CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761088Orig1s000

NON-CLINICAL REVIEW(S)

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:	761088
Supporting document/s:	2, 34
Applicant's letter date:	28 May 2018
CDER stamp date:	29 May 2018
Product:	Truxima (rituximab-abbs, CT-P10)
Indication:	Non-Hodgkin's Lymphoma (NHL) • Relapsed or refractory, low grade or follicular, CD20-positive B-cell NHL as a single agent. • Previously untreated follicular, CD20-positive, B-cell NHL in combination with first line chemotherapy and, in patients achieving a complete or partial response to a rituximab product in combination with chemotherapy, as single-agent maintenance therapy. • Non-progressing (including stable disease), low-grade, CD20-positive, B-cell NHL as a single agent after first-line cyclophosphamide, vincristine, and prednisone (CVP) chemotherapy.
Applicant:	Celltrion Inc
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer:	Michael L Manning, PhD
Supervisor/Team Leader:	Christopher M Sheth, PhD
Division Director:	John Leighton, PhD (DHOT) Ann Farrell, MD (DHP)
Project Manager:	Esther Park, PharmD

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Celltrion developed Truxima (rituximab-abbs, CT-P10) as a proposed biosimilar product to US-licensed Rituxan (rituximab). Rituxan was approved by FDA in 1997 (BLA 103705). Rituximab is approved in the European Union (EU) and marketed under the trade name MabThera. BLA 761088 was submitted on 28 April 2017 for the purpose of licensure of rituximab-abbs under section 351(k) of the Public Health Service Act. A Complete Response (CR) letter was sent on 28 February 2018. BLA 761088 was resubmitted on 28 May 2018.

The proposed formulation and dosing regimen of rituximab-abbs are identical to that of US-licensed Rituxan. The applicant conducted a stepwise biosimilar development program intended to demonstrate that rituximab-abbs is highly similar to US-licensed Rituxan, with no clinically meaningful differences in terms of safety, purity, or potency. The biosimilar development program consists of a definitive analytical similarity assessment and abbreviated nonclinical and clinical programs.

1.2 Brief Discussion of Nonclinical Findings

Rituximab is a chimeric murine/human monoclonal antibody that binds CD20, which is primarily found on the surface of B cells. After binding to CD20 on the cell surface, rituximab is believed to mediate B cell lysis, possibly through antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), and/or direct induction of apoptosis. The applicant conducted an in vitro 3-way similarity assessment including studies for cell-based CD20 binding affinity, FcγR binding affinity, FcRn binding affinity, C1q binding affinity, ADCC, ADCP, CDC, and apoptosis to demonstrate similarity between CT-P10, US-licensed Rituxan, and EU-MabThera. No issues were identified that would affect the nonclinical safety assessment for CT-P10.

The tissue cross-reactivity profiles of CT-P10 and EU-MabThera were evaluated in a panel of tissues of human origin. The staining profiles of CT-P10 and EU-MabThera were comparable with only minor differences in staining intensity and consistent with the known expression of CD20 on B cells.

The comparative toxicity, toxicokinetics (TK), and pharmacodynamics (PD) of CT-P10 and EU-MabThera were evaluated in an 8-week repeat-dose toxicity study in cynomolgus monkeys. The study was terminated early following the premature sacrifice of two animals in moribund condition; females received 5 of 8 planned doses and males received 6 of 8 planned doses. Morbidity was attributed to immune reactions which are expected in cynomolgus monkeys; the overall incidence of immunogenicity was similar between treatment arms. Among the surviving animals, clinical signs, clinical pathology, organ weights, and histopathological findings were similar between treatment arms. Systemic exposure was similar between treatment arms on Day 1. B cell depletion, the expected PD activity of rituximab, was of similar magnitude between treatment arms.

The results of the in vitro 3-way similarity assessment, the tissue cross-reactivity study, and the comparative repeat-dose toxicity study in cynomolgus monkeys support the demonstration of biosimilarity between rituximab-abbs and US-licensed Rituxan.

1.3 Recommendations

1.3.1 Approvability

From the perspective of nonclinical pharmacology and toxicology rituximab-abbs may be approved for the proposed indications.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

The applicant is not seeking all the indications currently approved for US-licensed Rituxan; otherwise, the rituximab-abbs label is comparable to the label of US-licensed Rituxan.

2 Drug Information

2.1 Drug

CAS Registry Number	174722-31-7
Proprietary Name	Truxima
Generic Name	rituximab-abbs
Code Name	CT-P10
Molecular Formula	Light chains: $C_{1016}H_{1577}N_{273}O_{328}S_6$ Heavy chains: $C_{2191}H_{3377}N_{575}O_{675}S_{16}$
Molecular Weight	~147 kDa (glycosylated)
Biochemical Description	CT-P10 is a chimeric murine/human monoclonal antibody consisting of a glycosylated IgG1 kappa immunoglobulin with murine light and heavy chain variable regions (Fab domain) and human kappa and gamma-1 constant regions (Fc domain) (see Figure 1). Each light and heavy chain contains 213 and 450 amino acids, respectively; the amino acid sequences are presented in Figure 2. Each heavy chain contains a single N-linked glycosylation site (asparagine-301). CT-P10 is directed against the CD20 antigen.
Pharmacologic Class	CD20-directed cytolytic antibody

Figure 1: Schematic diagram of CT-P10

(Adapted from applicant's submission)

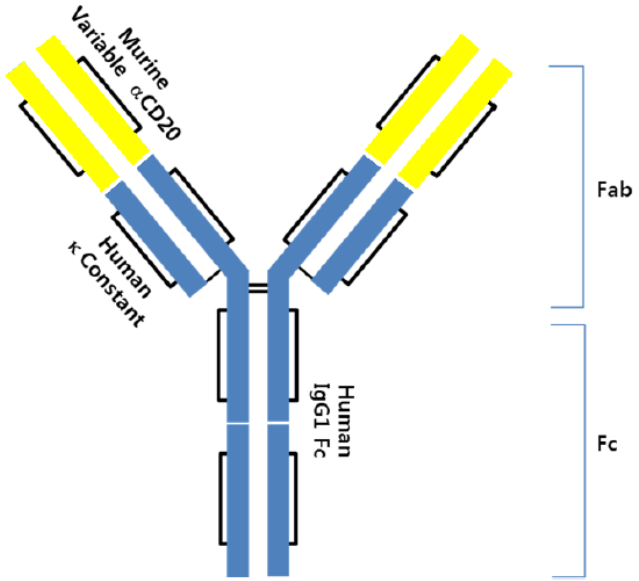

Figure 2: Amino acid sequence of CT-P10 heavy and light chains

(Adapted from applicant's submission)

Heavy chain:

```
  1  QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNMHWVKQT PGRGLEWIGA
 51  IYPGNGDTSY NQKFKGKATL TADKSSSTAY MQLSSLTSED SAVYYCARST
101  YYGGDWYFNV WGAGTTVTVS AASTKGPSVF PLAPSSKSTS GGTAALGCLV
151  KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ
201  TYICNVNHKP SNTKVDKKAE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK
251  PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
301  N#STYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
351  QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP
401  VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG
451  (K*)
```

Light chain:

```
  1  QIVLSQSPAI LSASPGEKVT MTCRASSSVS YIHWFQQKPG SSPKPWIYAT
 51  SNLASGVPVR FSGSGSGTSY SLTISRVEAE DAATYYCQQW TSNPPTFGGG
101  TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD
151  NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL
201  SSPVTKSFNR GEC
```

Yellow = variable region; blue = constant region; # glycosylation site; (K*) = C terminal lysine which is predominantly removed post-translationally

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 120885 (CT-P10)
BLA 103705 (Rituxan)

2.3 Drug Formulation

Rituximab-abbs is supplied as a sterile liquid solution in single-use vials containing 500 mg (50 mL) or 100 mg (10 mL) rituximab at pH 6.5. The formulation of rituximab-abbs is identical to US-licensed Rituxan. The composition of the rituximab-abbs drug product, including the function and reference standard of each component, is listed in Table 1.

Table 1: Composition of the rituximab-abbs drug product

Ingredient	Quantity		Function	Compendial reference
	500 mg / vial	100 mg / vial		
Rituximab	500 mg	100 mg	Active ingredient	In house
Sodium Chloride	450 mg	90 mg	(b) (4)	USP/Ph.Eur.
Tri-Sodium Citrate Dihydrate	368 mg	73.5 mg		USP/Ph.Eur.
Polysorbate 80	35 mg	7 mg		NF/Ph.Eur.
Water for Injection	q.s. to 50 mL	q.s. to 10 mL		USP/Ph.Eur.

