CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761053Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
BLA: 761053
Submission date: 4/28/2016
Drug: ocrelizumab
Applicant: Roche/Genentech
Indication: relapsing and primary progressive forms of multiple sclerosis

Reviewing Division: Division of Neurology Products

Discussion:
Toxicity studies of ocrelizumab were conducted in cynomolgus monkeys with up to 8 doses given every 3 weeks. Effects were related to the pharmacologic activity of B-cell depletion. Full to partial recovery was observed after a treatment-free period of 23 weeks.

Developmental and reproductive toxicity studies showed fetal effects related to the pharmacologic activity of ocrelizumab. B-cell depletion was observed in offspring of females treated from gestation day 20 through lactation day 28. B-cell depletion persisted through postnatal day 90 but was recovered by postnatal day 180. Adequate assessment of recovery of B-cell function was not conducted.

An appropriate established pharmacologic class for ocrelizumab is the existing term, “CD20-directed Cytolytic Antibody” which is the term used for other members of this class.

The adequacy of the nonclinical program has been called into question by a lack of sufficient comparability between the drug material used in the nonclinical studies and the to-be-marketed drug product. In particular, the comparability of the material used in the chronic toxicity study and the embryofetal study has not been established.

Conclusions:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. There appears to be a lack of comparability of the drug material used in the chronic toxicity and reproductive toxicity studies and the to-be-marketed product. In addition, there is concern that immune function in offspring of drug-treated dams has not been adequately assessed.

The reproductive toxicity studies may be acceptable if adequate comparability can be demonstrated between the drug material used in the study and the to-be-marketed clinical drug product. Repeat of the chronic toxicity study was not considered necessary given the available clinical data. Additional data

addressing recovery of B-cell function in offspring of dams treated with ocrelizumab appears warranted.

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/s/

PAUL C BROWN
12/13/2016

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: December 5, 2016

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: BLA 761053 (ocrelizumab; Ocrevus)

BLA 761053 has been submitted by the sponsor (Genentech, Inc.) for use of ocrelizumab as a treatment for relapsing forms of multiple sclerosis (MS) and primary progressive MS. Submission of Module 4, the nonclinical portion of Module 2, and part of Module 1 on April 4, 2016, initiated a rolling BLA submission. The remainder of the information (completion of Module 1 and Modules 2, 3, and 5) was received on April 28, 2016, and the BLA was filed (*Filing Communication, June 24, 2016*). Clinical development was conducted under IND 100593.

The nonclinical studies submitted in support of the BLA include the following:

- Pharmacology (in vitro, in vivo [monkey])
- PK/tissue distribution (mouse, rat, monkey)
- Toxicology (4- and 24-week studies in monkey)
- Reproductive and developmental toxicology (fertility assessments [male, female] and embryofetal and pre- and postnatal development studies in monkey)
- Tissue cross-reactivity (monkey, human)

This battery of nonclinical studies is consistent with the division's recommendations conveyed to the sponsor during clinical development. The data from these studies have been reviewed by Dr. Wilcox (*cf. Review and Evaluation of Pharmacology/Toxicology Data, Barbara J. Wilcox, Ph.D., BLA 761053, DATE*). Detailed description of the nonclinical studies and discussion of the findings are provided in Dr. Wilcox's review. Dr. Wilcox has concluded that the data provided by the sponsor do not support approval of the BLA because of the lack of sufficient data to document comparability between the product used in the pivotal reproductive and developmental toxicology studies and either the product used in the pivotal Phase 3 clinical trials or the to-be-marketed product.

This issue and selected nonclinical data are discussed below.

Pharmacology

Ocrelizumab is a humanized IgG_1 monoclonal antibody designed to bind CD20, a cell-surface antigen present on all B cells except early pro-B cells, plasma, or memory B cells, resulting in depletion of CD20+ B-cell populations through one or more mechanisms (i.e., "antibody-dependent cellular phagocytosis [ADCP], antibody-dependent cellular cytotoxicity [ADCC], complement-dependent cytotoxicity [CDC], and [or] apoptosis"). The pharmacological activity of ocrelizumab was characterized in a series of in vitro studies, compared to rituximab. (Rituximab is a CD20-directed monoclonal antibody approved for oncology and non-oncology indications but not for multiple sclerosis.) Data from selected in vitro studies are summarized in the following table (n/t = not tested):

IN VITRO SYSTEM	UNITS	OCRELIZUMAB	RITUXIMAB
WIL2-S cells (CD20-expressing human lymphoblastoid B-cell line)	K_d (nM)	0.84 ± 0.37	0.99 ± 0.49
human $F_{c\gamma}$ receptors $F_{c\gamma}$RIa $F_{c\gamma}$RIIa $F_{c\gamma}$RIIb $F_{c\gamma}$RIIIa (F158) $F_{c\gamma}$RIIIIa (V158)	EC_{50} (µg/mL)	 0.0036 ± 0.0005 1.65 ± 0.76 9.17 ± 1.77 3.28± 1.59 0.35 ± 0.14	 0.0037 ± 0.0002 1.68 ± 0.54 10.9 ± 1.52 10.8 ± 2.64 0.67 ± 0.15
Human Complement C1q	EC_{50} (µg/mL)	0.75 ± 0.015	0.62 ± 0.14
NK cell lysis of WIL2-S cells (ADCC)	EC_{50} (pM)	5.2 ± 2.6	23 ± 6.2
ADCC activity with PBMC	EC_{50} (nM)	0.12 ± 0.08	0.31 ± 0.23
	maximum lysis (%)	46.5 ± 8.8	34.1 ± 12.5
CDC (targets: WIL2-S cells and human complement)	EC_{50} (nM)	4.04 ± 1.87	1.09 ± 0.32
human B lymphoma cell lines (ADCP)	maximum (%)	≥20 (vs <10 for C)	n/t
Ramos cells (apoptotic activity)	crosslinking (%) (vs 2.5 for C)	18.5-29	16.5-38
	live cells/10 sec (vs 8600 for C)	5200	3100

In vivo characterization of pharmacological activity in animals was assessed as part of PK and toxicity studies in cynomolgus monkey and will be discussed under PK/ADME and Toxicology. Although PK/ADME studies were conducted in rodent, ocrelizumab is not pharmacologically active in rodent species (mouse, rat); therefore, those data are of limited relevance to humans and will not be discussed.

PK/ADME

PK/ADME studies were conducted in huCD20 transgenic and CD-1 mice, Sprague-Dawley rat, and cynomolgus monkey. Only the PK/ADME data for selected studies in cynomolgus monkey will be discussed (Studies 02-182-0352 and 03-0235-0349). The toxicokinetic (TK) data collected in the toxicity studies will be discussed under Toxicology.

In Study 02-082-0352, the PK/PD of ocrelizumab was assessed in cynomolgus monkey (total of 4 males/group) administered intravenous (IV) doses of 0, 0.05, and 10 mg/kg over a 5-min period. Each monkey received 2 weekly doses (Day 1 and Day 8), followed by an 8-week recovery period. Flow cytometry analysis indicated dose-related decreases in B cells (65 and >99% at LD and HD, respectively, on Day 10). At the last recovery sampling time (Day 67), B-cell count had normalized at the LD and in 1 HDM but was still decreased (57%) in the other HDM. The PK data are summarized in the sponsor's table, below.

