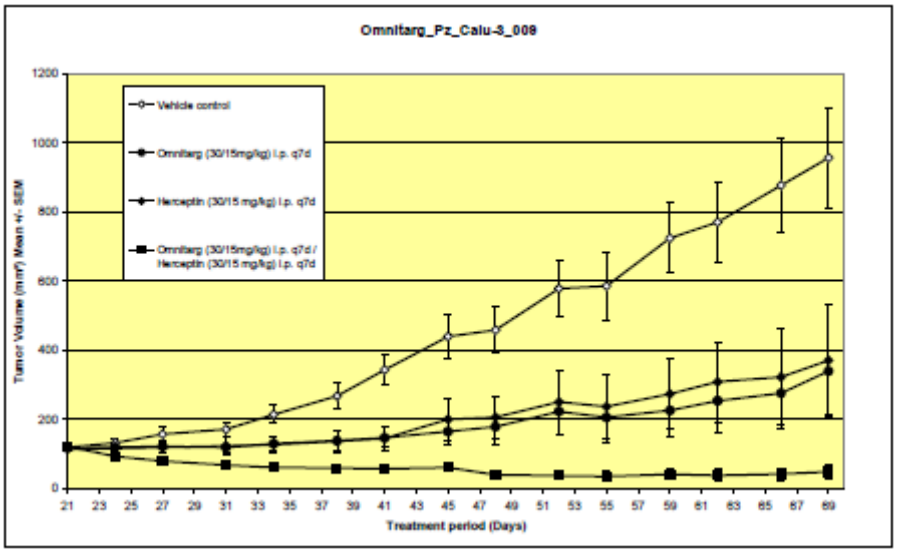

Summary: Both Pertuzumab and trastuzumab as single agents were significantly active against HER2 overexpressing (3^+) Calu-3 NSCLC xenografts (85% and 82% TGI, respectively). The combination of pertuzumab and trastuzumab was greater in activity (100 % TGI) compared to either single agent effects.

4.5 Overall Discussion and Conclusions

Pertuzumab is a recombinant humanized MAb produced in CHO cells. Pertuzumab binds to the dimerization domain of HER2 and blocks HER2 dimerization with other members of the HER receptor family, thereby inhibiting multiple HER signaling pathways that mediate cancer cell proliferation and survival. The binding of pertuzumab to the human and monkey ErbB2 extracellular domain was compared. The applicant showed that the amino acid homology of human and monkey ErbB2 was 99%. Therefore, the monkey was selected as the appropriate model for nonclinical evaluation. The applicant also evaluated the antitumor activity of pertuzumab in the Founder 2-134R tumor xenograft model, a model that is resistant to trastuzumab. The results show that tumor growth was inhibited by pertuzumab at doses of 30 - 90 mg/kg in this model. The in vivo activity of pertuzumab in patient-derived mammary, ovarian, and non-small cell lung cancer models was also evaluated. The results show that pertuzumab exhibited anti-tumor activity in 1/6 of the mammary cancer models, 1/4 of the ovarian cancer models, and 4/18 of the NSCLC cancer models.

The sponsor also submitted a number of peer-reviewed articles to support the understanding of the mechanism of action of pertuzumab. From these articles the following information was derived.

1. Pertuzumab and trastuzumab, through differing mechanisms of action, act in a complementary manner to promote tumor regression in HER2-positive breast cancer and NSCLC xenograft models (Scheuer et al. 2009).
2. Pertuzumab activates ADCC with identical potency as trastuzumab (Scheuer et al. 2009).
3. Pertuzumab, but not trastuzumab, blocked Heregulin (HRG)-induced activation of the PI3K cell survival pathway, as indicated by a lack of phosphorylation of a key enzyme (Akt) in this pathway (Agus et al. 2002).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 Absorption

Study title: Single dose intravenous and subcutaneous pharmacokinetic study of Pertuzumab in cynomolgus monkeys
Study no.: 00-564-1821
Study report location: eCTD 4.2.2.2

This study was conducted to characterize the pharmacokinetics (PK) of rhuMab 2C4 in cynomolgus monkeys following a single intravenous dose of 15, 50, and 150 mg/kg, or subcutaneous (SC) administration at 50 mg/kg. Blood samples were collected at predose and day 29.

Treatment Group	Test Article Dose (mg/kg)	Route of Administration	Dose Concentration[a] (mg/mL)	Number of Animals Males	Females
1	15	Intravenous	2.50	2	2
2	50	Intravenous	8.33	2	2
3	50	Subcutaneous	8.33	2	2
4	150	Intravenous	25.0	2	2
[a]Dose volume will be 6 mL/kg					

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The results indicated that following intravenous administration, rhuMab 2C4 was eliminated from plasma with a half-life of approximately 10 days. The plasma clearance and volume of distribution following IV administration were low (clearance = 5 mL/day/kg and volume of distribution, Vss = 70 ml/kg). Following SC administration, the peak plasma level (t_{max}) was reached within 2.28 days, and rhuMab 2C4 was slowly eliminated from plasma with a half-life of approximately 10 days. The subcutaneous bioavailability was 81.5%.