USP = United States Pharmacopoeia; Ph.Eur. = European Pharmacopoeia; NF = National Formulary; q.s. = quantum satis

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The applicant is not seeking all the indications currently approved for US-licensed Rituxan. The proposed dosing regimen is identical to that of US-licensed Rituxan.

2.7 Regulatory Background

A pre-IND meeting (BPD-2) was held on 19 March 2014 to discuss development of CT-P10 as a proposed biosimilar to US-licensed Rituxan. The nonclinical team advised the applicant the comparative pharmacology and toxicology studies appeared sufficient to support clinical development of CT-P10 as a proposed biosimilar product under an IND. Data demonstrating comparability between the product used in the nonclinical studies and the product to be used in the proposed comparative clinical studies would be required.

IND 120885 was submitted on 5 June 2014; a Study May Proceed letter was sent to the applicant on 3 July 2014.

No nonclinical issues were discussed at the following meetings:

- BPD-2 meeting held on 13 July 2015.
- BPD-2 meeting held on 12 February 2016.
- BPD-2 and BPD-3 meetings held on 17 January 2017.

- BPD-4 meeting scheduled for 26 April 2017, but cancelled at applicant's request after receiving preliminary comments dated 20 April 2017.

BLA 761088 was submitted on 28 April 2017. The nonclinical team agreed rituximab-abbs could be approved for the proposed indication using a totality-of-the-evidence approach; however, BLA 761088 received a CR letter on 28 February 2018.

BLA 761088 was resubmitted on 28 May 2018.

3 Studies Submitted

3.1 Studies Reviewed

Study number	Study title	eCTD location
ZIP0002	CT-P10: Cross Reactivity Study in Human Tissues	4.2.1.1.
ZIP0003	CT-P10: Toxicity Study by Weekly Intravenous (Bolus) Administration to Cynomolgus Monkeys for 8 Weeks	4.2.3.2.

3.2 Studies Not Reviewed

Study number	Study title	eCTD location
2290/003a	Validation of Automated Gyros Immunoassay to Measure CT-P10 and Rituximab in Cynomolgus Monkey Serum	4.2.2.1.
2290/003b	Validation for the Detection of Anti-CT-P10/Rituximab Antibodies in Cynomolgus Monkey Serum by an Electrochemiluminescent Assay (ECLA)	4.2.2.1.
2290/003e	Long Term Stability Assessment of CT-P10 and Rituximab in Cynomolgus Monkey Serum at -20 ± 10°C by an Automated Gyros Immunoassay	4.2.2.1.
2290/009	Validation for the Detection of Neutralizing Anti-CT-P10/Rituximab Antibodies in Cynomolgus Monkey Serum by a Complement-Dependent Cytotoxicity Assay	4.2.2.1.

3.3 Previous Reviews Referenced

Nonclinical review for IND 120885 by Dr. Marcie Wood dated 2 July 2014.

4 Pharmacology

4.1 Primary Pharmacology

Celltrion conducted an in vitro 3-way similarity assessment including studies for cell-based CD20 binding affinity, FcγR binding affinity, FcRn binding affinity, C1q binding affinity, ADCC, ADCP, CDC, and apoptosis to demonstrate similarity between CT-P10, US-licensed Rituxan, and EU-MabThera. Refer to the Product Quality review for the review of these studies. No issues were identified that would affect the nonclinical safety assessment for CT-P10.

The in vivo PD effect of CT-P10 and EU-MabThera was assessed by evaluating changes in B cell count as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

4.2 Secondary Pharmacology

No studies were submitted for review.

4.3 Safety Pharmacology

Standalone safety pharmacology studies were not conducted. Electrocardiograms (ECGs) were recorded as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Standalone PK studies were not conducted. The TK of CT-P10 and EU-MabThera were assessed as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

6 General Toxicology

6.1 Single-Dose Toxicity

No studies were submitted for review.

6.2 Repeat-Dose Toxicity

Study title: CT-P10: Toxicity Study by Weekly Intravenous (Bolus) Administration to Cynomolgus Monkeys for 8 Weeks

Study no.:	ZIP0003
Study report location:	4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12 October 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CT-P10, lot # CP0502, purity=99% (HPLC-SEC) Rituximab (EU-MabThera), lot # B6036, purity=99% (HPLC-SEC)

Reviewer note: Portions of this review were excerpted from the nonclinical review for IND 120885 (Reference ID 3536596).

Key Study Findings

- Two animals in the EU-MabThera arm were prematurely sacrificed in moribund condition; morbidity was attributed to an immune response characterized by low serum concentrations of rituximab and the presence of neutralizing anti-drug antibodies (ADAs). After the two premature sacrifices, the study was terminated early such that females received 5 of 8 planned doses and males received 6 of 8 planned doses.
- In surviving animals the clinical signs, clinical pathology, organ weights, and histopathology were similar between the CT-P10 and EU-MabThera arms.
- Immunophenotyping revealed marked depletion of B cells in the peripheral blood, spleen, lymph node, and bone marrow. B cell depletion was of similar magnitude between arms, and is consistent with the expected PD activity of rituximab.
- Systemic exposure (AUC_{168}) was similar between arms on Day 1; on Day 22, systemic exposure of CT-P10 was higher in males, while systemic exposure of EU-MabThera was higher in females. Three animals in each arm developed neutralizing ADAs.

Methods

Doses:	0 or 20 mg/kg/week (see Table 2)
Frequency of dosing:	Once weekly for 6 (male) or 5 (female) doses: Males: Days 1, 8, 15, 22, 29, and 36 Females: Days 1, 8, 15, 22, and 29
Route of administration:	Intravenous (bolus) injection
Dose volume:	2 mL/kg/week
Formulation/Vehicle:	0.9% Sodium Chloride injection, BP (sterile saline)
Species/Strain:	Cynomolgus monkey (purpose bred, Belgrave Services, Vietnam)
Number/Sex/Group:	3/sex/group
Age:	32 to 36 months (at study initiation)
Weight:	Males: 2.17 to 3.25 kg Females: 2.44 to 2.73 kg
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	Deviations from the study protocol did not adversely affect study outcome or data interpretation

Table 2: 8-week monkey study, experimental design

Group	Test material	Dose level (mg/kg/week)	Number of animals	Animal identifying number	
				Male	Female
1	Control article	0	3/sex	351, 353, 355	352, 354, 356
2	CT-P10	20	3/sex	357, 359, 361	358, 360, 362
3	EU-MabThera	20	3/sex	363, 365, 367	364, 366, 368

Observations and times

The first day of dosing was designated as Day 1. Animals were sacrificed 14 days after the last dose (Day 43 for females or Day 50 for males).

Mortality and morbidity:	• Twice daily
Clinical observations:	• Dosing days: during and immediately after dosing, 1/2 to 2 hours after dosing, and as late as possible in the working day • Non-dosing days: in the morning and as late as possible in the working day
Injection site observations:	• Observed daily until resolution of any changes
Body weight:	• Weekly prestudy • On Day 1 • Weekly thereafter
Food consumption:	• Qualitative
Ophthalmoscopy:	• Once prestudy • Weeks 6 (females) and 7 (males)
Body temperature:	• Once prestudy • Weeks 4, 6 (females) and 7 (males)
Electrocardiography:	• Once prestudy • Day 1, 24 hours post dose • Weeks 6 (females) and 7 (males)
Clinical pathology: (hematology, coagulation, clinical chemistry, urinalysis)	• See Table 3
TK and immunogenicity:	• See Table 3
Immunophenotyping and PD:	• See Table 3
Sacrifice:	• Day 43 (females) or 50 (males)

Table 3: 8-week monkey study, sample collection time points

Parameter	Time point
Hematology	Pretreatment: All animals
	Day 3: All animals
	Day 3 of Week 4: All males, EDTA sample only
	Day 3 of Week 4: All females, EDTA and citrate sample
	Day 7 of Week 4: Female 366, EDTA only
	Day 3 of Week 5: All males, citrate sample only
	Week 7: All female animals
	Week 8: All male animals
Clinical chemistry	Pretreatment: All animals

Parameter	Time point
	Day 3: All animals
	Week 7: All females
	Week 8: All males
Urinalysis	Pretreatment: All animals
	Week 7: All animals
TK	Day 1 and 22: All animals, pre-dose, 15 mins, 1, 6, 12, 24, 72, 96, 120 and 168 hours post dose
	7th day of Week 6: All females, single time point approximately 14 days post final dose
	1st day of Week 8: All males, single time point approximately 14 days post final dose
Immunogenicity	Pretreatment: All animals
	Day 22: All animals
	Week 7: Females
	Week 8: Males
Immunophenotyping and PD	Pretreatment: All animals, two occasions
	Day 1 and 22: All animals, 24, 72 and 168 hours
	Week 7: Females, single time point
	Week 8: Males, single time point

Results

Mortality

Two EU-MabThera-treated animals were prematurely sacrificed in moribund condition on Days 29 and 36.