Parameter [a]	Ocrelizumab (10 mg/kg)
CL (mL/day/kg)	10.3 (1.28)
V_{ss} (mL/kg)	126 (7.34)
$t_{1/2}$ (days)	6.58 (1.13)
C_{max} (µg/mL)	231 (12.3)
AUC_{inf} (µg • day/mL)	1963 (279)
AUC_{0-9} (µg • day/mL)	821 (55.4)

In Study 03-0235-0349, the PK/PD of ocrelizumab was assessed in cynomolgus monkey (4 males/group) administered IV bolus doses of 0.2, 0.5, and 2.0 mg/kg; each monkey received two doses (Day 1 and Day 8). There was a 12-week recovery period following the second dose. Flow cytometry analysis indicated marked decrease ("close to zero") in total B-cell number at 1 hr after each dose, at all dose levels. Partial recovery of B-cell number was observed at all doses, although to a lesser extent at the highest dose. The PK data are summarized in the sponsor's table, below (R_{Cmax} is the accumulation ratio: Dose 2 C_{max}/Dose 1 C_{max}).

Parameters	Dose Level (mg/kg)		
	0.2	0.5	2
C_{max} (µg/mL)	3.48±1.15	12.2±1.75	61.5±9.24
AUC_{last} (µg • day/mL)	3.56±4.66	14.5±6.66	90.2±37.9
$t_{1/2z}$ (day)	0.981±1.22	1.12±0.427	1.73±0.192
AUC_{inf} (µg • day/mL)	3.71±4.83	17.3±5.75	140±12.0
CL_{ss} (mL/day/kg)	154±122	32.2±10.6	15.4±1.46
V_{ss} (mL/kg)	88.4±42.1	49.0±8.21	39.8±6.76
R_{Cmax} (µg/mL)	1.43±0.188	1.25±0.121	1.29±0.142

Toxicology

Toxicology studies of ocrelizumab were conducted in cynomolgus monkey, the only pharmacologically relevant species, using a variety of dosing regimens.

The proposed clinical dosing regimen is:

- Initial doses: 300 mg IV infusion, followed by a second 300-mg IV infusion 2 weeks later
- Subsequent doses: 600 mg as a single IV infusion every 6 months

The same clinical dosing regimen was used during clinical development in studies of relapsing forms of MS. In studies of primary progressive MS, ocrelizumab was administered as two 300 mg IV infusions, given 2 weeks apart, every 24 weeks throughout the treatment period.

In monkey, early studies were conducted at doses of 0, 10, 50, and 100 mg/kg. The 10-mg/kg dose was most consistently associated with ADAs; therefore, later studies were conducted at doses of 0, 50, and 100 mg/kg, including the pivotal chronic toxicity study. (The presence of drug may have masked the presence of ADAs at the higher doses.)

In Study 03-0113-0349, ocrelizumab was administered to cynomolgus monkeys (4/sex/group) at doses of 0, 10, 50, and 100 mg/kg, as two IV bolus injections, one on Day 1 and one on Day 15. Animals were sacrificed on Day 29 or after a recovery period (Day 113; an additional 2/sex for C and HD).

In Study 03-0114-0349, ocrelizumab was administered to cynomolgus monkeys at doses of 0, 10, 50, or 100 mg/kg by IV bolus injection, according to the following study design (from the sponsor):

Group No.	Number of M/F	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Treatment Cycles	Number Released from Study M/F (SDay)[b]	Number Euthanized: M/F	
						Day 141	Day 297/296
1	4/4	0 (control)	5	2	-	2/2	2/2
2	2/2	10	5	1[a]	2/2 (108)[b]	-	-
3	2/2	50	5	2	-	2/2	-
4	4/4	100	5.1	2	-	2/2	2/2
5	2/2	100	5.1	1[c]	-	-	2/2

M/F = male/female; SDay = study day

[a] The animals were not administered a re-treatment of another two doses of rhuMAb 2H7 (second treatment cycle) because more than 50% of the animals within this group were positive for anti-drug antibodies.

[b] Evaluation of the development of anti-drug antibodies and the study day (noted in parenthesis) the animals were released from study and returned to the SBi animal colony.

[c] Only one treatment cycle was administered to Group 5 based on the original study design.

Groups 1, 3, and 4 received one dose (placebo or ocrelizumab) on Day 1 and a second dose two weeks later (Cycle 1). 14 weeks later, animals received two additional doses (Cycle 2) according to the same regimen used in Cycle 1. Animals were sacrificed as noted in the table above. Group 2 received one cycle of dosing (Cycle 1) only because ADAs were detected in >50% of the animals, associated with decrease in ocrelizumab

exposure. Animals were released back to the colony on Day 108 but were observed for clinical signs until Day 112. Group 5 received one cycle of dosing (Cycle 1), followed by a 40-week recovery period. Animal were sacrifice as noted in the table above. Terminal studies (histopathology, immunohistochemistry) were conducted but were not adequate because of the small number of animals at the scheduled sacrifice times. ADAs were detected primarily at the LD but, as previously noted, may have been masked at the higher doses by the presence of drug.

In Study 03-0684-0134, ocrelizumab (PRO70769, rhuMAb 2H7) was administered to cynomolgus monkeys (2/sex for C, 4/sex/group for LD and HD groups; 4-7 yrs) at doses of 0, 50, and 100 mg/kg as 4 IV injections, given once weekly. Half of the animals (1/sex for C, 2/sex/group for LD and HD) were sacrificed on Day 28; the remaining animals were followed for a 32-week recovery period. This study is not considered pivotal because of the inadequate number of control animals for in-life examinations and for all groups at the two sacrifice times.

In these studies, the primary toxicities observed were generally consistent among studies, i.e., decreases in absolute lymphocyte counts and decreases in peripheral and tissue (lymph nodes, spleen) B cell depletion, which tended to be greater at 50 and 100 mg/kg, with partial (>20% of baseline) to near complete recovery at 100 mg/kg, depending on the duration of dosing and of the recovery period. The one notable difference was the presence of microscopic findings in the eye (lymphoid or lymphoplasmacytic cell infiltrate in ciliary body, vacuolation of the optic nerve, and lymphoplasmacytic cell infiltrate of the choroid), which were observed in main-study (not recovery) animals at 50 and 100 mg/kg in Study 03-0114-0349.

In the pivotal chronic toxicity study (04-0192-0134), ocrelizumab was administered to cynomolgus monkeys (4/sex/group for main study; 2/sex C and HD for recovery) by IV bolus injection at doses of 0, 50, and 100 mg/kg every 3 weeks (total of 8 injections). Main-study animals were sacrificed on Day 149 (Week 22); the remaining animals were sacrificed after a 23-week recovery period, or "until B-cells recovered to greater than 25% of baseline levels." The study was conducted under U.S. FDA GLP regulations but with a number of exceptions, including the lack of a validated method for dose solution analysis and a number of analyses conducted by Genentech (i.e., dose formulation stability analyses, whole blood analysis by flow cytometry, and ICH and flow cytometry analysis of lymphoid tissues). Also, a signed and dated Study Pathology Report was not provided. Peer review involved only examination of target organs identified, presumably, by the study pathologist. (A signed and dated "Pathology Peer Review Documentation" was provided.)