Table 11: Summary of pharmacokinetics parameters
(Excerpted from applicant's submission)

	Study 00-564-1821			
Species:	Cynomolgus Monkey (*Macaca fascicularis*)			
Gender (M/F)/Number of Animals:	M/2, F/2	M/2, F/2	M/2, F/2	M/2, F/2
Method of Administration:	IV	IV	SC	IV
Dose (mg/kg):	15.0	50.0	50.0	150
Sample:	Serum	Serum	Serum	Serum
Analyte:	Pertuzumab	Pertuzumab	Pertuzumab	Pertuzumab
Assay:	ELISA	ELISA	ELISA	ELISA
PK parameters (Mean± SD)				
t_{max} (day)	–	–	2.28 (0.286)	–
C_{max} (µg/mL)	403 (33.0)	1620 (122)	536 (41.2)	4580 (992)
Ka-HL (µg/mL)	–	–	0.986 (0.370)	–
α-HL (day)	0.893 (0.572)	0.513 (0.257)	1.62 (0.965)	0.320 (0.180)
β-HL (day)	10.4 (1.52)	9.89 (0.759)	10.6 (1.46)	10.0 (0.973)
AUC_{inf} (day • µg/mL)	3050 (405)	9640 (941)	7860 (673)	28700 (1600)
CL/F (mL/day/kg)	–	–	6.40 (0.561)	–
CL (mL/day/kg)	4.98 (0.625)	5.23 (0.553)	–	5.24 (0.287)
V_z/F (mL/kg)	–	–	53.0 (17.6)	–
V_c (mL/kg)	37.4 (3.06)	30.9 (2.32)	–	33.9 (7.07)
V_{ss} [mL/kg]	68.1 (6.25)	68.7 (5.87)	–	72.7 (6.34)
MRT (day)	13.8 (1.97)	13.2 (0.876)	–	13.9 (1.17)
Bioavailability (%)	–	–	81.5	–

Study title: Twenty-six week intravenous toxicity and toxicokinetic study with pertuzumab in cynomolgus monkeys with an 8-week recovery period
Study no.: 00-458-1821
Study report location: eCTD 4.2.2.2

The purpose of this study was to evaluate multiple dose PK of pertuzumab in cynomolgus monkeys following IV administration once per week for 26 weeks. Blood samples were collected at pre-dose (day 1) and at approximately 18, 24 (Day 2), 48 (Day 3), 72 (Day 4), and 120 (Day 6) hours post-dose, and on Days 8, 29, 71, 113, 148, and 183 pre-dose and approximately 1 hour post-dose.

Group	No. of Animals Male	No. of Animals Female	rhuMAb 2C4 Nominal Dose Level (mg/kg/dose)	Dose Concentration[a] (mg/mL)
1 (Control)[b]	6[c]	6[c]	0	0
2 (Low)	4	4	15	2.5
3 (Mid)	4	4	50	8.33
4 (High)	6[c]	6[c]	150	25

a The dose volume was 6.0 mL/kg.
b The control monkeys received the vehicle only.
c Two monkeys/sex in Groups 1 and 4 (based on survival) were designated as recovery animals and were dosed weekly with test article or vehicle for 26 weeks (183 days), after which dosing was discontinued, and the monkeys were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 8 weeks posttreatment (Day 240).

The results indicated that following intravenous administration, rhuMab 2C4 serum levels increased in a dose-proportional manner during days 0 – 7 and 0 - 182. Faster clearance was apparently observed in the 150 mg/kg dose group.

Table 12: Summary of pharmacokinetics parameters
(Excerpted from applicant's submission)

	Study 01-458-1821		
Species:	Cynomolgus Monkey (*Macaca fascicularis*)		
Gender (M/F)/Number of Animals:	M/4, F/4	M/4, F/4	M/6 [a], F/6 [a]
Method of Administration:	IV	IV	IV
Dose (mg/kg):	15.0	50.0	150
Sample:	Serum	Serum	Serum
Analyte:	Pertuzumab	Pertuzumab	Pertuzumab
Assay:	ELISA	ELISA	ELISA
Non-Compartmental PK parameters (Mean± SD)			
$t_{max\text{-}obs}$ (day)	119 (38.7)	135 (67.6)	118 (37.1)
$C_{max\text{-}obs}$ (µg/mL)	862 (101)	2820 (335)	7310 (1190)
C_{trough} Day 182 (µg/mL)	340 (86.8)	993 (373) [b]	1610 (599)
C_{peak} Day 182.04 (µg/mL)	789 (90.1)	2830 (318) [b]	5630 (787)
AUC (day • µg/mL)			
0–7 (day)	1270 (157)	3990 (330)	11000 (1030)
0–182 (day)	97100 (13300)	282000 (67100)	723000 (86600)
0–τ (day)	3730 (618)	12200 (2220) [b]	22400 (5210)
CL (mL/day/kg)	4.03 (0.598)	4.20 (0.657) [b]	7.18 (2.31)
Compartmental PK parameters (Mean± SD)			
α-HL (day)	–	–	1.12 (0.175)
β-HL (day)	–	–	10.6 (2.23)
Compartmental PK parameters (Mean± SD) (cont'd)			
AUC_{inf} (day • µg/mL)	–	–	24300 (4180)
CL (mL/day/kg)	–	–	6.31 (1.04)
V_c (mL/kg)	–	–	38.4 (1.79)
V_{ss} (mL/kg)	–	–	79.6 (8.26)
MRT (day)	–	–	13.0 (3.34)

Additional information: Pertuzumab exposure for the first cycle (Day 0–7) appears to increase linearly with increasing dose. However, based on the $AUC_{0\text{-}182}$ ratio between the 15 and the 150 mg/kg dose groups, multiple IV bolus administration of pertuzumab resulted in a faster clearance for the 150 mg/kg group, suggesting nonlinear PK following multiple IV bolus administration of pertuzumab at 150 mg/kg. Results are consistent with previous studies (Studies 00-564-1821 and 00-377-1821).