Female animal 366 exhibited abnormal clinical signs after the fourth dose administration on Day 22, including hunched posture, underactivity, piloerection, body tremors, and bruising and/or swelling of the wrists, ankles, face, muzzle, shoulders, and tail. After dosing on Day 29 (fifth dose), additional clinical signs were observed including vomiting, unresponsiveness, salivation, partially close eyelids, and unsteadiness. The animal was sacrificed after further deterioration. Day 24 and Day 28 hematology findings included reduced hematocrit, hemoglobin, and red blood cell (RBC) counts. Increased white blood cell (WBC) and platelet counts were also observed on Day 28 (versus reduced counts on Days 3 and 24). At necropsy, pale kidneys and increased kidney weights (versus background control data) were observed, as well as increased liver and spleen weights. A cause of death was not identified histopathologically.

Male animal 365 was found unconscious after the sixth dose administration on Day 36, approximately 15 minutes post-dose. The animal recovered briefly upon oxygen administration, but exhibited signs including salivation, abnormal breathing, body tremors, and bruising of the hindlimbs. The animal lost consciousness again and could not be resuscitated. Congested thymus and increased thymus weight (versus Day 50 control animals) were observed at necropsy. A cause of death was not identified histopathologically.

Day 22 serum EU-MabThera concentrations for these animals were significantly decreased versus group means, indicating an increased immune response in these animals. Both animals tested positive for neutralizing ADAs (see below).

The study initially planned for 8 weeks of dosing with no recovery period, but following the premature sacrifice of two EU-MabThera animals in moribund condition on Days 29 and 36, dosing was terminated early due to welfare concerns for the remaining animals; remaining animals were sacrificed 14 days following the last dose. In total, females and males on study received a total of five and six doses, respectively.

Clinical signs

Two CT-P10 male animals (359 and 361) exhibited piloerection on Day 1. Animal 361 exhibited body tremors 0.5 to 2 hours post dose on Day 15. By Day 29, only piloerection was observed in this animal.

Clinical signs for the EU-MabThera animals included piloerection in one male on Day 1 (animal 367) and vomiting in one female on Day 22 (animal 368).

Body weights

Unremarkable

Food consumption

Unremarkable

Ophthalmoscopy

Unremarkable

Electrocardiography

Unremarkable

Hematology

The following changes in hematology parameters were considered treatment-related (see Table 4):

- Day 3: Decreased WBC (↓38-50%) and lymphocytes (LYM, ↓50-58%) in male and female CT-P10 and EU-MabThera treatment groups, and decreased eosinophils (EOS, ↓70-81%) in CT-P10 and EU-MabThera males and EU-MabThera females. Additional Day 3 changes included decreased basophils (BASO, ↓50-67%) and large unstained cells (LUC, ↓54-77%) in CT-P10 and EU-

MabThera females, and decreased monocytes (MONO, ↓56%) in EU-MabThera females.

- Week 4: WBC, LYM, and EOS in CT-P10 and EU-MabThera males returned to control values, though decreased WBC (↓37-50%), LYM (↓38-50%), EOS (↓45-66%), and BASO (↓60%) were still observed in females. Decreased neutrophils (NEUT) were also observed in EU-MabThera males (52%) and females (72%).
- Weeks 7/8: Hematology parameters had generally returned to control values; however, RBC was slightly decreased in CT-P10 and EU-MabThera females (↓7-8%).

Table 4: 8-week monkey study, mean percent change in hematology parameters versus concurrent vehicle control

(Table adapted from IND review)

Parameter	Timepoint[#]	Males		Females	
		CT-P10	EU-MabThera	CT-P10	EU-MabThera
RBC	Day 3	-	-	-	-
	Week 4 (D3)	-	-	-	-
	Week 7/8	-	-	-7%*	-8%*
WBC	Day 3	-38%*	-50%**	-40%*	-44%*
	Week 4 (D3)	-	-	-37%	-50%*
	Week 7/8	-	-	-	-
NEUT	Day 3	-	-	-	-
	Week 4 (D3)	-	-52%*	-	-72%
	Week 7/8	-	-	-	-
LYM	Day 3	-50%*	-50%*	-58%**	-57%**
	Week 4 (D3)	-	-	-50%**	-38%*
	Week 7/8	-	-	-	-
EOS	Day 3	-70%	-80%*	-	-81%*
	Week 4 (D3)	-	-	-45%	-66%*
	Week 7/8	-	-	-	-
BASO	Day 3	-	-	-50%*	-67%*
	Week 4 (D3)	-	-	-60%*	-60%*
	Week 7/8	-	-	-	-
MONO	Day 3	-	-	-	-56%*
	Week 4 (D3)	-	-	-	-
	Week 7/8	-	-	-	-
LUC	Day 3	-	-	-54%**	-77%**
	Week 4 (D3)	-	-	-	-
	Week 7/8	-	-	-	-

[#] Week 7/8: Week 7 (females) / Week 8 (males); * P<0.05; ** P< 0.01

Coagulation

- Day 3: Statistically significant (p<0.05) increase in prothrombin time (PT) was observed in EU-MabThera females versus controls (13.1 versus 11.2 seconds).
- Week 7: Statistically significant (p<0.05) increase in PT was observed in EU-MabThera females versus controls (12.8 versus 10.8 sec).

Clinical chemistry

Statistically significant changes in clinical chemistry parameters were observed (see Table 5); however, these changes were sporadic and were not considered toxicologically significant.

Table 5: 8-week monkey study, mean percent change in clinical chemistry parameters versus concurrent vehicle control

(Table adapted from IND review)

Parameter	Time point[#]	Males		Females	
		CT-P10	EU-MabThera	CT-P10	EU-MabThera
Total bilirubin	Day 3	-	-	-	-
	Week 7/8	-33%	-67%*	-	-
Urea	Day 3	-	-	-	-
	Week 7/8	-30%*	-32%*	-	-
Total cholesterol	Day 3	-	-	-	-
	Week 7/8	-	-	-22%**	-14%*
Glucose	Day 3	-	-	-13%	-27%*
	Week 7/8	-	-	-	-
Inorganic phosphorus	Day 3	-	-	-	-
	Week 7/8	-21%*	-39%**	-	-

[#] Week 7/8: Week 7 (females) / Week 8 (males); * P<0.05; ** P< 0.01

Urinalysis

Unremarkable

Gross pathology

Unremarkable

Organ weights

Treatment-related increase in absolute thymus and kidney weights were observed (see Table 6).

- Increased thymus weights were observed in all treatment groups relative to controls (males: ↑21-44%, females: ↑95-125%).
- Increased kidney weights were observed in CT-P10 and EU-MabThera females relative to controls (↑18-23%).

Table 6: 8-week monkey study, mean percent change in absolute organ weights versus vehicle control

(Table adapted from IND review)

Organ	Males		Females	
	CT-P10	EU-MabThera	CT-P10	EU-MabThera
Thymus	+21%	+44%*	+95%	+125%*
Kidney	-	-	+18%	+23%

* P<0.05

Histopathology

Adequate Battery: Yes
Peer Review: Yes, but statement not submitted

At the end of the treatment period, treatment-related histopathology findings were observed in the mesenteric lymph node, spleen, and sublingual salivary gland (see Table 7). Histopathology findings for the two animals sacrificed in moribund condition are not included in the table below.

Table 7: 8-week monkey study, histopathology findings at end of treatment period

Observation	Severity	Males			Females		
		Group*			Group*		
		1	2	3	1	2	3
LN mesenteric (No. examined)		3	3	2	3	3	2
Decreased germinal center development	Minimal	0	2	2	0	2	2
	Slight	0	1	0	0	0	0
	Total	0	3	2	0	2	2
Spleen (No. examined)		3	3	2	3	3	2
Decreased germinal center development	Minimal	0	1	1	0	2	0
	Slight	0	2	1	0	0	0
	Total	0	3	2	0	2	0
Sublingual SG (No. examined)		3	3	2	3	3	2
Inflammatory Cell Infiltrate	Minimal	0	0	1	0	0	2
	Slight	0	0	0	0	1	0
	Total	0	0	1	0	1	2

*Group 1: Vehicle control; Group 2: CT-P10; Group 3: EU-MabThera

Toxicokinetics and immunogenicity

Serum TK parameters were calculated after the first (Day 1) and fourth (Day 22) doses of CT-P10 or EU-MabThera (see Table 8). The TK analysis excludes data for animals with evidence of ADA production and animals that were prematurely sacrificed in moribund condition; therefore, due to the small number of animals analyzed, data from Day 22 should be interpreted cautiously.