The only potentially ocrelizumab-related clinical signs (4/8 LD and 3/12 HD) were red or clear nasal discharge and associated sneezing, accompanied in some animals by conjunctival discharge, which were successfully treated with penicillin. There were no ocrelizumab-related effects on body weight, ophthalmology/ERG, or other in-life parameters except for hematology. Absolute lymphocyte count was decreased in a dose-related manner in males and in LD females but not consistently in HD females. The

sponsor also reported a decrease in circulating rbc mass, accompanied by an increase in reticulocyte count and bone marrow erythroid hypercellularity in 1 M and 2 F at each dose; these findings were considered ocrelizumab-related.

Terminal evaluations revealed no macroscopic or organ weight findings. Ocrelizumab-related histopathology findings in main-study animals consisted of lymphoid follicular atrophy in lymph nodes (inguinal, mandibular, and mesenteric) and spleen and erythroid cell hypercellularity of bone marrow at both doses, with similar incidence. The eye findings observed in Study 03-0114-0349 were not detected. No ocrelizumab-related findings were detected in recovery animals (Day 315).

Additional measurements conducted by Genentech (rather than the CRO) included: flow cytometry analysis was conducted on bone marrow aspirates and whole blood and tissue (spleen and inguinal and submandibular lymph node) samples, immunohistochemistry evaluation of spleen and mandibular lymph nodes, and toxicokinetics (TK).

The TK data are summarized in the table below (mean ± SD; SD not calculated for values based on n = 2). AUC values were calculated from 0 to Day 148 (AUC_{last}) or from 0 to infinity (AUC_{inf}).

DOSES (mg/kg)	**C_{max} (µg/mL)**	**AUC_{last} (µg*day/mL)**	**AUC_{inf} (µg*day/mL)**	**$t_{1/2}$ (day)**	**Cl_{ss} (mL/day/kg)**	**V_{ss} (mL/kg)**
MALES						
50	1450 ± 43.3	100000 ± 7140	--	--	--	--
100	3240 ± 344	229000 ± 17700	274000	15.7	4.22	92.9
FEMALES						
50	1540 ± 121	115000 ± 13100	--	--	--	--
100	3020 ± 327	208000 ± 21500	221000	12.7	5.57	92.3

ADAs were detected in 2 animals (1 at each dose); no effect on serum concentrations was observed.

Results of the flow cytometry analysis demonstrated nearly complete depletion of peripheral B cells at both doses of ocrelizumab. B-cell levels remain near zero throughout the dosing period. B-cell depletion was also observed in tissues (spleen, lymph nodes, and bone marrow) at necropsy on Day 149. In HD recovery animals, peripheral B cells had increased to 58% of baseline by Day 307. At necropsy, B-cell depletion was observed in lymphoid tissue at both doses (0.4-0.1, 2.8-1.6, 1.4-1.1, and 20.3-23.9% of C in spleen, mandibular lymph node, inguinal lymph node, and bone marrow [LD-HD]). Mean % B cells in mandibular and inguinal lymph nodes was 27 and 55%, respectively, lower in HD recovery animals compared to controls (8.98 ± 4.50 vs 20.2 ± 5.79; males and females combined). Immunohistochemistry analysis of tissues (mandibular lymph node and spleen) revealed marked to complete depletion of B cells at both doses in main-study animals; tissue from HD recovery animals were either within normal limits or exhibited a slight-to-mild increase in B cells.

Reproductive and Developmental Toxicology

Fertility: effects of ocrelizumab on fertility were assessed in separate studies in male and female cynomolgus monkey. In the male fertility study, ocrelizumab was administered to sexually mature animals (6-8 years) at doses of 0, 15/20, and 75/100 mg/kg. Loading doses of 0, 15, and 75 mg/kg were administered daily for three days; thereafter, doses of 0, 20, and 100 mg/kg were administered weekly for 8 weeks, followed by a 16-week recovery period. Animals were necropsied after 9 weeks of dosing (5/group) or at the end of the 16-week recovery period (3/group) for evaluation of male reproductive organs (in a stage-aware manner). The only ocrelizumab-related finding was a dose-dependent decrease in sperm count during the recovery period (36 and 63% at LD and HD, respectively, at Week 8; 52 and 83% at LD and HD, respectively, at Week 16). In the absence of other findings and the high variability evident in the sperm data, the biological relevance of this finding is uncertain.

Serum and semen ocrelizumab exposures are summarized in the tables below (mean ± SD). Blood samples were collected at various intervals during the dosing and recovery periods. (All control samples were reported to be "lower than reporting range" of 20 ng/mL serum.) Semen samples were collected during Week 9 in main-study animals and during Week 8 of recovery. (Methods and sampling times for semen collection were not otherwise specified.)

Serum (selected data)

DOSE (mg/kg)	LOADING		MAINTENANCE		RECOVERY	
	$C_{max\ 3rd\ dose}$ (µg/mL)	$AUC_{(2\text{-}9\ days)}$ (µg*day/mL)	$C_{max\ 11th\ dose}$ (µg/mL)	$AUC_{(58\text{-}61\ days)}$ (µg*day/mL)	$AUC_{(55\text{-}t\ days)}$ (µg*day/mL)	$t_{1/2}$ (day)
15	1020 ± 72.1	5120 ± 676				
75	5180 ± 520	24600 ± 3060				
20			1670 ± 184*	3980 ± 584*	20010	13.5
100			5890 ± 737	13700 ± 2060	83255 ± 10044	18.7 ± 2.05

*ADA-positive animal excluded

Semen (µg/mL; mean ± SD [range]; LTR = lower than reporting range [nos])

SAMPLING TIME	DOSE (mg/kg)		
	0	20	100
MAIN STUDY (W9)	0.0394 ± 0.0394 [LTR*-0.0859]	22.1 ± 29.5 [2.69-88.0]	113 ± 131 15.5-353]
RECOVERY (W8)	LTR	0.809 ± 0.0374 [LTR-0.836]	3.37 ± 1.97 [2.06-5.64]
RECOVERY (W16)	0.00725 [LTR-0.00725]	0.107 ± 0.0695 [LTR-0.156]	1.30 ± 1.24 [0.176-2.62]

In the female fertility study, ocrelizumab was administered to sexually mature animals (≥6 years; 8/group) for three consecutive menstrual cycles at doses of 0, 15/20, and 75/100 mg/kg, given as weekly IV injections. Loading doses of 0, 15, and 75 mg/kg were administered daily for three days; thereafter (Day 10-105), doses of 0, 20, and 100 mg/kg

were administered weekly for up to 105 days, followed by a 3-menstrual cycle recovery period. Three LD and 2 HD animals exhibited a lack of menstruation either throughout the study (1 HD) or from recovery cycle 2-3 on (2 LD, 1 HD), although the mean menstrual cycle length was similar among groups during the dosing and recovery periods (affected animals excluded). No effects were observed on body weight (except for body weight loss in 1 HDF) or on hematology or clinical chemistry parameters. Immunophenotyping analysis revealed a marked decrease in CD40+ B cells at both doses, with near or complete depletion from Day 2 on, with no evidence of recovery. Microscopic findings were detected in lymphoid organs ("hypocellularity of lymphoid follicles in spleen and lymph nodes") but not in female reproductive organs. A nasal cavity carcinoma was detected in 1 LD animal, which was considered an incidental finding.