α-HL = initial half-life; β-HL = terminal half-life; AUC = area under the serum concentration–time curve (also: AUC from Day 0 to Day 7, Day 0 to Day 182, and Day 0 to τ); CL = clearance; $C_{max\text{-}obs}$ = observed maximum concentration; C_{peak} = peak plasma concentrations; C_{trough} = trough plasma concentrations; IV = intravenous; MRT = mean residence time; PK = pharmacokinetic; $t_{max\text{-}obs}$ = observed time to reach maximum concentration; V_c = volume of distribution of the central compartment; V_{ss} = volume of distribution at steady state.

[a] Two animals/sex (based on survival) were designated as recovery animals.

[b] n = 7; Animal I53886 euthanized on Day 126.

5.2 Distribution

No distribution studies were conducted with pertuzumab per current ICH S6(R1) guidance on the Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

5.3 Metabolism

No metabolism studies were conducted with Pertuzumab per current ICH S6(R1) guidance on the Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

5.4 Excretion

No excretion studies were conducted with Pertuzumab per current ICH S6(R1) guidance on the Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

5.5 Discussion and Conclusions

The pharmacokinetic profile of rhuMab 2C4 was studied in single-dose and multiple-dose studies in the cynomolgus monkey, the non-clinical species used for toxicity testing. Following single-dose IV and SC administration of rhuMab 2C4 in monkeys (15, 50, and 150 mg/kg IV and 50 mg/kg SC; 292 mg/m^2, 972 mg/m^2, 2916 mg/m^2, respectively) the half-life was approximately 10 days. At 50 mg/kg, exposure was lower and the rate of absorption was slower with SC administration (C_{max}= 536 μg/mL; t_{max} = 2 days; $AUC_{(inf)}$= 7860 day·μg/mL) than with intravenous administration (C_{max}= 1620 μg/mL; $AUC_{(inf)}$= 9640 μg/mL). The subcutaneous bioavailability was 81.5%.

Following multiple-dose administration of rhuMab 2C4 in monkeys, exposure increased in a dose-proportional manner during days 0 – 7 and 0 – 182. Faster clearance was apparently observed in the 150 mg/kg dose group.

6 General Toxicology

6.1 Single-dose Toxicity

A single-dose study was conducted to determine the PK and tolerability following IV and SC administration and to determine the bioavailability data of rhuMAb 2C4 administered subcutaneously. The PK and bioavailability data are detailed in the pharmacokinetic section, above. No significant drug-related clinical signs were noted. Local tolerance reactions were observed with SC administration, which were limited to scab/bleeding at the injection site.

6.2 Repeat-dose Toxicity

Study title: Twenty Six-Week Intravenous Toxicity and Toxicokinetic Study with rhuMAb 2C4* in Cynomolgus Monkeys with an 8-Week Recovery Period

Study no.:	6281-504
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	13 March 2002
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	rhuMAb 2C4 (lot No. M3-TOX57), 100 %

Key Study Findings

- Mortalities: 1 animal at 50 mg/kg dose was euthanized in extremis on day 126 with clinical signs including hunched posture and hypoactivity.
- Most notable clinical sign was non-formed feces (diarrhea)
- ↑ BUN was observed at all doses
- Inflammation was noted microscopically in the lungs, pancreas, and cecum at the 150 mg/kg dose

Methods

This study was designed to determine the toxicity and toxicokinetics (TK) of rhuMAb 2C4 when given intravenously to cynomolgus monkeys once weekly for 26 weeks, with an 8 week recovery period. The animals were assigned according to the table below.

(Excerpted from applicant's submission)

Group Designation and Dose Levels

Group	No. of Animals Male	No. of Animals Female	rhuMAb 2C4 Nominal Dose Level (mg/kg/dose)	Dose Concentration[a] (mg/mL)
1 (Control)[b]	6[c]	6[c]	0	0
2 (Low)	4	4	15	2.5
3 (Mid)	4	4	50	8.33
4 (High)	6[c]	6[c]	150	25

a The dose volume was 6.0 mL/kg.
b The control monkeys received the vehicle only.
c Two monkeys/sex in Groups 1 and 4 (based on survival) were designated as recovery animals and were dosed weekly with test article or vehicle for 26 weeks (183 days), after which dosing was discontinued, and the monkeys were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 8 weeks posttreatment (Day 240).

Observations and Results

Mortality

Animals were checked twice daily for mortality and moribundity, and at least once daily on non-dosing days

Animal #	Dose (mg/kg)	Sex	Day of Death	Observations	
				Reason	General (including pathology)
I53886	50	F	126	Euthanized *in extremis*	Hunched posture and hypoactive, no food consumption, diarrhea for 3 days, low body temperature, dehydration, and diarrhea. Mottled lung, material in lumen of colon/cecum

Clinical observations

Animals were checked at least once daily (AM) during acclimation on non-dosing days, and three times daily during dosing.

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	15	50	150	0	15	50	150
No. animals (T/R)	6	4	4	6	6	4	4	6
Non-formed feces	2	3	3	5	3	4	4	3

Bodyweight

Body weights were recorded at least twice prior to initiation of treatment (Weeks -2 and -1), on each day of dosing (prior to dosing), and weekly during recovery. There were no treatment-related effects.

Food consumption

Qualitative food consumption was assessed daily beginning at least 1 week prior to initiation of treatment. There were no treatment-related effects.

Physical Examination

Rectal body temperature, respiration rate, and heart rate data (from anesthetized monkeys) were examined once prior to study initiation (Week -1), and during Weeks 4, 16, and 26. There were no treatment-related effects.

Blood Pressure and Electrocardiograms

Blood pressure was measured once during Weeks 4, 16, and 26. Electrocardiogram measurements were taken twice prior to initiation of treatment (Days -7 and -4) and once during Weeks 4, 16, and 26. There were no treatment-related effects.

Ophthalmic Examinations

Examinations were performed once prior to initiation of treatment and prior to the terminal necropsy. There were no treatment-related effects.