- Day 1: Systemic exposure was similar in males and females receiving CT-P10 or EU-MabThera.
- Day 22: Systemic exposure of CT-P10 was higher in males, while systemic exposure of EU-MabThera was higher in females.

Table 8: 8-week monkey study, mean TK parameters for CT-P10 and EU-MabThera

Group	Day 1		Day 22	
	Males	Females	Males	Females
	C_{max} (µg/mL)			
CT-P10	650 (331)	529 (56)	991 (-)	737 (189)
EU-MabThera	566 (133)	508 (55)	777 (-)	1230 (-)
	AUC_{168} (µg*hr/mL)			
CT-P10	30700 (2300)	32500 (5200)	57600 (-)	28800 (9200)
EU-MabThera	34200 (5200)	34500 (2800)	37000 (-)	81500 (-)
	C_{max} ratio			
N/A	1.1	1.0	1.3	0.60
	AUC_{168} ratio			
N/A	0.90	0.94	1.6	0.35

Standard deviations are in parentheses; C_{max} ratio refers to C_{max} of CT-P10 relative that of EU-MabThera; AUC_{168} ratio refers to AUC_{168} of CT-P10 relative that of EU-MabThera

A total of six animals (three in each the CT-P10 and EU-MabThera groups) tested positive for neutralizing ADAs:

CT-P10 group:
- Animal 358 (Week 7)
- Animal 359 (Day 22 and Week 8)
- Animal 362 (Week 7)

EU-MabThera group:
- Animal 364 (Day 22 and Week 7)
- Animal 365 (Day 22)
- Animal 366 (Day 22 and Day 29 terminal sporadic sample)

Special evaluation

Major cell populations in the peripheral blood, spleen, lymph node, and bone marrow were evaluated by flow cytometry. The following cell populations were analyzed:
- Total T cells ($CD3^+$)
- Total B cells ($CD20^+$)
- CD4 T cells ($CD3^+CD4^+CD8^-$)
- CD8 T cells ($CD3^+CD4^-CD8^+$)
- Monocytes ($CD14^+$) [peripheral blood only]
- NK cells ($CD3^-CD16^+CD14^-$)
- Activated T cells (CD3, CD25, and CD69)
- Activated B cells ($CD20^+$HLA-DR^+)

At the end of the study, the total number of B cells in the peripheral blood, spleen, lymph node, and bone marrow were greatly reduced in both male and female cynomolgus monkeys administered either CT-P10 or EU-MabThera (see Table 9). Changes in other cell populations were sporadic and not considered toxicologically significant.

Table 9: 8-week monkey study, mean percent change in total B cells at terminal bleed versus vehicle control

Tissue/cell type	Absolute or %	Males		Females	
		CT-P10	EU-MabThera	CT-P10	EU-MabThera
Blood					
Total B cells	Absolute	-99%***	-99%***	-90%*	-71%
	%	-99%***	-99%***	-83%*	-71%*
Spleen					
Total B cells	Absolute	-95%*	-64%	-77%	-49%
	%	-97%	-75%	-77%**	-64%*
Lymph node					
Total B cells	Absolute	-99%**	-98%*	-76%	-82%
	%	-98%***	-92%***	-86%**	-90%**
Bone marrow					
Total B cells	Absolute	-66%	-92%*	-67%	-34%
	%	-66%	-83%*	-57%	-48%

* P<0.05; ** P< 0.01; ***P<0.001

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Not applicable.

7.2 *In Vitro* Assays in Mammalian Cells

Not applicable.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Not applicable.

7.4 Other Genetic Toxicity Studies

Not applicable.

8 Carcinogenicity

Not applicable.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not applicable.

9.2 Embryonic Fetal Development

Not applicable.

9.3 Prenatal and Postnatal Development

Not applicable.

10 Special Toxicology Studies

Study title: CT-P10: Cross reactivity study in human tissue

Study no.:	ZIP0002
Study report location:	eCTD, Module 4.2.1.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	29 September 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CT-P10, lot # CP0502, purity=99% (HPLC-SEC) Rituximab (EU-MabThera), lot # B6036, purity=99% (HPLC-SEC)

Key Study Findings

- CT-P10 and EU-MabThera demonstrated similar staining profiles with only minor differences in staining intensity.
- CT-P10 and EU-MabThera specifically and reproducibly stained the tonsil, lymph node, thymus, and spleen, consistent with the known expression of CD20 on B cells.

The objective of this GLP study was to assess the potential tissue cross-reactivity of CT-P10 in comparison to EU-MabThera.

Tissue samples were previously obtained and stored frozen at -80ºC until ready for use. The tissue panel contained 37 different tissues and blood smears from three unrelated human donors (see Table 10). The immunohistochemical method was developed and validated previously in a series of method development studies (study reports not submitted). Human tonsil and liver tissue were used for positive and negative control material, respectively. Three concentrations of CT-P10 and MabThera were evaluated: 0.05 µg/mL, 0.1 µg/mL, and 0.25 µg/mL. Human IgG1 isotype control (0.25 µg/mL) was used to distinguish between specific and non-specific binding. Slides were evaluated by light microscopy. A peer review statement was not submitted. Staining intensity was graded on a scale of negative (-), weak positive (+), moderate positive (++), or strong positive (+++).

Table 10: Tissue cross-reactivity study, tissues examined

Tissue (each tissue from three unrelated human donors)		
Adrenal gland	Kidney	Stomach
Urinary bladder	Liver	Striated muscle
Blood	Lung	Testis
Bone marrow	Lymph node	Thymus
Breast	Ovary	Thyroid
Cerebellum	Pancreas	Ureter
Cerebral cortex	Parathyroid	Cervix
Colon	Placenta	Endometrium
Endothelium	Pituitary gland	Tonsil
Eye	Prostate	Parotid gland
Fallopian tube	Skin	Peripheral nerve
Heart	Spinal cord	
Ileum	Spleen	

Results

Similar staining profiles were observed for CT-P10 and EU-MabThera with only minor differences in staining intensity. CT-P10 and EU-MabThera both stained the positive control material, and staining of negative control material was similar to isotype control. CT-P10 and EU-MabThera specifically and consistently stained the tonsil, lymph node, thymus, and spleen. Lymphocytes in the colon, stomach, ileum, and endothelial tissue were also stained by CT-P10 and EU-MabThera.

11 Integrated Summary and Safety Evaluation

The applicant conducted a stepwise approach to support the demonstration of similarity between CT-P10 and US-licensed Rituxan; this approach consisted of a definitive analytical similarity assessment and abbreviated nonclinical and clinical programs. The comparative in vitro studies conducted as part of the analytical similarity assessment supports the use of EU-MabThera as a comparator arm in the tissue cross-reactivity study and in the repeat-dose toxicity study in cynomolgus monkeys.

The tissue cross-reactivity profiles of CT-P10 and EU-MabThera were comparable between treatment arms and consistent with the known expression of CD20 on B cells.

The pivotal nonclinical study was the comparative 8-week repeat-dose toxicity study in cynomolgus monkeys. The choice of test species, dose level, route of administration, and study duration are supported by information available for EU-MabThera[1]. Two animals from the EU-MabThera treatment arm were prematurely sacrificed in moribund

[1] EMA, 2005, MabThera: EPAR - Scientific Discussion (accessible at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000165/WC500025817.pdf)

condition; morbidity was attributed to immune reactions which are expected in cynomolgus monkeys. The overall incidence of immunogenicity was similar between treatment arms. Among the surviving animals, clinical signs, clinical pathology, organ weights, and histopathological findings were similar between treatment arms. The predominant observation was B cell depletion, consistent with the expected PD activity of rituximab, and was of similar magnitude between treatment arms. Systemic exposure was similar between treatment arms on Day 1. On Day 22, systemic exposure of CT-P10 was higher in males, while systemic exposure of EU-MabThera was higher in females; due to the small number of animals analyzed TK data from Day 22 should be interpreted with caution.