Blood samples for TK analysis were collected at various intervals during the dosing and recovery periods. Selected data are summarized in the following table (mean ± SD; summary recovery data could not be located in the report):

DOSE (mg/kg)	LOADING		DOSE 11			DOSE 13		
	C_{max} (µg/mL)	$AUC_{(2\text{-}9\ days)}$ (µg*day/mL)	C_{max} (µg/mL)	$AUC_{(79\text{-}t\ days)}$ (µg*day/mL)	$t_{1/2}$ (day)	C_{max} (µg/mL)	$AUC_{(93\text{-}t\ days)}$ (µg*day/mL)	$t_{1/2}$ (day)
15	77.9 ± 72.5	4530 ± 349						
75	4870 ± 762	21300 ± 3430						
20			905 ± 197	13700	12.5	790 ± 236	12600	11.0
100			4100 ± 856	88000	15.7	3460 ± 480	54400 ± 8920	14.8 ± 2.20

In the embryofetal development study, ocrelizumab was administered to pregnant monkeys (12/group) at doses of 0, 15/20, and 75/100 mg/kg by IV injection. Loading doses of 0, 15, and 75 mg/kg were administered for three days (GDs 20, 21, and 22); thereafter, doses of 0, 20, and 100 mg/kg were administered weekly for 4 weeks (GDs 29, 36, 43, and 50). Fetuses were examined following sacrifice of dams on GD 100-103. There were no clear ocrelizumab-related findings in dams. In fetuses, the only ocrelizumab-related findings were microscopic changes in lymphoid organs. (Only bone marrow, liver, lung, spleen, duodenum, colon, and thymus were examined microscopically.) The primary finding was a "very slight to slight decrease of splenic white pulp…" in 0/10 C, 5/11 LD, and 6/11 HD fetuses. Immunohistochemistry analysis of spleen and lymph nodes (mandibular, mesenteric) revealed a dose-related decrease in B cells and B cell follicles; complete depletion of B cells in one or more tissues examined was detected in 4/11 HD fetuses. Flow cytometry analysis (peripheral or umbilical vein blood) revealed substantial, dose-related decreases in B-lymphocytes (CD3-CD40+) in dams (by GD51; ~90-95%) and fetuses (85-93%). For TK analysis, blood samples were collected from dams at 0 and 0.25 hr post dose at various times during gestation (GD20-100); fetal blood samples were collected at cesarean section. The data for dams (over the entire dosing period) and fetuses (GD 100-103; data available for only 8/11 LD fetuses) are summarized in the following tables:

Dams

DOSE (mg/kg)	C_{max} ((µg/mL)	AUC_{inf} (µg*day/mL)	$t_{1/2}$ (day)	Cl_{ss} (mL/day/kg)	V_{ss} (mL/kg)
15/20	1310 ± 356	29100 ± 7830	8.93 ± 1.41	6.08 ± 2.10	82.7 ± 48.2
75/100	5860 ± 777	137000 ± 19600	9.97 ± 1.35	5.58 ± 0.794	64.8 ± 7.72

Fetuses

DOSE (mg/kg)	CONCENTRATION (µg/mL)	
	MEAN	RANGE
15/20	26.90	3.64-153
75/100	50.65	31.8-115

In the pre- and postnatal development study, ocrelizumab was administered to pregnant monkeys (15/group) at doses of 0, 15/20, and 75/100 mg/kg by IV injection. Loading doses of 0, 15, and 75 mg/kg were administered for three days (GDs 20, 21, and 22); thereafter, doses of 0, 20, and 100 mg/kg were administered weekly, from GD29 "to at least LD28" (i.e., total of 25-31 doses). Dams were allowed to deliver offspring and were followed for up to LD215. Offspring were examined for viability, sex, physical development, clinical pathology, and in terminal studies (gross pathology, organ weights, and histopathology). There were numerous deviation from GLP, including (but not limited to) misdosing, deviations from scheduled sampling collection times (TK, clinical pathology), incorrect storing of samples (e.g., room temperature instead of under refrigeration). However, the only deviations consider to have impacted study validity were those involved in the TDAR analysis. According to the sponsor, multiple methodological issues precluded a valid assessment of TDAR.

Three dams (1/group) were sacrificed moribund (on LD128, GD44, and GD111 in C, LD, and HD groups, respectively). No COD was identified for the C or HD dam. "…multi-organ hemorrhage and marked bilateral acute renal cortical necrosis" was detected in the LD dam, which, according to the study report, was thought to be the result of an "adverse immune reaction" to ocrelizumab; however, the study pathologist only indicated that the multi-organ hemorrhage and renal necrosis were the presumed COD. Decreased cellularity in lymphoid organs, both B- and T-cell, was detected in all 3 dams. All other dams survived the study and were returned to the colony. Thirteen C, 11 LD, and 9 HD dams received a total of 25-31 doses. The number of doses administered per dam and fates of corresponding fetuses are provided in the following table (D# = dam number; SS = scheduled sacrifice; SM = sacrificed moribund; PND = postnatal day; GD = gestation day; LD = lactation day):

DOSE (mg/kg)								
0			15/20			75/100		
D#	**LAST DOSE**	**FATE**	**D#**	**LAST DOSE**	**FATE**	**D#**	**LAST DOSE**	**FATE**
101	LD32	SS	**201**	GD162	**stillborn (GD168)**	**301**	LD31	SS
102	LD29	SS	**202**	LD34	SS	**302**	LD33	SS
103	LD34	SS	**203**	GD162	**stillborn (GD168)**	**303**	LD2[#]	**death (PND6)**
104	LD31	SS	**204**	LD30	SS	**304**	LD30	SS
105	LD28	SS	**205**	GD43	**dam SM[**]**	**305**	GD22	**aborted (GD25)**
106	LD34	SS	**206**	LD32	SS	**306**	LD31	SS
107	GD22	**aborted (GD25)**	**207**	LD29	SS	**307**	LD30	SS
108	LD29[*]	SS	**208**	LD29	SS	**308**	GD155	**stillborn (GD161)**
109	LD34	SS	**209**	LD31	SS	**309**	LD29[#]	SS
110	LD28	SS	**210**	GD169	**death (GD171)**	**310**	GD29	**aborted (GD32)**
111	LD29	SS	**211**	LD30	SS	**311**	GD106	**dam SM[**]**
112	LD29	SS	**212**	LD33	SS	**312**	LD32[#]	SS
113	LD31	SS	**213**	LD29	SS	**313**	LD34	SS
114	GD29	**aborted (GD30)**	**214**	LD33	SS	**314**	GD120	**aborted (GD121)**
115	LD29	SS	**215**	LD34	SS	**315**	LD28	**SM (PND138)**

[*]dam sacrificed moribund; offspring hand-reared; [**]fetus died as a result; #premature delivery

The fate of fetuses is summarized in the following table:

FATE OF FETUS/OFFSPRING	DOSE (mg/kg)		
	0	**15/20**	**75/100**
scheduled sacrifice	13/15	11/15	8/15
aborted	2/15	0/15	3/15
fetal death	0/15	1/15	0/15
stillborn	0/15	2/15	1/15
neonatal death	0/15	0/15	1/15
neonate sacrificed moribund	0/15	0/15	1/15
death due to moribund sacrifice of dam	0/15	1/15	1/15

The COD in the 3 stillborn offspring could not be identified. The 2 HD offspring found dead or sacrificed moribund were found to have bacterial infections. Findings in offspring #303N (of dam #303; premature delivery) were described as follows:

> "…severe suppurative inflammation characterized microscopically by a mixture of neutrophils and mononuclear inflammatory cells effacing the cerebellum and adjacent meninges…Pericardial and pleural adhesions with hemorrhage in the lung was noted grossly…and correlated microscopically to pleural fibrosis, interstitial mononuclear cell infiltration and alveolar hemorrhage with

> macrophage infiltration. A minimal increase in lymphoid follicle cellularity was noted in the spleen."