Hematology

Samples were collected once during Week -1, Day 1 predose, and Days 30, 72, 114, 184, and 239 (recovery). There were no treatment-related effects.

Coagulation

Samples were collected once during Week -1, Day 1 predose, and Days 30, 72, 114, 184, and 239 (recovery). There were no treatment-related effects.

Clinical Chemistry

Samples were collected once during Week -1, Day 1 predose, and Days 30, 72, 114, 184, and 239 (recovery). Other than slight elevations in BUN that were not dose-related, there were no remarkable findings in the pertuzumab-treated monkeys when compared to the control group.

	Male				Female			
	Control	% change			Control	% change		
Dose (mg/kg)	0	15	50	150	0	15	50	150
No. animals (T/R)	4/2	4/0	4/0	4/2	6/2	4/0	4/0	6/2
BUN								
Day 30	18	-	-	-	-	-	-	-
Day 72	17	↑53*	↑53*	↑59*				
Day 114	20	↑30*	↑30	↑35*				
Day 184	18	↑44*	↑50*	↑61*				
Day 239	25	NA	NA	-				

T: Terminal necropsy; R: Recovery necropsy; NA: not applicable; BUN: blood urea nitrogen, *: statistically significant compared to controls (P≤0.05)

Thyroid function

Blood samples were obtained at pre-dose, day 5, weeks 13, 26, 39, and 43 (recovery). No drug-related changes were observed.

Troponin

Samples were collected from male monkeys scheduled for terminal necropsy (Day 184). No drug-related changes were observed

Urinalysis

Samples were collected from male monkeys scheduled for terminal necropsy (Day 184). No drug-related changes were observed

Antibody Analysis

(*Excerpted from applicant's submission*)

Samples were collected once during acclimation (within 1 week of initiation of treatment), pre-dose on Days 1, 29, 71, 113, and 183 (last day of dosing) during treatment, and Days 211 and 239 during recovery. Samples were subjected to a rhuMab 2C4 Antibody ELISA for the detection of antibody response to rhuMab 2C4. There were no positive antibody titers against rhuMab 2C4 detected.

The sponsor noted that they could not exclude the possibility of rhuMab 2C4 present in the serum samples interfering with the ELISA assay for anti-rhu Mab 2C4, due to rhuMab 2C4 levels in some test samples greater than 10 µg/mL (the limit of sensitivity of the ELISA assay).

Sperm Analysis

(*Excerpted from applicant's submission*)

Due to the young age and therefore sexual immaturity of the animals, there were only two male monkeys from which a sperm motility sample could be obtained (one male from the control and another male from the 150 mg/kg/day group). Sperm counts were low in both animals, and data were also highly variable between the two groups. For all other male animals, there were no sperm present for analysis. Therefore, an evaluation of the potential of adverse effects of rhuMab 2C4 on sperm motility, epididymal sperm count, and sperm morphology was not possible.

Testosterone

Samples were collected from males on Day 1 predose, and Days 114, 184, and 239 (recovery). There were no treatment-related effects, as values were consistent with those expected for sexually immature monkeys.

Gross Pathology

Macroscopic findings - Terminal		Male				Female			
Dose (mg/kg)		0	15	50	150	0	15	50	150
Terminal Sacrifice									
Lung	Failure to collapse	-	-	-	-	-	-	1	1
	Interlobar adhesion	-	-	-	-	-	-	-	1
	Adhesion	-	-	-	-	-	-	-	1
Recovery sacrifice									
There were no treatment-related effects									

Organ Weights
Measurements were taken at scheduled necropsies after 26 weeks of treatment (Days 184 and 185), and after approximately 8 weeks of recovery (Day 240). There were no treatment-related effects.

Histopathology

Adequate Battery (yes)
Peer Review (yes)

Histological Findings:

Macroscopic findings - Terminal		Male				Female			
Dose (mg/kg)		0	15	50	150	0	15	50	150
Terminal Sacrifice									
Lung	Granulomatous inflammation, focal, diaphragm musculature	-	-	-	-	-	-	-	1
Pancreas	Inflammation, chronic	-	-	-	-	-	-	-	2
	Focal congestion, islet cell	-	-	-	-	-	-	-	1
Cecum	Inflammation, chronic active	-	-	-	1	-	-	-	1
	Ulceration	-	-	-	1	-	-	-	1
Recovery sacrifice									
There were no treatment-related effects reported.									

Toxicokinetics

Results are reported in the PK section, above.

Study Summary:

RhuMab 2C4 was administered to male and female monkeys via IV, bolus injection, once weekly for 26 weeks at doses of 0, 15, 50, and 150 mg/kg, followed by a 8-week recovery period. RhuMab 2C4 exposure generally increased with dose. Faster clearance was apparently observed in the 150 mg/kg dose group.

One female in the 50 mg/kg dose group was euthanized in extremis on day 126, with clinical signs including hunched posture and hypoactivity, diarrhea, low body temperature, and dehydration. Pathology changes included a mottled lung, and material in the lumen of the colon and cecum.

In surviving animals, non-formed feces (diarrhea) were the most notable clinical signs. Increased blood urea nitrogen levels were observed in all treatment groups. Gross pathology changes were noted in the lungs of 1-2 females. These changes included failure to collapse upon opening of the chest cavity, and interlobular adhesions. Inflammation was noted microscopically in the lungs, pancreas, and cecum at the 150 mg/kg dose. Effects were generally reversible, as there were no treatment-related histopathology findings after the 8-week recovery period.

6.3 Discussion and Conclusion

The toxicological profile of pertuzumab suggests that pertuzumab is well-tolerated in monkeys. Nonclinical findings in the monkey show target organ toxicities in the lung and gastrointestinal tract, which are expected based on the distribution of the Her2/neu target antigen shown in the tissue cross-reactivity studies (see below).