Conclusion

The results of the in vitro 3-way similarity assessment, the tissue cross-reactivity study, and the comparative repeat-dose toxicity study in cynomolgus monkeys support the demonstration of biosimilarity between rituximab-abbs and US-licensed Rituxan. From the perspective of nonclinical pharmacology and toxicology rituximab-abbs may be approved for the proposed indications.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

MICHAEL L MANNING
10/22/2018

CHRISTOPHER M SHETH
10/22/2018
I concur

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:	761088
Supporting document/s:	2
Applicant's letter date:	28 April 2017
CDER stamp date:	28 April 2017
Product:	Truxima (rituximab-abbs, CT-P10)
Indication:	– Non-Hodgkin's Lymphoma (NHL) (b) (4) [redacted]
Applicant:	Celltrion Inc
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer:	Michael L Manning, PhD
Supervisor/Team Leader:	Christopher M Sheth, PhD
Division Director:	John Leighton, PhD (DHOT) Ann Farrell, MD (DHP)
Project Manager:	Esther Park, PharmD

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Celltrion developed Truxima (rituximab-abbs, CT-P10) as a proposed biosimilar product to US-licensed Rituxan (rituximab). Rituxan was approved by FDA in 1997 (BLA 103705). Rituximab is approved in the European Union (EU) and marketed under the trade name MabThera. Truxima was approved in South Korea in November 2016 and in the EU in February 2017 for the same indications as MabThera.

BLA 761088 was submitted for the purpose of licensure of CT-P10 under section 351(k) of the Public Health Service Act. The proposed formulation and dosing regimen of CT-P10 are identical to that of Rituxan, (b) (4) The applicant conducted a stepwise biosimilar development program intended to demonstrate that CT-P10 is highly similar to Rituxan, with no clinically meaningful differences in terms of safety, purity, or potency. The biosimilar development program consists of a definitive analytical similarity assessment and abbreviated nonclinical and clinical programs.

1.2 Brief Discussion of Nonclinical Findings

Rituximab is a chimeric murine/human monoclonal antibody that binds CD20, which is primarily found on the surface of B cells. After binding to CD20 on the cell surface, rituximab is believed to mediate B cell lysis, possibly through antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), and/or direct induction of apoptosis. The applicant conducted an in vitro 3-way similarity assessment including studies for cell-based CD20 binding affinity, FcγR binding affinity, FcRn binding affinity, C1q binding affinity, ADCC, ADCP, CDC, and apoptosis to demonstrate similarity between CT-P10, Rituxan, and MabThera. No issues were identified that would affect the nonclinical safety assessment for CT-P10. The results of the in vitro 3-way similarity assessment create a scientific bridge between CT-P10, Rituxan, and MabThera.

The tissue cross-reactivity profiles of CT-P10 and MabThera were evaluated in a panel of tissues of human origin. The staining profiles of CT-P10 and MabThera were comparable with only minor differences in staining intensity and consistent with the known expression of CD20 on B cells.

The comparative toxicity, toxicokinetics (TK), and pharmacodynamics (PD) of CT-P10 and MabThera were evaluated in an 8-week repeat-dose toxicity study in cynomolgus monkeys. The study was terminated early following the premature sacrifice of two animals in moribund condition; females received 5 of 8 planned doses and males received 6 of 8 planned doses. Morbidity was attributed to immune reactions which are expected in cynomolgus monkeys; the overall incidence of immunogenicity was similar between treatment arms. Among the surviving animals, clinical signs, clinical pathology, organ weights, and histopathological findings were similar between treatment arms.

Systemic exposure was similar between treatment arms on Day 1. B cell depletion, the expected PD activity of rituximab, was of similar magnitude between treatment arms.

The results of the in vitro 3-way similarity assessment, the tissue cross-reactivity study, and the comparative repeat-dose toxicity study in cynomolgus monkeys support the demonstration of biosimilarity between CT-P10 and Rituxan.

1.3 Recommendations

1.3.1 Approvability

From the perspective of nonclinical pharmacology and toxicology Truxima may be approved for the proposed indications.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

Section 8 of the proposed Truxima label is in Pregnancy and Lactation Labeling Rule format. The nonclinical sections of the Truxima label are otherwise comparable to the label of Rituxan.

2 Drug Information

2.1 Drug

CAS Registry Number	174722-31-7
Proprietary Name	Truxima
Generic Name	rituximab-abbs
Code Name	CT-P10
Molecular Formula	Light chains: $C_{1016}H_{1577}N_{273}O_{328}S_6$ Heavy chains: $C_{2191}H_{3377}N_{575}O_{675}S_{16}$
Molecular Weight	~147 kDa (glycosylated)
Biochemical Description	CT-P10 is a chimeric murine/human monoclonal antibody consisting of a glycosylated IgG1 kappa immunoglobulin with murine light and heavy chain variable regions (Fab domain) and human kappa and gamma-1 constant regions (Fc domain) (see Figure 1). Each light and heavy chain contains 213 and 450 amino acids, respectively; the amino acid sequences are presented in Figure 2. Each heavy chain contains a single N-linked glycosylation site (asparagine-301). CT-P10 is directed against the CD20 antigen.
Pharmacologic Class	CD20-directed cytolytic antibody

Figure 1: Schematic diagram of CT-P10

(Adapted from applicant's submission)

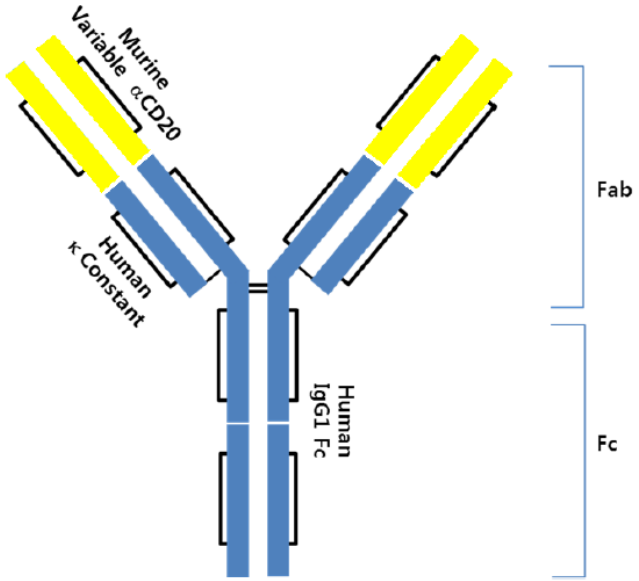

Figure 2: Amino acid sequence of CT-P10 heavy and light chains

(Adapted from applicant's submission)

Heavy chain:

```
  1  QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYNMHWVKQT PGRGLEWIGA
 51  IYPGNGDTSY NQKFKGKATL TADKSSSTAY MQLSSLTSED SAVYYCARST
101  YYGGDWYFNV WGAGTTVTVS AASTKGPSVF PLAPSSKSTS GGTAALGCLV
151  KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ
201  TYICNVNHKP SNTKVDKKAE PKSCDKTHTC PPCPAPELLG GPSVFLFPPK
251  PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
301  N#STYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
351  QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP
401  VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG
451  (K*)
```

Light chain:

```
  1  QIVLSQSPAI LSASPGEKVT MTCRASSSVS YIHWFQQKPG SSPKPWIYAT
 51  SNLASGVPVR FSGSGSGTSY SLTISRVEAE DAATYYCQQW TSNPPTFGGG
101  TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD
151  NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL
201  SSPVTKSFNR GEC
```

Yellow = variable region; blue = constant region; # glycosylation site; (K*) = C terminal lysine which is predominantly removed post-translationally

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 120885 (CT-P10)
BLA 103705 (Rituxan)

2.3 Drug Formulation

CT-P10 is supplied as a sterile liquid solution in single-use vials containing 500 mg (50 mL) or 100 mg (10 mL) CT-P10 at pH 6.5. The formulation of CT-P10 is identical to Rituxan. The composition of the CT-P10 drug product, including the function and reference standard of each component, is listed in Table 1.

Table 1: Composition of the CT-P10 drug product

Ingredient	Quantity		Function	Compendial reference
	500 mg / vial	100 mg / vial		
CT-P10	500 mg	100 mg	Active ingredient	In house
Sodium Chloride	450 mg	90 mg	(b) (4)	USP/Ph.Eur.
Tri-Sodium Citrate Dihydrate	368 mg	73.5 mg		USP/Ph.Eur.
Polysorbate 80	35 mg	7 mg		NF/Ph.Eur.
Water for Injection	q.s. to 50 mL	q.s. to 10 mL		USP/Ph.Eur.