Findings in offspring #315N (of dam #315) were described as follows:

> "…inflammation of the meninges…with intralesional bacteria involving the brain and spinal cord, acute centrilobular hepatocellular necrosis, acute gall bladder *[sic]* necrosis, intralesional bacteria colonization with vascular invasion in the gallbladder, inflammation of the mesentery…decreased cellularity in the thymic cortex, splenic lymphoid follicle, and periarteriolar lymphoid sheath…"

There were no clear ocrelizumab-related clinical signs in dams or offspring, although "swollen skin in the breast area" resulting from bacterial infection (milk was positive for *Staphylococcus*) was detected in 1 LD (#202) and 1 HD (#315) dam. Gestation length was slightly reduced at the HD compared to C (157.7 ± 7.3 vs 165.4 ± 8.9 days); in 3 dams (#303, #309, #312), gestation length was consistent with preterm birth (generally defined, according to the sponsor, as birth on GD141 to GD155). No ocrelizumab-related adverse developmental effects were observed, as measured by body weight, growth (e.g., head width/circumference, crown-to-rump length, and anogenital distance), and neurological examination (e.g., reflexes, grip strength, and pain response).

In dams, wbc and lymphocyte counts were reduced at both doses during gestation (GD 141: 15 and ~20%, respectively) and lactation (LD 28-180: 11-24 and 20-44%, respectively). In neonates, wbc and lymphocyte counts were reduced at both doses on PND 28-64 (14-23 and 19-31%, respectively) but tended to normalize by PND 90 (wbc count) or PND 180-187 (lymphocyte count). Immunophenotyping on serum samples collected from dams (GD 20-LD 180) and neonates (PND 28-180) indicated no clear effect on T-cells; however, B-cells were depleted throughout gestation and lactation in dams (↓98%) and to at least PND 90 in neonates. At PND 180, recovery of B-cell counts was observed, with B-cell counts similar or only slightly lower (↓15%) than C in LD and HD neonates. However, functional recovery was not adequately assessed because of methodological problems encountered by the sponsor.

There were no clear gross pathology findings. The only ocrelizumab-related effect on organ weights was a 36-38% decrease in absolute and relative (to body weight) testis weight in HD neonates. The only histopathology finding in testes was immaturity (all males examined), consistent with the age of the offspring.

Ocrelizumab-related histopathology findings in neonates were also detected in kidney and bone marrow. The kidney findings presented in the summary histopathology table and in the individual data table were inconsistent and the severity of the kidney findings described by the Study Pathology were inconsistent with the severities listed in the summary and individual data tables. The table below provides the incidence of selected findings based on the individual data.

TISSUE	FINDING	DOSE (mg/kg)		
		0	15/20	75/100
kidney	lymphoplasmacytic inflammation (slight)	**0/13**	**0/13**	**2/11**
	glomerulopathy			
	very slight	0/13	3/13	2/11
	slight	0/13	0/13	2/11
	total	**0/13**	**3/13**	**4/11**
	lymphoplasmacytic, tubulointerstitial inflammation (slight)	**0/13**	**0/13**	**2/11**
bone marrow (sternum)	lymphoid follicle formation			
	very slight	0/13	4/13	3/11
	slight	0/13	0/13	2/11
	total	**0/13**	**4/13**	**5/11**

The renal findings were characterized by the study pathologist ((b) (4) DVM, DAVCP) as follows:

> "Minimal to mild glomerulopathy…comprised a spectrum of glomerular changes ranging from small immature (fetal) glomeruli [2 HD] with concentric fibrosis of the Bowman's capsule (crescent formation) to severely contracted sclerotic glomeruli…Mild lymphoplasmacytic inflammation of the kidneys was observed in 2 of 11 animals in the 75/100 mg/kg group. The extent of lymphoplasmacytic infiltrates was greater than concurrent control animals, and the infiltrating cells were present in nodular aggregates in the interstitium resembling lymphoid follicles."

Upon evaluation of kidney tissue with PAS staining, the kidney findings were characterized ((b) (4) M.D.) as a "…very mild excess of global glomerulosclerosis…suggestive of possible ischemic injury to developing glomeruli."

The study pathologist concluded that both the glomerulopathy and lymphoplasmacytic inflammation of the kidney were ocrelizumab-related, but noted that "…a possible association with the pharmacological effect of Ocrelizumab cannot be ruled out" only for lymphoplasmacytic inflammation. The possibility that increased protein clearance may have contributed to the kidney effects was not suggested by the study pathologist.

Immunohistochemistry (CD20 and CD3) evaluation of spleen, thymus, lymph nodes, tonsil and bone marrow from neonates indicated an increase in CD20- and CD3-positive cell aggregates (lymphoid follicle formation) in bone marrow at both doses (CD20+: 1/13 C, 7/11 LD, and 8/8 HD; CD3+: 0/13 C, 2/10 LD, and 3/6 HD).

For dams, toxicokinetic parameters were calculated only on GD22 data (15/group; mean ± SD).

DOSE (mg/kg)	C_{max} (ng/mL)	T_{max} (day)	$AUC_{(22\text{-}29\ d)}$ (ng*day/mL)
15	959000 ± 113000	22.0104*	3970000 ± 350000
75	4490000 ± 714000	22.1	17700000 ± 3100000

*0.25 hr post dose

Mean (± SD) serum levels in dams at 0.25 hr post dose on GD 71 were 1230000 ± 978000 and 1380000 ± 1060000 ng/mL at the LD and HD, respectively.

Serum ocrelizumab levels (ng/mL) in dams and neonates during the postnatal period are summarized in the following table (the sponsor did not provide summary data):

DOSE (mg/kg)	DAM	NEONATE	N:D RATIO
FINAL DOSE (pre-dose)			
20	606640 (373000-1290000)	141400 (80400-229000)	0.252 (0.139-0.434)
100	2257000 (1510000-3020000)	689111 (371000-1240000)	0.298 (0.233-0.411)
PND 99			
20	48881 (6190-70900)	2442 (1580-5900)	0.0895 (0.0205-0.285)
100	191956 (84400-471000)	22866 ((4890-42300)	0.138 (0.0283-0.234)
PND 180			
20	2457* (434-6300)	228* (109-368)	0.187* (0.0360-0.848)
100	5834** (1270-21800)	484** (146-1370)	0.138** (0.0428-0.345)

*does not include 2 pairs for which either dam or neonate data were not available
**does not include 1 pair for which data were not available for either dam or neonate

Ocrelizumab levels in milk were assessed during PNDs 25-28. Ocrelizumab was detected in milk of 3 (of 12) C dams (460-625 ng/mL). Ocrelizumab levels in LD and HD dams were 296-1370 ng/mL (8/11; in 3 LD dams, levels were undetectable) and 2360-7770 ng/mL (9/9), respectively.

The results of this study demonstrate an effect on gestation length in dams and adverse effects of ocrelizumab on the neonate, including an increase in stillbirths and neonatal death, B-cell depletion, and microscopic changes in kidney and bone marrow. While the cause(s) of the stillbirths was not identified, the neonatal deaths were attributed to bacterial infections, transferred from the dam and possibly exacerbated by B-cell depletion in the neonate.

Genetic Toxicology and Carcinogenicity

Genetic toxicology studies were not conduct for ocrelizumab, consistent with ICH S6 guidance.