7 Genetic Toxicology

No genotoxicity studies were conducted with pertuzumab, per current ICH S6(R1) guidance on the Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. Pertuzumab is a protein (monoclonal antibody) and is not expected to interact directly with DNA.

8 Carcinogenicity

No carcinogenicity studies with pertuzumab were conducted or are planned to support the current indication, as per the recent ICH S6(R1) guidance on the Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

9 Reproductive and Developmental Toxicology

9.2 Embryonic Fetal Development

Study title: Embryo-Fetal Development Study of Pertuzumab Administered by Intravenous Injection to Pregnant Cynomolgus Monkeys

Study no.:	07-0925
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	04 Feb 08
GLP compliance:	Statement included and signed
QA statement:	Statement included and signed
Drug, lot #, and % purity:	rhuMab 2C4, Lot # 704321, 100%
Route of administration:	intravenous

Key Study Findings

- Pharmacological effects of the drug were observed at all doses
- Fetal lethality was observed at all dose levels, between gestation day (GD) 25-70
- Fetal effects included reduced fetal weight at ≥ 30/10 mg/kg; decreased head width, circumference, hindfoot length at 100/33.3 mg/kg, decreased crown rump

and tail length ≥ 30/10 mg/kg, as well as decreased heart, lung and kidney weights at ≥ 30/10 mg/kg

- Adverse embryo-fetal effects included the following: paw hyperextension/hyperflexion, microtia, small lungs, thin walls in the ventricular regions of the heart, fused caudal and sacral vertebra, and supernumerary lumber vertebra
- Malformations & variations were observed at ≥ 100/33 mg/kg dose level
- Exposure was reported at delivery in offspring at levels of 30 to 86 % of the maternal blood levels
- A NOAEL for embryofetal or teratogenic effects could not be identified

Design:

This study was designed to assess the maternal and fetal effects of pertuzumab when administered to pregnant monkeys. Forty-eight females weighing 2.54 – 4.45 kg and 4 – 7 years of age were naturally impregnated by 40 breeder males and randomly distributed among 4 treatment groups. Presumed pregnant females were administered pertuzumab for 9 doses beginning at gestation day (GD) 19 with a loading dose, with subsequent doses given on GDs 26, 29, 33, 36, 40, 43, 47 and 50. Planned caesarean sections performed on GD 100-102. Group assignments are shown in the following table.

Group Assignments: One control group and three test article groups

Group	Test and Control Articles	Loading Dose (mg/kg) on GD19	Subsequent Dose (mg/kg)	Loading Dose Concentration (mg/mL)	Subsequent Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Pregnant Animals* (Animal No.)
1	Vehicle [a)]	0	0	0	0	5	12 (10001 to 10012)
2	Pertuzumab	30	10	6	2	5	12 (10101 to 10112)
3	Pertuzumab	100	33.3	20	6.66	5	12 (10201 to 10212)
4	Pertuzumab	150	100	30	20	5	12 (10301 to 10312)

a) Control article was administered in the same manner as test article

*: The total number of animals in each group was 12. Additional animals were not added to any group to replace animals that had aborted.

GD: Day of gestation

On 11 occasions, deviations in administration volumes due to calculation errors were reported. The actual administration volumes were ± 0.05 mL for 10 of the animals treated. However, one animal was given an additional 2.20 mL. The applicant suggests that this deviation did not appear to affect study outcome.

Observations and Results

Mortality

Animals were checked twice daily for clinical signs and mortality.

Dams: No treatment-related mortalities occurred during this study.

F1: Twenty abortions or embryo-fetal deaths were observed in total (LD: 4/12; MD: 6/12; HD: 10/12). No abortions were reported in the vehicle control-treated animals.

(Excerpted from applicant)

Dose group	Animal No.	GD	Type	Dose group	Animal No.	GD	Type
30/10 mg/kg	10101	70	F	150/100 mg/kg	10301	70	A
	10103	70	F		10303	30	A
	10105	25	A		10305e	35	E
	10112	40	E		10306f	51	F
100/33.3 mg/kg	10201	30	A		10307e	40	E
	10202	40	A		10308f	70	F
	10204	40	E		10309	40	A
	10205	51	F		10310	51	F
	10207	40	E		10311	25	A
	10209	35	E		10312e	35	E

GD: gestation day; A: abortion; E: embryonic death; F: fetal death

Clinical Signs

Unremarkable

Body Weights

Unremarkable

Food Consumption

Unremarkable

Hematology

Dams: Blood was collected from peripheral vein before dose administration (GDs 19 & 50) and at Caesarean section (GD 100)

	Dams			
	Control	% change		
Dose (mg/kg)	0	30/10	100/33.3	150/100
Leukocytes (10^6/mm)				
GD19	-	-	-	-
GD50	-	-	-	-
Caesarean	8.6	-	↑31*	↑32
Basophils (10^3/mm;%)				
GD19	-	-	-	-
GD50	0.33	-	↑11	↑27
Caesarean	0.30	-	↑25	↑45*
Basophils (10^3/mm;abs)				
GD19	-	-	-	-
GD50	0.04	-	↑22	↑26
Caesarean	0.03	-	↑48	↑66*
Lymphocytes (10^3/mm;%)				
GD19	-	-	-	-
GD50	-	-	-	-
Caesarean	49.06	-	-	↑33
Lymphocytes (10^3/mm;abs)				
GD19	-	-	-	-
GD50	-	-	-	-
Caesarean	4.17	↑11	↑32	↑56**
Monocytes (10^3/mm;abs)				
GD19	-	-	-	-
GD50	-	-	-	-
Caesarean	6.80	↓29	↓35	↓109