USP = United States Pharmacopoeia; Ph.Eur. = European Pharmacopoeia; NF = National Formulary; q.s. = quantum satis

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population and dosing regimen are identical to that of Rituxan.

2.7 Regulatory Background

A pre-IND meeting (BPD-2) was held on 19 March 2014 to discuss development of CT-P10 as a proposed biosimilar to US-licensed Rituxan. The nonclinical team advised the applicant the comparative pharmacology and toxicology studies appeared sufficient to support clinical development of CT-P10 as a proposed biosimilar product under an IND. Data demonstrating comparability between the product used in the nonclinical studies and the product to be used in the proposed comparative clinical studies would be required.

IND 120885 was submitted on 5 June 2014; a Study May Proceed letter was sent to the applicant on 3 July 2014.

No nonclinical issues were discussed at the following meetings:

- BPD-2 meeting held on 13 July 2015.
- BPD-2 meeting held on 12 February 2016.
- BPD-2 and BPD-3 meetings held on 17 January 2017.
- BPD-4 meeting scheduled for 26 April 2017, but cancelled at applicant's request after receiving preliminary comments dated 20 April 2017.

3 Studies Submitted

3.1 Studies Reviewed

Study number	Study title	eCTD location
ZIP0002	CT-P10: Cross Reactivity Study in Human Tissues	4.2.1.1.
ZIP0003	CT-P10: Toxicity Study by Weekly Intravenous (Bolus) Administration to Cynomolgus Monkeys for 8 Weeks	4.2.3.2.

3.2 Studies Not Reviewed

Study number	Study title	eCTD location
2290/003a	Validation of Automated Gyros Immunoassay to Measure CT-P10 and Rituximab in Cynomolgus Monkey Serum	4.2.2.1.
2290/003b	Validation for the Detection of Anti-CT-P10/Rituximab Antibodies in Cynomolgus Monkey Serum by an Electrochemiluminescent Assay (ECLA)	4.2.2.1.
2290/003e	Long Term Stability Assessment of CT-P10 and Rituximab in Cynomolgus Monkey Serum at -20 ± 10°C by an Automated Gyros Immunoassay	4.2.2.1.
2290/009	Validation for the Detection of Neutralizing Anti-CT-P10/Rituximab Antibodies in Cynomolgus Monkey Serum by a Complement-Dependent Cytotoxicity Assay	4.2.2.1.

3.3 Previous Reviews Referenced

Nonclinical review for IND 120885 by Dr. Marcie Wood dated 2 July 2014.

4 Pharmacology

4.1 Primary Pharmacology

Celltrion conducted an in vitro 3-way similarity assessment including studies for cell-based CD20 binding affinity, FcγR binding affinity, FcRn binding affinity, C1q binding affinity, ADCC, ADCP, CDC, and apoptosis to demonstrate similarity between CT-P10, Rituxan, and MabThera. Refer to the Product Quality review for the review of these studies. No issues were identified that would affect the nonclinical safety assessment for CT-P10. The results of the in vitro 3-way similarity assessment create a scientific bridge between CT-P10, Rituxan, and MabThera.

The in vivo PD effect of CT-P10 and MabThera was assessed by evaluating changes in B cell count as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

4.2 Secondary Pharmacology

No studies were submitted for review.

4.3 Safety Pharmacology

Standalone safety pharmacology studies were not conducted. Electrocardiograms (ECGs) were recorded as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Standalone PK studies were not conducted. The TK of CT-P10 and MabThera were assessed as part of the repeat-dose toxicity study in cynomolgus monkeys (refer to section 6.2 Repeat-Dose Toxicity).

6 General Toxicology

6.1 Single-Dose Toxicity

No studies were submitted for review.

6.2 Repeat-Dose Toxicity

Study title: CT-P10: Toxicity Study by Weekly Intravenous (Bolus) Administration to Cynomolgus Monkeys for 8 Weeks

Study no.:	ZIP0003
Study report location:	4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 12, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CT-P10, lot # CP0502, purity=99% (HPLC-SEC) Rituximab (MabThera), lot # B6036, purity=99% (HPLC-SEC)

Reviewer note: Portions of this review were excerpted from the nonclinical review for IND 120885 (Reference ID 3536596).

Key Study Findings

- Two animals in the MabThera arm were prematurely sacrificed in moribund condition; morbidity was attributed to an immune response characterized by low serum concentrations of rituximab and the presence of neutralizing anti-drug antibodies (ADAs). After the two premature sacrifices, the study was terminated early such that females received 5 of 8 planned doses and males received 6 of 8 planned doses.
- In surviving animals the clinical signs, clinical pathology, organ weights, and histopathology were similar between the CT-P10 and MabThera arms.

- Immunophenotyping revealed marked depletion of B cells in the peripheral blood, spleen, lymph node, and bone marrow. B cell depletion was of similar magnitude between arms, and is consistent with the expected PD activity of rituximab.
- Systemic exposure (AUC_{168}) was similar between arms on Day 1; on Day 22, systemic exposure of CT-P10 was higher in males, while systemic exposure of MabThera was higher in females. Three animals in each arm developed neutralizing ADAs.

Methods

Doses:	0 or 20 mg/kg/week (see Table 2)
Frequency of dosing:	Once weekly for 6 (male) or 5 (female) doses: Males: Days 1, 8, 15, 22, 29, and 36 Females: Days 1, 8, 15, 22, and 29
Route of administration:	Intravenous (bolus) injection
Dose volume:	2 mL/kg/week
Formulation/Vehicle:	0.9% Sodium Chloride injection, BP (sterile saline)
Species/Strain:	Cynomolgus monkey (purpose bred, Belgrave Services, Vietnam)
Number/Sex/Group:	3/sex/group
Age:	32 to 36 months (at study initiation)
Weight:	Males: 2.17 to 3.25 kg Females: 2.44 to 2.73 kg
Satellite groups:	No
Unique study design:	No
Deviation from study protocol:	Deviations from the study protocol did not adversely affect study outcome or data interpretation

Table 2: 8-week monkey study, experimental design

Group	Test material	Dose level (mg/kg/week)	Number of animals	Animal identifying number	
				Male	Female
1	Control article	0	3/sex	351, 353, 355	352, 354, 356
2	CT-P10	20	3/sex	357, 359, 361	358, 360, 362
3	MabThera	20	3/sex	363, 365, 367	364, 366, 368

Observations and times

The first day of dosing was designated as Day 1. Animals were sacrificed 14 days after the last dose (Day 43 for females or Day 50 for males).

Mortality and morbidity:	• Twice daily
Clinical observations:	• Dosing days: during and immediately after dosing, 1/2 to 2 hours after dosing, and as late as possible in

	the working day • Non-dosing days: in the morning and as late as possible in the working day
Injection site observations:	• Observed daily until resolution of any changes
Body weight:	• Weekly prestudy • On Day 1 • Weekly thereafter
Food consumption:	• Qualitative
Ophthalmoscopy:	• Once prestudy • Weeks 6 (females) and 7 (males)
Body temperature:	• Once prestudy • Weeks 4, 6 (females) and 7 (males)
Electrocardiography:	• Once prestudy • Day 1, 24 hours post dose • Weeks 6 (females) and 7 (males)
Clinical pathology: (hematology, coagulation, clinical chemistry, urinalysis)	• See Table 3
TK and immunogenicity:	• See Table 3
Immunophenotyping and PD:	• See Table 3
Sacrifice:	• Day 43 (females) or 50 (males)

Table 3: 8-week monkey study, sample collection time points

Parameter	Time point
Hematology	Pretreatment: All animals
	Day 3: All animals
	Day 3 of Week 4: All males, EDTA sample only
	Day 3 of Week 4: All females, EDTA and citrate sample
	Day 7 of Week 4: Female 366, EDTA only
	Day 3 of Week 5: All males, citrate sample only
	Week 7: All female animals
	Week 8: All male animals
Clinical chemistry	Pretreatment: All animals
	Day 3: All animals
	Week 7: All females
	Week 8: All males
Urinalysis	Pretreatment: All animals
	Week 7: All animals
TK	Day 1 and 22: All animals, pre-dose, 15 mins, 1, 6, 12, 24, 72, 96, 120 and 168 hours post dose
	7th day of Week 6: All females, single time point approximately 14 days post final dose
	1st day of Week 8: All males, single time point approximately 14 days post final dose

Parameter	Time point
Immunogenicity	Pretreatment: All animals
	Day 22: All animals
	Week 7: Females
	Week 8: Males
Immunophenotyping and PD	Pretreatment: All animals, two occasions
	Day 1 and 22: All animals, 24, 72 and 168 hours
	Week 7: Females, single time point
	Week 8: Males, single time point

Results

Mortality

Two MabThera-treated animals were prematurely sacrificed in moribund condition on Days 29 and 36.