The sponsor submitted a carcinogenicity assessment waiver request under IND 100593 (Serial No. 0675, February 4, 2016). The waiver was granted (*IND 100593, email communication, dated May 2, 2016*).

Comparability Issues

During clinical development, there were a number of changes in the drug substance manufacturing process. When a change was made from (b) (4) as part of the comparability assessment. A similar study was not conducted when subsequent changes were made.

Based on the available information, general toxicity studies (including the pivotal chronic study) and the embryofetal development study were conducted using v 0.1 drug substance (DS), the pre- and postnatal study was conducted using v 0.2 DS, the male fertility study was conducted using v 0.3 DS, and the female fertility study was conducted using v 0.4 DS. The pivotal clinical studies were conducted using v 0.4 DS.

According to the CMC team, nonclinical studies using v 0.2-0.4 DS may be acceptable, based on ADCC activity, which was highest in the v 0.2 DS (204-231%; compared to ~65-100% (estimated) in v 0.1 DS, 117-202% in v 0.3 DS, and 77-133% in v 0.4 DS), and on the activity levels for other mechanisms of action (e.g., CDC, apoptosis, and ADCP). Therefore, the chronic toxicity (04-0192-0134) and the embryofetal development studies were conducted using DS material that does not appear to be sufficiently comparable to that used in the pivotal clinical studies, based on ADCC activity.

Conclusions and Recommendations

The nonclinical studies conducted to support clinical development and a BLA for ocrelizumab are consistent with recommendations provided by the division, although the format of the study reports (e.g., the lack of separate summary data tables) made it unnecessarily difficult to conduct a thorough review of the data.

Pharmacology data demonstrated the intended pharmacological effect of ocrelizumab in cynomolgus monkey and verified that monkey was the only biologically relevant species for further nonclinical safety testing. Toxicity studies in cynomolgus monkey were conducted using a variety of IV dosing regimens, all involving more frequent dosing than is proposed for humans. In general, findings were similar among studies, with circulating B cells and lymphoid tissue being the primary targets, consistent with intended pharmacological activity of ocrelizumab in the treatment of multiple sclerosis, i.e., depletion of peripheral CD20+ B-cells. The nonclinical data suggest reversal of these effects, although complete reversibility was not always obtained following chronic administration.

The reproductive and developmental studies identified no clear effect on male or female reproductive organs or direct developmental toxicity. However, effects on the fetus and offspring were observed, including B-cell depletion in peripheral blood and lymphoid tissues, indicating exposure to drug during development. An increase in stillbirths and postnatal death or morbidity leading to premature sacrifice was attributed to adverse

effects of B-cell depletion in dams and, possibly, neonates. In surviving neonates, kidney (glomerulopathy) and bone marrow (lymphoid follicle formation) were observed at both doses evaluated.

Genetic toxicology studies were not required or conducted. Assessment of carcinogenic potential was deemed not feasible and the sponsor's waiver request was granted.

Overall, there are two primary deficiencies in the nonclinical data conducted to support approval:

- The DS material used in the embryofetal development study (v 0.1) does not appear to be sufficiently comparable (based on information provided by the CMC team) to that (v 0.4) used in the pivotal clinical studies.
- The lack of an adequate TDAR evaluation in the pre- and postnatal development (PPND) study precluded an evaluation of potential adverse effects on immune function.

It is recommended that an expanded pre- and postnatal development (ePPND) study in cynomolgus monkey be conducted (*cf. Stewart J. Repro Toxicol 28:220-225, 2009*), to include assessment of immune function. While the pivotal chronic toxicity study was also conducted using v 0.1 DS material, it is possible that there are now sufficient human safety data so that a repeat study in cynomolgus monkey may not be necessary. Alternatively, if the sponsor is able to provide additional data demonstrating sufficient comparability between v 0.1 DS and v 0.4 DS, then the completed embryofetal development study would be adequate; however, concerns regarding the second deficiency remain.

At this time, it appears that there are insufficient CMC data to support approval. If the to-be-marketed drug product is determined by the CMC team to not be comparable to the drug product(s) used in the pivotal nonclinical studies, additional nonclinical studies may need to be repeated.

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/s/

LOIS M FREED
12/05/2016

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:	761053
Supporting document/s:	0000/SDN1
Applicant's letter date:	4/28/2016
CDER stamp date:	12/1/2015, 4/28/2016
Product:	Ocrelizumab
Indication:	Multiple sclerosis
Applicant:	Roche/Genentech
Review Division:	Division of Neurology Products
Reviewer:	Barbara J. Wilcox, Ph. D.
Supervisor	Lois M. Freed, Ph.D.
Division Director:	Billy Dunn, M.D.
Project Manager:	Nahleen Lopez, Pharm.D.

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All tables and figures in this review are from the sponsor unless otherwise designated.

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1 Executive Summary

1.1 Introduction

Ocrelizumab is a humanized IgG1 monoclonal antibody directed against the CD20 antigen expressed on B lymphocytes and is under development for treatment of multiple sclerosis ((b) (4) and PPMS). CD20 is expressed on the surface of pre-B cells, mature B cells, and memory cells but is not found on lymphoid stem cells or plasma cells. Binding of ocrelizumab to CD20 results in rapid depletion of CD20+ B cells but does not affect the capacity for B cell repopulation.

1.2 Brief Discussion of Nonclinical Findings

Ocrelizumab specifically binds CD20 in humans and nonhuman primates. It does not bind CD20 in rodents. Therefore, cynomolgus monkey was selected as the relevant species for nonclinical investigation. A tissue cross-reactivity study confirmed that binding of ocrelizumab to cynomolgus monkey tissues was similar to that observed in human tissues. *In vivo* pharmacology studies demonstrated that B cell depletion to near undetectable levels was observed by 1 hour post-dose.

The mechanism through which ocrelizumab acts to deplete CD20+ B cells is believed to be via antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), and/or apoptosis. Each mechanism was investigated *in vitro*. The results demonstrated that ocrelizumab binds to CD20 with high affinity, binds to 5 subtypes of the Fc gamma receptor with relatively high affinity, and is capable of mediating ADCC, ADCP, CDC, apoptosis, and binds to the human FcRn.

PK/TK: The pharmacokinetic (PK) characteristics of ocrelizumab were assessed in a study in cynomolgus monkeys using relatively low doses, as well as in the toxicokinetic (TK) analyses conducted as part of the general toxicology studies. In the monkey PK study, the exposure increased in proportion with dose (0.2, 0.5, or 2.0 mg/kg IV). Clearance was biphasic with a rapid initial distribution phase followed by a slower elimination phase. ADAs were detected in all animals by 2 weeks after the first dose. Results of TK analyses confirmed the immunogenicity of ocrelizumab at all doses levels, but ADAs were most evident at doses of 10 mg/kg or lower. Therefore, the lower doses were not used in toxicology studies. At the higher doses used in the toxicology studies (20, 50, or 100 mg/kg), ADAs were detected in all dose groups; however, exposure was not significantly affected. Exposures increased dose-proportionally, and the elimination $t_{1/2}$ ranged generally from 15 to 20 hours at the higher doses used in the toxicology studies.