Abs: absolute; -: no change, *: statistically significant compared to controls (P≤0.05); **: statistically significant compared to controls (P≤0.01)

Clinical chemistry

Dams: Blood was collected from peripheral vein before dose administration (GDs 19 & 50) and at Caesarean section (GD 100)

	Dams			
	Control	% change		
Dose (mg/kg)	0	30/10	100/33.3	150/100
Glucose				
GD19	-	-	-	-
GD50	59	↑15	↑24	↑33**
Caesarean	49	↑18*	↑32**	↑34*
Blood Urea Nitrogen				
GD19	-	-	-	-
GD50	19	↑17	↑19	↑44**
Caesarean	19	↑12	↑33**	↑23*

-: no change, *: statistically significant compared to controls (P≤0.05); **: statistically significant compared to controls (P≤0.01)

Fetal Examinations and External Measurements

	Fetus			
	VC	% change		
Dose (mg/kg)	0	30/10	100/33.3	150/100
Fetal Weight (gm)	114	↓11	↓31*	↓47**
Head width (mm)	36	-	↓12*	↓11
Head circumference	144	-	↓7*	↓8**
Crown rump length	125	↓6*	↓12*	↓15**
Tail length	117	-	↓12*	↓33**
Chest circumference	85	-	↓14**	↓17**
Hindfoot length	36	↓9*	↓18**	↓19*
Amniotic fluid				
-volume (mL)	66	↓72	↓18-fold**	↓18-fold*
-oligohydramnios (incidence)	0	↑25	↑100*	↑100
-yellow color (incidence)	0	↑12.5	↑67	↑100

-: no change, *: statistically significant compared to controls (P≤0.05); **: statistically significant compared to controls (P≤0.01); external measurements are reported as mm for the control group and % change for the treatment groups (mL)

Relative Fetal Organ Weight

	Fetus			
	Weight (mg/g BW)	% change		
Dose (mg/kg)	0	30/10	100/33.3	150/100
Brain	131	↑12*	↑18**	↑24**
Lungs	21	↓17	↓75**	↓2-fold**
Kidney (left)	3.5	↓62**	↓87**	↓89**
Adrenal (left)	0.29	↑12	↑12	↑42*
Adrenal (Right)	0.26	-	↑21	↑42*

-: no change, *: statistically significant compared to controls (P≤0.05); **: statistically significant compared to controls (P≤0.01)

Fetal Malformations

	Fetus			
Dose (mg/kg)	0	30/10	100/33.3	150/100
No. of fetuses	12	8	6	2
External abnormalities				
Paw hyperextension *%*	-	-	3 *50%*	1 *50%*
Paw hyperflexion *%*	-	-	1 *16.7%*	1 *50%*
Microtia *%*	-	-	-	1 *50%*
Visceral abnormalities				
Small Lung *%*	-	-	1 *16.7%*	1 *50%*
Thin ventricular wall in the heart *%*	-	-	1 *16.7%*	1 *50%*
Skeletal abnormalities				
Fused caudal and sacral vertebrae *%*	-	-	-	1 *50%*
Skeletal variations				
Supernumerary lumber vertebrae *%*	-	-	-	1 *50%*
Total abnormalities	-	-	4	6
Total variation	-	-	-	1

-: no change

Fetal Histopathology

Microscopic findings -		Fetus			
Dose (mg/kg)		0	30/10	100/33.3	150/100
Kidney (left)	Hypoplasia, renal tubule				
	-very slight		1	-	-
	-slight	-	5	1	-
	-moderate		4	3	-
	-marked		-	2	2
	Hypoplasia, pelvis				
	-very slight		6	-	-
	-slight	-	2	4	-
	-moderate		-	1	-
	-marked		-	1	2
	Hypoplasia, glomerulus				
	-very slight		7	-	-
	-slight	-	1	4	-
	-moderate		-	2	2
	-marked		-	-	-
	Hypoplasia, collecting tube				
	-very slight		1	-	-
	-slight	-	-	-	-
	-moderate		2	3	-
	-marked		-	3	2
Kidney (right)	Hypoplasia, renal tubule				
	-very slight		2	-	-
	-slight	-	-	1	-
	-moderate		4	4	-
	-marked		2	1	2
	Hypoplasia, pelvis				
	-very slight		6	-	-
	-slight	-	2	4	-
	-moderate		-	1	-
	-marked		-	1	2
	Hypoplasia, glomerulus				
	-very slight		6	-	-
	-slight	-	2	4	-
	-moderate		-	2	2
	-marked		-	-	-
	Hypoplasia, collecting tube				
	-very slight		1	-	-
	-slight	-	-	-	-
	-moderate		2	3	-
	-marked		-	3	2