Female animal 366 exhibited abnormal clinical signs after the fourth dose administration on Day 22, including hunched posture, underactivity, piloerection, body tremors, and bruising and/or swelling of the wrists, ankles, face, muzzle, shoulders, and tail. After dosing on Day 29 (fifth dose), additional clinical signs were observed including vomiting, unresponsiveness, salivation, partially close eyelids, and unsteadiness. The animal was sacrificed after further deterioration. Day 24 and Day 28 hematology findings included reduced hematocrit, hemoglobin, and red blood cell (RBC) counts. Increased white blood cell (WBC) and platelet counts were also observed on Day 28 (versus reduced counts on Days 3 and 24). At necropsy, pale kidneys and increased kidney weights (versus background control data) were observed, as well as increased liver and spleen weights. A cause of death was not identified histopathologically.

Male animal 365 was found unconscious after the sixth dose administration on Day 36, approximately 15 minutes post-dose. The animal recovered briefly upon oxygen administration, but exhibited signs including salivation, abnormal breathing, body tremors, and bruising of the hindlimbs. The animal lost consciousness again and could not be resuscitated. Congested thymus and increased thymus weight (versus Day 50 control animals) were observed at necropsy. A cause of death was not identified histopathologically.

Day 22 serum MabThera concentrations for these animals were significantly decreased versus group means, indicating an increased immune response in these animals. Both animals tested positive for neutralizing ADAs (see below).

The study initially planned for 8 weeks of dosing with no recovery period, but following the premature sacrifice of two MabThera animals in moribund condition on Days 29 and 36, dosing was terminated early due to welfare concerns for the remaining animals; remaining animals were sacrificed 14 days following the last dose. In total, females and males on study received a total of five and six doses, respectively.

Clinical signs

Two CT-P10 male animals (359 and 361) exhibited piloerection on Day 1. Animal 361 exhibited body tremors 0.5 to 2 hours post dose on Day 15. By Day 29, only piloerection was observed in this animal.

Clinical signs for the MabThera animals included piloerection in one male on Day 1 (animal 367) and vomiting in one female on Day 22 (animal 368).

Body weights

Unremarkable

Food consumption

Unremarkable

Ophthalmoscopy

Unremarkable

Electrocardiography

Unremarkable

Hematology

The following changes in hematology parameters were considered treatment-related (see Table 4):

- Day 3: Decreased WBC (↓38-50%) and lymphocytes (LYM, ↓50-58%) in male and female CT-P10 and MabThera treatment groups, and decreased eosinophils (EOS, ↓70-81%) in CT-P10 and MabThera males and MabThera females. Additional Day 3 changes included decreased basophils (BASO, ↓50-67%) and large unstained cells (LUC, ↓54-77%) in CT-P10 and MabThera females, and decreased monocytes (MONO, ↓56%) in MabThera females.
- Week 4: WBC, LYM, and EOS in CT-P10 and MabThera males returned to control values, though decreased WBC (↓37-50%), LYM (↓38-50%), EOS (↓45-66%), and BASO (↓60%) were still observed in females. Decreased neutrophils (NEUT) were also observed in MabThera males (52%) and females (72%).
- Weeks 7/8: Hematology parameters had generally returned to control values; however, RBC was slightly decreased in CT-P10 and MabThera females (↓7-8%).

Table 4: 8-week monkey study, mean percent change in hematology parameters versus concurrent vehicle control

(Table adapted from IND review)

Parameter	Timepoint[#]	Males		Females	
		CT-P10	MabThera	CT-P10	MabThera
RBC	Day 3	-	-	-	-
	Week 4 (D3)	-	-	-	-
	Week 7/8	-	-	-7%*	-8%*
WBC	Day 3	-38%*	-50%**	-40%*	-44%*
	Week 4 (D3)	-	-	-37%	-50%*
	Week 7/8	-	-	-	-
NEUT	Day 3	-	-	-	-
	Week 4 (D3)	-	-52%*	-	-72%
	Week 7/8	-	-	-	-
LYM	Day 3	-50%*	-50%*	-58%**	-57%**
	Week 4 (D3)	-	-	-50%**	-38%*
	Week 7/8	-	-	-	-
EOS	Day 3	-70%	-80%*	-	-81%*
	Week 4 (D3)	-	-	-45%	-66%*
	Week 7/8	-	-	-	-
BASO	Day 3	-	-	-50%*	-67%*
	Week 4 (D3)	-	-	-60%*	-60%*
	Week 7/8	-	-	-	-
MONO	Day 3	-	-	-	-56%*
	Week 4 (D3)	-	-	-	-
	Week 7/8	-	-	-	-
LUC	Day 3	-	-	-54%**	-77%**
	Week 4 (D3)	-	-	-	-
	Week 7/8	-	-	-	-

[#] Week 7/8: Week 7 (females) / Week 8 (males); * P<0.05; ** P< 0.01

Coagulation

- Day 3: Statistically significant (p<0.05) increase in prothrombin time (PT) was observed in MabThera females versus controls (13.1 versus 11.2 seconds).
- Week 7: Statistically significant (p<0.05) increase in PT was observed in MabThera females versus controls (12.8 versus 10.8 sec).

Clinical chemistry

Statistically significant changes in clinical chemistry parameters were observed (see Table 5); however, these changes were sporadic and were not considered toxicologically significant.

Table 5: 8-week monkey study, mean percent change in clinical chemistry parameters versus concurrent vehicle control

(Table adapted from IND review)

Parameter	Time point[#]	Males		Females	
		CT-P10	MabThera	CT-P10	MabThera
Total bilirubin	Day 3	-	-	-	-
	Week 7/8	-33%	-67%*	-	-
Urea	Day 3	-	-	-	-

Parameter	Time point[#]	Males		Females	
		CT-P10	MabThera	CT-P10	MabThera
	Week 7/8	-30%*	-32%*	-	-
Total cholesterol	Day 3	-	-	-	-
	Week 7/8	-	-	-22%**	-14%*
Glucose	Day 3	-	-	-13%	-27%*
	Week 7/8	-	-	-	-
Inorganic phosphorus	Day 3	-	-	-	-
	Week 7/8	-21%*	-39%**	-	-

[#] Week 7/8: Week 7 (females) / Week 8 (males); * P<0.05; ** P< 0.01

Urinalysis

Unremarkable

Gross pathology

Unremarkable

Organ weights

Treatment-related increase in absolute thymus and kidney weights were observed (see Table 6).

- Increased thymus weights were observed in all treatment groups relative to controls (males: ↑21-44%, females: ↑95-125%).
- Increased kidney weights were observed in CT-P10 and MabThera females relative to controls (↑18-23%).

Table 6: 8-week monkey study, mean percent change in absolute organ weights versus vehicle control

(Table adapted from IND review)

Organ	Males		Females	
	CT-P10	MabThera	CT-P10	MabThera
Thymus	+21%	+44%*	+95%	+125%*
Kidney	-	-	+18%	+23%

* P<0.05

Histopathology

Adequate Battery: Yes
Peer Review: Yes, but statement not submitted

At the end of the treatment period, treatment-related histopathology findings were observed in the mesenteric lymph node, spleen, and sublingual salivary gland (see Table 7). Histopathology findings for the two animals sacrificed in moribund condition are not included in the table below.

Table 7: 8-week monkey study, histopathology findings at end of treatment period

Observation	Severity	Males			Females		
		Group*			Group*		
		1	2	3	1	2	3
LN mesenteric (No. examined)		3	3	2	3	3	2
Decreased germinal center development	Minimal	0	2	2	0	2	2
	Slight	0	1	0	0	0	0
	Total	0	3	2	0	2	2
Spleen (No. examined)		3	3	2	3	3	2
Decreased germinal center development	Minimal	0	1	1	0	2	0
	Slight	0	2	1	0	0	0
	Total	0	3	2	0	2	0
Sublingual SG (No. examined)		3	3	2	3	3	2
Inflammatory Cell Infiltrate	Minimal	0	0	1	0	0	2
	Slight	0	0	0	0	1	0
	Total	0	0	1	0	1	2

*Group 1: Vehicle control; Group 2: CT-P10; Group 3: MabThera

Toxicokinetics and immunogenicity

Serum TK parameters were calculated after the first (Day 1) and fourth (Day 22) doses of CT-P10 or MabThera (see Table 8). The TK analysis excludes data for animals with evidence of ADA production and animals that were prematurely sacrificed in moribund condition; therefore, due to the small number of animals analyzed, data from Day 22 should be interpreted cautiously.