Toxicology: A series of repeat-dose toxicology studies were conducted in cynomolgus monkeys in which various dosing regimens were used. In all studies, cynomolgus monkeys received IV doses of ocrelizumab at dose levels of up to 100 mg/kg. No unexpected toxicities were observed. All studies showed near total depletion of B cells

from blood and lymphoid tissues. Histopathology typically showed follicular atrophy in spleen and lymph nodes. Recovery of B cell populations was variable and dose-related. Full recovery to baseline after repeat dosing required more than 6 months. In the longest duration study (Study #04-0192-0134), monkeys received ocrelizumab every three weeks for 24 weeks (total of 8 total doses) at doses of 50 or 100 mg/kg IV. The results showed the expected effects of the pharmacological activity of ocrelizumab, including depletion of B lymphocytes. Histopathology demonstrated follicular atrophy in spleen and lymph nodes of both dose groups, dose-related in incidence and severity. At the end of the 23-week recovery period, spleen and lymph nodes appeared similar to control.

Immunophenotyping of whole blood, lymphoid tissues, and bone marrow using FACS analysis was conducted for each toxicology study. Marked depletion of B lymphocytes was consistently demonstrated in all compartments assessed. Recovery was variable, but at least partial recovery was demonstrated in all studies, depending on the length of the recovery period. No dose-related effects on T cell populations were observed in any study.

Reproductive toxicology: A full battery of reproductive and developmental toxicology studies was conducted in cynomolgus monkey. In all studies, the pharmacological activity of ocrelizumab was confirmed by immunophenotyping using FACS analysis, and exposure was confirmed through TK analysis. Fertility was assessed in separate studies in males and females. No adverse effects on fertility parameters were observed in either sex.

In an embryofetal development study, pregnant cynomolgus monkeys received IV doses of ocrelizumab, with loading doses of 15 or 75 mg/kg for 3 days beginning on GD20, followed by weekly doses of 20 or 100 mg/kg through GD50. Cesarean section was conducted on GD100-103. No adverse effects on embryonic development related to ocrelizumab were observed. However, spleen and lymph nodes of fetuses from both dose groups showed reduced numbers of B cell follicles, dose-related in incidence. Reduced white pulp area was also observed in fetal spleen from both dose groups (graded slight or very slight). These data demonstrate trans-placental exposure to ocrelizumab in monkey fetuses.

In a pre-and postnatal study, pregnant monkeys received IV doses of ocrelizumab (loading doses of 15 or 75 mg/kg for 3 days beginning on GD20, followed by weekly doses of 20 or 100 mg/kg through 28 days of lactation [PND28]). Embryofetal viability was monitored with regular ultrasound evaluations. Results indicated that embryofetal loss was slightly greater in the HD group (13.3% in control, 6.7% in LD, and 20.0% in the HD groups). The sponsor asserts that the rate of embryofetal death is within the historical background rate at the testing facility. However, due to the apparent effect in the HD group, a relationship to ocrelizumab cannot be ruled out. An increase in stillbirths was also observed in the ocrelizumab groups. No stillbirths occurred in the control group. Two stillbirths (of 13) occurred in the LD group and 1 (of 11) occurred in

the HD group. Although not clearly dose-related in incidence, because none occurred in the control group, a relationship to ocrelizumab should be considered.

Three pre-term births (defined as birth GD141 to 155) occurred in the HD group compared to none in either the control or LD groups. Therefore, the mean gestation length was slightly shorter for the HD group, relative to the other groups. Two deaths occurred in offspring of HD dams. Both of these deaths were due to opportunistic infection (one case involved transfer from the dam), which could have been related to ocrelizumab via the immune suppression induced by the B cell depletion. Immunophenotyping demonstrated that B cells were nearly completely depleted at PND28 and remained at near undetectable levels through PND 90. By PND 180, B cell levels in offspring from both dose groups were similar to control.

Ocrelizumab was detected in milk in both dose groups (range of 428-3970 ng/mL).

No ocrelizumab-related adverse effects were observed on postnatal development. Physical developmental parameters and neurological parameters were monitored on a regular basis, but a definitive assessment of learning and memory was not conducted (not feasible in monkeys under the conditions of the pre- postnatal study design).

There were no visible developmental abnormalities (visceral or skeletal) in offspring of ocrelizumab-dosed dams. However, histopathology of the offspring demonstrated an increased incidence of glomerulopathy (graded minimal to mild) at both doses. The incidence of glomerular changes was 3 of 13 in the LD group and 4 of 11 (number includes the 2 neonatal deaths) in the HD group. Mild lymphoplasmacytic inflammation of the kidneys was also noted at low incidence in HD neonates. The mechanism for these findings was not identified, but the data suggest an ocrelizumab-related injury to the developing kidney.

Concerns regarding lot-to-lot potency were raised by the CMC team that could invalidate some nonclinical studies (of most concern would be the validity of the reproductive toxicology studies). The sponsor should be asked to confirm that the ocrelizumab used for the reproductive toxicology studies is comparable to the product used in the pivotal clinical studies and the product intended for market.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical perspective, ocrelizumab is not considered approvable for the proposed indication due to lack of information regarding whether the product used in the reproductive toxicology studies is comparable to the product used in the pivotal clinical studies and the product intended for market. If the product used in the pivotal clinical studies is not comparable (as determined by the CMC team) to the product used in the reproductive toxicology studies and the product intended for market, some studies may need to be repeated.

1.3.3 Labeling Recommendations

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no adequate (b) (4) data (b) (4) OCREVUS (b) (4) in pregnant women (b) (4) B-cell levels in human neonates following maternal exposure to OCREVUS (b) (4). However, transient peripheral B-cell depletion and lymphocytopenia have been reported in infants born to mothers exposed to other anti-CD20 antibodies during pregnancy *[see Data]*. (b) (4) OCREVUS is a humanized monoclonal antibody of an immunoglobulin G1 subtype and immunoglobulins are known to cross the placental barrier. (b) (4)

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

Data

Animal Data

(b) (4)

(b) (4)

8.2 Lactation

Risk Summary

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity studies have been (b) (4) to assess the carcinogenic potential of OCREVUS.

No studies have been (b) (4) to assess the mutagenic potential of OCREVUS. As an antibody, OCREVUS is not expected to interact directly with DNA (b) (4)

(b) (4) 15 or 75 mg/kg (b) (4) followed by weekly IV doses of 20 or 100 mg/kg (b) (4)

2 Drug Information

2.1 Drug

Generic Name: Ocrelizumab

Code Name: RO4964913, PRO70769, rhuMAb 2H7

Molecular Formula/Molecular Weight:
$C_{6482}H_{9952}N_{1712}O_{2014}S_{46}$
145,564 Da

Structure or Biochemical Description: *Monoclonal antibody, humanized IgG1*

Pharmacologic Class: Anti-CD20 recombinant humanized monoclonal antibody

2.2 Relevant INDs, NDAs, BLAs, and DMFs

IND 100593 (DNP/multiple sclerosis), other INDs have been withdrawn or are inactive.

2.3 Drug Formulation

Table P.1-1 Composition of Drug Product

Ingredient	Nominal Amount per Vial	Target Concentration	Function	Standard Specifications
Ocrelizumab	300 mg	30 mg/mL	Active ingredient	Section S.4.1 *Specification*.
Sodium Acetate Trihydrate	21.4 mg[a]	(b) (4)		USP/Ph. Eur./JP
Glacial Acetic Acid	2.5 mg[a]			USP/Ph. Eur./JP
(b) (4) Trehalose Dihydrate	400.0 mg			NF/Ph. Eur./JP
Polysorbate 20	2.0 mg			NF/Ph. Eur./JPE
				(b) (4)

Note: The allowable excess volume is in accordance with USP <1151>. For details see Section P.2.2 *Drug Product*.