Toxicokinetics

Methods

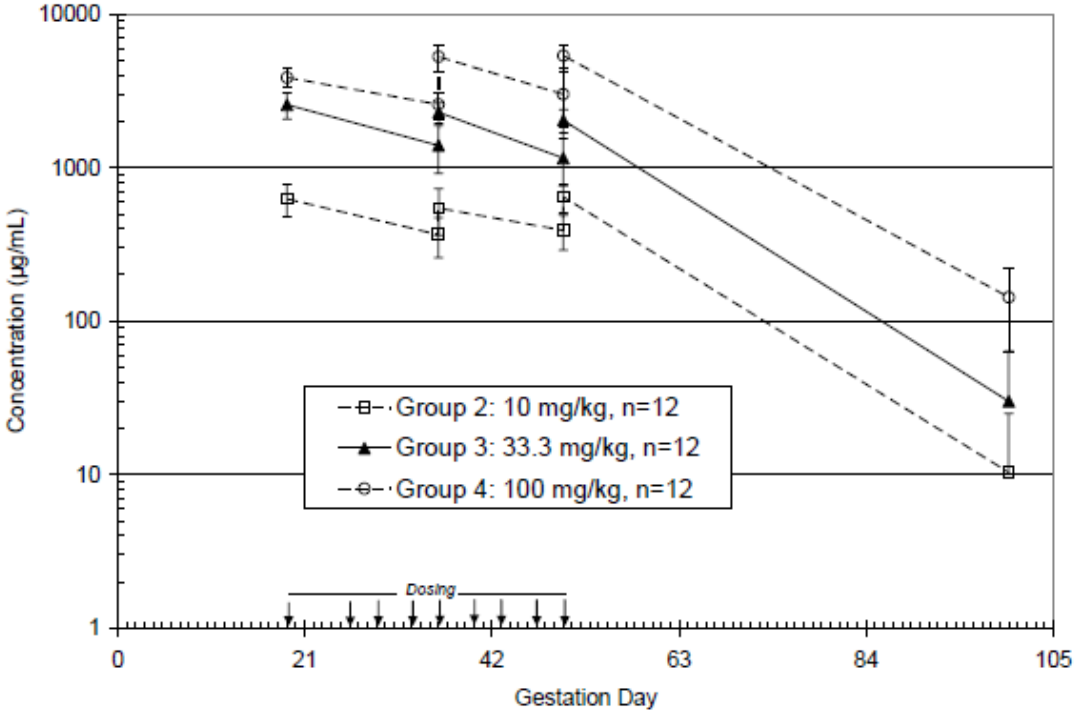
Twelve pregnant cynomolgus monkeys in each of four groups were given eight intravenous (IV) doses of pertuzumab vehicle (Group 1) or 10, 33.3, or 100 mg/kg pertuzumab (Groups 2-4, respectively) on Days 26, 29, 33, 36, 40, 43, 47, and 50 of gestation, following an initial loading dose (vehicle, 30, 100, or 150 mg/kg for Groups 1, 2, 3, and 4, respectively) administered on Day 19 of gestation.

Blood samples for TK analysis were collected pre-dose and 30 minutes post-dose on Days 19, 36, and 50 of gestation, and on the day of Caesarean sectioning (between Days 100 and 102 of gestation). Fetal serum levels were also measured.

Results

- Serum pertuzumab concentrations increased in a dose proportional manner between all doses tested in pregnant monkeys and fetuses
- Maternal serum levels of pertuzumab declined by the time of Caesarean section
- Ratios of fetal to maternal pertuzumab levels were comparable (0.294, 0.399, and 0.338, respectively)

Mean (±SD) Serum Pertuzumab Concentrations following IV Administration in Pregnant Cynomolgus Monkeys



Group Mean (±SD) Summary of Fetal Serum Exposure to Pertuzumab

Dose Group	Dose Level (mg/kg)	C_{fetal} (µg/mL)	$C_{last, maternal}$ (µg/mL)	Ratio of Fetal to Maternal Serum Concentration
2	10	8.88±10.1	10.3±14.7	0.294±0.235
3	33.3	10.5±10.7	30.0±34.0	0.399±0.243
4	100	42.5±6.29	143±80.5	0.338±0.146

C_{fetal} = fetal serum concentration at the time of Caesarean sectioning; $C_{last, maternal}$ = final maternal observed serum concentration.

Animal to Human Exposure Multiples

Dose (mg/kg)	AUC 0 - ∞ (µg/mL)	Human Exposure AUC 0 - ∞ (µg/mL)	Multiples of Human Exposure
10	644	2660	0.242
33.3	2030		0.763
100	5360		2.02

Study Summary

RhuMab 2C4 was administered to pregnant monkeys via IV administration on GD 19 at doses of 0, 30, 100, and 150 mg/kg with subsequent, bi-weekly doses of 10, 33.3, and 100 mg/kg, respectively, until GD 50. No maternal toxicity was noted. Fetal lethality and effects were observed at doses ≥ 30/10 mg/kg, beginning on GD 25. Fetal effects included reduced fetal weight at ≥ 30/10 mg/kg; head width, circumference, hindfoot length ≥ 100/33.3 mg/kg, crown rump and tail length ≥ 100/33.3 mg/kg, decreased lung and kidney weight ≥ 100/33.3 mg/kg, and hypoplasia in the kidney ≥ 30/10 mg/kg. Malformations & variations were observed at ≥ 100/33.3 mg/kg and included paw hyperextension/hyperflexion, microtia, small lungs, thin walls in the ventricular, fused caudal and sacral vertebra, and supernumerary lumber vertebra. Exposure was reported at delivery in offspring at levels of 30 - 86 % of the maternal blood levels. Due to fetal toxicities, a NOAEL was not established.

9.4 Discussion and Conclusions:

Administration of pertuzumab by IV injection to monkeys during gestation was associated with embryo-fetal deaths and malformations. Beginning on GD 25, fetal lethality was observed at doses ≥ 30/10 mg/kg (31 % lower than the expected exposure estimated in humans after IV administration at therapeutic doses, by AUC). Fetal effects were also noted at dose levels ≥ 30/10 mg/kg. Malformations were observed at both the 100/33.3 and the 150/100 mg/kg dose levels; the latter dose level provides an

exposure which is approximately 2-fold greater than the expected therapeutic exposure in patients, based on AUC. These malformations included paw hyperextension/hyperflexion, microtia, small lungs, thin walls in the ventricular regions of the heart, fused caudal and sacral vertebra, and supernumerary lumber vertebra. Thus, administration of pertuzumab during pregnancy may pose a risk to the human fetus.