- Day 1: Systemic exposure was similar in males and females receiving CT-P10 or MabThera.
- Day 22: Systemic exposure of CT-P10 was higher in males, while systemic exposure of MabThera was higher in females.

Table 8: 8-week monkey study, mean TK parameters for CT-P10 and MabThera

Group	Day 1		Day 22	
	Males	Females	Males	Females
	C_{max} (µg/mL)			
CT-P10	650 (331)	529 (56)	991 (-)	737 (189)
MabThera	566 (133)	508 (55)	777 (-)	1230 (-)
	AUC_{168} (µg*hr/mL)			
CT-P10	30700 (2300)	32500 (5200)	57600 (-)	28800 (9200)
MabThera	34200 (5200)	34500 (2800)	37000 (-)	81500 (-)
	C_{max} ratio			
N/A	1.1	1.0	1.3	0.60
	AUC_{168} ratio			
N/A	0.90	0.94	1.6	0.35

Standard deviations are in parentheses; C_{max} ratio refers to C_{max} of CT-P10 relative that of MabThera; AUC_{168} ratio refers to AUC_{168} of CT-P10 relative that of MabThera

A total of six animals (three in each the CT-P10 and MabThera groups) tested positive for neutralizing ADAs:

CT-P10 group:

- Animal 358 (Week 7)
- Animal 359 (Day 22 and Week 8)
- Animal 362 (Week 7)

MabThera group:

- Animal 364 (Day 22 and Week 7)
- Animal 365 (Day 22)
- Animal 366 (Day 22 and Day 29 terminal sporadic sample)

Special evaluation

Major cell populations in the peripheral blood, spleen, lymph node, and bone marrow were evaluated by flow cytometry. The following cell populations were analyzed:

- Total T cells ($CD3^+$)
- Total B cells ($CD20^+$)
- CD4 T cells ($CD3^+CD4^+CD8^-$)
- CD8 T cells ($CD3^+CD4^-CD8^+$)
- Monocytes ($CD14^+$) [peripheral blood only]
- NK cells ($CD3^-CD16^+CD14^-$)
- Activated T cells (CD3, CD25, and CD69)
- Activated B cells ($CD20^+$HLA-DR^+)

At the end of the study, the total number of B cells in the peripheral blood, spleen, lymph node, and bone marrow were greatly reduced in both male and female cynomolgus monkeys administered either CT-P10 or MabThera (see Table 9). Changes in other cell populations were sporadic and not considered toxicologically significant.

Table 9: 8-week monkey study, mean percent change in total B cells at terminal bleed versus vehicle control

Tissue/cell type	Absolute or %	Males		Females	
		CT-P10	MabThera	CT-P10	MabThera
Blood					
Total B cells	Absolute	-99%***	-99%***	-90%*	-71%
	%	-99%***	-99%***	-83%*	-71%*
Spleen					
Total B cells	Absolute	-95%*	-64%	-77%	-49%
	%	-97%	-75%	-77%**	-64%*
Lymph node					
Total B cells	Absolute	-99%**	-98%*	-76%	-82%
	%	-98%***	-92%***	-86%**	-90%**
Bone marrow					
Total B cells	Absolute	-66%	-92%*	-67%	-34%
	%	-66%	-83%*	-57%	-48%

* P<0.05; ** P< 0.01; ***P<0.001

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Not applicable.

7.2 *In Vitro* Assays in Mammalian Cells

Not applicable.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Not applicable.

7.4 Other Genetic Toxicity Studies

Not applicable.

8 Carcinogenicity

Not applicable.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not applicable.

9.2 Embryonic Fetal Development

Not applicable.

9.3 Prenatal and Postnatal Development

Not applicable.

10 Special Toxicology Studies

Study title: CT-P10: Cross reactivity study in human tissue

Study no.:	ZIP0002
Study report location:	eCTD, Module 4.2.1.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	29 September 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CT-P10, lot # CP0502, purity=99% (HPLC-SEC) Rituximab (MabThera), lot # B6036, purity=99% (HPLC-SEC)

Key Study Findings

- CT-P10 and MabThera demonstrated similar staining profiles with only minor differences in staining intensity.
- CT-P10 and MabThera specifically and reproducibly stained the tonsil, lymph node, thymus, and spleen, consistent with the known expression of CD20 on B cells.

The objective of this GLP study was to assess the potential tissue cross-reactivity of CT-P10 in comparison to MabThera.

Tissue samples were previously obtained and stored frozen at -80ºC until ready for use. The tissue panel contained 37 different tissues and blood smears from three unrelated human donors (see Table 10). The immunohistochemical method was developed and validated previously in a series of method development studies (study reports not submitted). Human tonsil and liver tissue were used for positive and negative control material, respectively. Three concentrations of CT-P10 and MabThera were evaluated: 0.05 µg/mL, 0.1 µg/mL, and 0.25 µg/mL. Human IgG1 isotype control (0.25 µg/mL) was used to distinguish between specific and non-specific binding. Slides were evaluated by light microscopy. A peer review statement was not submitted. Staining intensity was graded on a scale of negative (-), weak positive (+), moderate positive (++), or strong positive (+++).

Table 10: Tissue cross-reactivity study, tissues examined

Tissue (each tissue from three unrelated human donors)		
Adrenal gland	Kidney	Stomach
Urinary bladder	Liver	Striated muscle
Blood	Lung	Testis
Bone marrow	Lymph node	Thymus
Breast	Ovary	Thyroid
Cerebellum	Pancreas	Ureter
Cerebral cortex	Parathyroid	Cervix
Colon	Placenta	Endometrium
Endothelium	Pituitary gland	Tonsil
Eye	Prostate	Parotid gland
Fallopian tube	Skin	Peripheral nerve
Heart	Spinal cord	
Ileum	Spleen	

Results

Similar staining profiles were observed for CT-P10 and MabThera with only minor differences in staining intensity. CT-P10 and MabThera both stained the positive control material, and staining of negative control material was similar to isotype control. CT-P10 and MabThera specifically and consistently stained the tonsil, lymph node, thymus, and spleen. Lymphocytes in the colon, stomach, ileum, and endothelial tissue were also stained by CT-P10 and MabThera.

11 Integrated Summary and Safety Evaluation

The applicant conducted a stepwise approach to support the demonstration of similarity between CT-P10 and Rituxan; this approach consisted of a definitive analytical similarity assessment and abbreviated nonclinical and clinical programs. The comparative in vitro studies conducted as part of the analytical similarity assessment create a scientific bridge between CT-P10, Rituxan, and MabThera. The scientific bridge supports the use of MabThera as a comparator arm in the tissue cross-reactivity study and in the repeat-dose toxicity study in cynomolgus monkeys.

The tissue cross-reactivity profiles of CT-P10 and MabThera were comparable between treatment arms and consistent with the known expression of CD20 on B cells.

The pivotal nonclinical study was the comparative 8-week repeat-dose toxicity study in cynomolgus monkeys. The choice of test species, dose level, route of administration, and study duration are supported by information available for MabThera[1]. Two animals

[1] EMA, 2005, MabThera: EPAR - Scientific Discussion (accessible at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000165/WC500025817.pdf)

from the MabThera treatment arm were prematurely sacrificed in moribund condition; morbidity was attributed to immune reactions which are expected in cynomolgus monkeys. The overall incidence of immunogenicity was similar between treatment arms. Among the surviving animals, clinical signs, clinical pathology, organ weights, and histopathological findings were similar between treatment arms. The predominant observation was B cell depletion, consistent with the expected PD activity of rituximab, and was of similar magnitude between treatment arms. Systemic exposure was similar between treatment arms on Day 1. On Day 22, systemic exposure of CT-P10 was higher in males, while systemic exposure of MabThera was higher in females; due to the small number of animals analyzed TK data from Day 22 should be interpreted with caution.

Conclusion

The results of the in vitro 3-way similarity assessment, the tissue cross-reactivity study, and the comparative repeat-dose toxicity study in cynomolgus monkeys support the demonstration of biosimilarity between Truxima and Rituxan. From the perspective of nonclinical pharmacology and toxicology Truxima may be approved for the proposed indications.

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

/s/

MICHAEL L MANNING
01/17/2018

CHRISTOPHER M SHETH
01/17/2018
I concur with the primary review.