10 Special Toxicology Studies

10.1 Other Toxicity Studies

Study title: Hemolytic Potential and Blood Compatibility of rhuMAb 2C4*
Study no.: 00-562-1821
Study report location: eCTD 4.2.3.7

Methods: Hemolytic potential and blood compatibility tests were performed with rhuMAb 2C4 (Lot # M3-TOX28) at concentrations of 21.6, 10.8, 5.4 mg/mL. After equal dilution with whole blood, serum, or plasma, the final concentrations were 10.8, 5.4, and 2.7 mg rhuMAb 2C4/mL. Testing was also performed with rhuMAb 2C4 Vehicle (Lot # M3-TOX30).

The hemolytic potential was evaluated by measuring the concentration of soluble hemoglobin in the supernatant after mixing equal volumes of rhuMAb 2C4 or rhuMAb 2C4 vehicle with cynomolgus monkey or human whole blood.

Test mixtures were incubated for 40 minutes at 37°C.

Compatibility with serum and plasma was determined by the absence of precipitation or coagulation in mixtures of equal volumes of rhuMAb 2C4 or rhuMAb 2C4 Vehicle and cynomolgus monkey or human serum or plasma that had been incubated for 30 minutes at room temperature (21.7°C).

Results: RhuMAb 2C4 did not cause hemolysis or precipitation/coagulation when mixed with an equal volume of cynomolgus monkey or human whole blood, serum or plasma.

Conclusion: RhuMAb 2C4 is compatible with cynomolgus monkey and human erythrocytes, plasma and serum at concentrations as high as 21.6 mg rhuMAb 2C4/mL.

Study title: Cross-Reactivity of rhuMAb 2C4 with Normal Human Tissues
Study no.: 01-014-1821
Study report location: eCTD 4.2.3.7

Objective: To evaluate the cross-reactivity of Pertuzumab (FITC-conjugated) with cryosections of normal human tissues.

Method: Immunoperoxidase Staining (using horseradish peroxidase conjugated 2° Ab directed against FITC)

Controls:
-positive control pellets (SKBR-3, MDA 231, MDA 175)
-positive control tissue: human mammary gland carcinoma (HT314)
-negative control tissue: human kidney glomeruli (HT267)
-negative control antibody: IgG_1-FITC

Results: Reactivity of pertuzumab was noted on the positive control pellets and tissue, but not on the negative control tissue nor with the negative control antibody, confirming the validity assay. Reactivity was observed at membrane surfaces of the human tonsil, parathyroid, mammary gland, haired skin, ureter, urinary bladder, placenta, and kidney tissues. Cytoplasmic staining was seen in the salivary and prostate glands, the stomach, haired skin, and thymic cyst.

Study title: Cross-Reactivity of rhuMAb 2C4* with Normal Cynomolgous Monkey Tissues
Study no.: 01-015-1821
Study report location: eCTD 4.2.3.7

Objective: To evaluate the cross-reactivity and tissue binding patterns of Pertuzumab in normal cynomolgus monkeys.

Method: Immunoperoxidase Staining

Controls:
-positive control pellets: (SKBR-3, MDA 231, MDA 175)
-positive control tissue: human mammary gland carcinoma (HT314)
-negative control tissue: cynomolgus monkey kidney glomeruli (HT267)
-negative control antibody: IgG_1-myeloma protein

Results: *(Excerpted from applicant's submission)*

Reactivity of pertuzumab was noted on the positive control pellets, but not on the negative control tissue/antibody, confirming the validity of the assay. Reactivity at the cell surface was observed in the haired skin (sweat glands; acinar and ductal epithelium, 4 of 4 donors); sebaceous gland epithelium, (2 of 2 donors at high dose only); mammary gland (acinar and ductal epithelium, 4 of 4 donors); placenta chorionic

epithelium and syncytiotrophoblasts, (2 of 2 donors); ureter (uretal transitional epithelium, 3 of 3 donors); renal tubular epithelium, (3 of 3 donors); urinary bladder (transitional epithelium, 3 of 3 donors); and prostate gland ductal and acinar epithelium, (1 of 3 donors). Predominantly cytoplasmic staining reactivity of the test article, rhuMAb 2C4, with normal cynomolgus monkey tissues was also identified in the following tissues: adenohypophysis secretory cells, (3 of 3 donors) and prostate gland ductal and acinar epithelium, (2 of 3 donors).

10.3 Discussion and Conclusions:

Pertuzumab did not cause lysis of cynomolgus monkey or human erythrocytes and was compatible with cynomolgus monkey and human serum and plasma in in vitro test systems. Cell surface staining with pertuzumab in human tissues was noted in the haired skin, placenta, parathyroid gland, tonsil, mammary gland, ureter, and urinary bladder tissues. Cytoplasmic staining was noted in the salivary gland and prostate gland as well as in the stomach, haired skin, and thymic cyst of human tissues. In monkey tissues, cell surface staining was noted in the sweat and sebaceous gland, mammary gland, placenta, ureter, urinary bladder, and prostate gland. Cytoplasmic staining was noted in adenohypophysis and salivary gland.

11 Integrated Summary and Safety Evaluation

The non-clinical studies submitted to this BLA support the use of pertuzumab intravenously for the treatment of HER2-positive metastatic or locally recurrent, unresectable breast cancer.

See the EXECUTIVE SUMMARY, Page 4, for an overall summary of nonclinical findings.

12 Appendix/Attachments

None

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

/s/

KIMBERLY R RINGGOLD
05/15/2012

ANNE M PILARO
05/15/2012
I concur with the reviewer's conclusion and recommendation that based on the nonclinical data submitted with this application, this BLA may be approved for its intended indication.