Abbreviations: NA = not applicable; NF = National Formulary; QS = quantity sufficient.

(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

Ocrelizumab is intended for treatment of patients with relapsing and primary progressive forms of MS.

Initial dose: 600 mg administered as two separate IV infusions of 300 mg each two weeks apart. Subsequent doses are to be administered as 600 mg IV infusion every 6 months.

2.7 Regulatory Background

IND 100593 was submitted on January 9, 2008. The IND was placed on full clinical hold on February 11, 2008 because of clinical protocol deficiencies. The sponsor responded to the hold concerns and the full clinical hold was removed (Remove Full Clinical Hold letter dated May 21, 2008). A pre-BLA meeting was held on February 4, 2016.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology:
Study #03-0387-0349 In vitro binding and activity of humanized anti-CD20 2H7
Study #02-182-0352 A two week (non-GLP) pharmacokinetic and pharmacodynamic study of two humanized 2H7 monoclonal antibodies in cynomolgus monkeys with an 8-week recovery period

Pharmacokinetics:
PK in rat, mouse, monkey.
Tissue cross-reactivity in human and cynomolgus monkey tissues

Repeat-dose toxicology:
Subchronic study in cynomolgus monkey: 4 weekly IV doses.
Chronic studies: Conducted in cynomolgus monkey, IV dosing.

Reproductive toxicology:
Fertility studies in male and female cynomolgus monkeys.
Embryofetal development in cynomolgus monkey
Pre- and postnatal development in cynomolgus monkey.

No stand-alone safety pharmacology studies were conducted.
Carcinogenicity studies were not considered feasible and were waived.

APPEARS THIS WAY ON ORIGINAL

4 Pharmacology

4.1 Primary Pharmacology

Ocrelizumab is a humanized IgG1 monoclonal antibody that selectively targets the CD20 antigen expressed on B lymphocytes. The product is under development for treatment of relapsing-remitting and primary progressive multiple sclerosis. CD20 is expressed on the surface of pre-B cells, mature B cells, and memory cells but is not found on lymphoid stem cells or plasma cells. Binding of ocrelizumab to CD20 results in rapid depletion of CD20+ B cells but does not affect the capacity for B cell reconstitution. (Recent published data indicate that CD20 may also be expressed on a small population of CD4+ and CD8+ T lymphocytes in humans. The presence of CD20+ T cells in monkeys has not been demonstrated.)

The pharmacology of ocrelizumab was investigated in a series of *in vitro* and *in vivo* studies. Because ocrelizumab binds only to human and non-human primate CD20, *in vitro* studies were conducted using human-derived cell lines and *in vivo* studies were conducted in cynomolgus monkey. The mechanism of action through which ocrelizumab acts to deplete CD20+ B cells is believed to be via antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), and apoptosis. The various mechanisms were investigated *in vitro* (except ADCP) to determine if any or all could be responsible for ocrelizumab-mediated B cell depletion *in vivo.*

Primary Pharmacodynamics:

Study #02-182-0352

Title: A two week (non-GLP) pharmacokinetic and pharmacodynamic study of two humanized 2H7 monoclonal antibodies in cynomolgus monkeys with an 8-week recovery period

Testing Facility: (b) (4)

GLP Compliance: No

Drug, lot #: ocrelizumab, 16hu2H7, lot #38903-25A; ocrelizumab, 31hu2H7, lot #38903-28

Methods:

Animals: cynomolgus monkey

Number/group: 4 males/group

Doses: 0, 0.05, or 10 mg/kg for each test article

Route of administration: IV

Regimen: Weekly for 2 doses

Study design: This study was conducted early in development to evaluate two clones of ocrelizumab. The report did not indicate whether either clone was carried forward as the definitive product.

Group Assignments

Treatment Group / Color Code	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Study Specific Animal Numbers
1 / white, hu2H7 Vehicle	0	0	0.5	1 – 4 (1, 3)*
2 / yellow, v.16 hu2H7	0.05	0.1	0.5	5 – 8 (7, 8)*
3 / green, v.16 hu2H7	10	20	0.5	9 – 12 (11, 12)*
4 / blue, v.31 hu2H7	0.05	0.1	0.5	13 – 16 (14, 15)*
5 / red, v.31 hu2H7	10	20	0.5	17 – 20 (17, 18)*

*Recovery Animals: for each treatment group, the Sponsor assigned the two animals with pre-study B-Cell counts nearest the mean of their respective groups to recovery.

Observations/Results

Mortality: (Observations were made twice daily.)
All animals survived to scheduled necropsy.

Clinical Observations: (Data were recorded twice daily.)

- No clinical signs related to the test articles were observed.

Body weight: (Data were recorded once prior to initiation of dosing, the day prior to each dose, and weekly during the recovery period.)

- No test article-related effects on body weight were observed.

Food Consumption: (Data were recorded twice per week.)

- No test article-related effects on food consumption were observed.

Hematology and immunophenotyping: (Samples were collected twice prior to initiation of dosing, on SD1 pre dose, and 6, 24, and 72 hours post-dose. Samples were collected prior to dosing on SD8, prior to necropsy on SD10, and from recovery animals on SD36 and 67.)

- Total lymphocyte counts were reduced by approximately 50% within 6 hours of dosing in the LD and HD groups for each test article. In the LD groups, a slight trend toward recovery was observed prior to the second dose.
- Both test articles produced dose-related reductions in B lymphocytes. The HD groups showed nearly total depletion of circulating B cells.
- B cell depletion by the 2H7v.31 drug variant appeared to persist longer than the v.16 variant

Clinical chemistry: (Samples were collected prior to dosing on SD1, 8, and 10. Recovery samples were collected on SD67 prior to the recovery euthanasia.)

- No effects on clinical chemistry parameters related to ocrelizumab were observed.

Cytokine analysis: (Samples were collected once prior to initiation of dosing, then 2 and 4 hours post-dose on SD1 and 8. Samples were analyzed for levels of IL-6.)

- IL-6 levels were increased on SD1 at 2 hours post-dose in 15 of 16 animals that received test article (all dose groups). IL-6 levels decreased at 4 hours post-dose but remained elevated compared to the pre dose baseline. After dosing on SD8, the incidence and magnitude of the elevations in IL-6 (6 of 16) were reduced.

Table 2
Results of Serum Assay for Interleukin 6

Group/ Dose/Test Material	No./ Sex	Animal ID	Interleukin 6 Levels (pg/mL)					
			Day 1			Day 8		
			Predose	2 Hours	4 Hours	Predose	2 Hours	4 Hours
1 0 mg/kg Vehicle	4/M	1	LTS	14.7	14.8	LTS	LTS	6.5
		2	LTS	LTS	LTS	LTS	LTS	17–29[a]
		3	LTS–80[a]	LTS	21–43[a]	LTS–35[a]	10–129[a]	6–95[a]
		4	LTS	LTS	LTS	LTS	LTS	LTS
2 0.05 mg/kg hu2H7 v.16	4/M	5	LTS	11.3	LTS	LTS	6.6	LTS
		6	LTS	22.9	6.1	LTS	LTS	LTS
		7	LTS	35.7	9.2	LTS	LTS	LTS
		8	LTS	11.3	LTS	LTS	6.6	LTS
3 10.0 mg/kg hu2H7 v.16	4/M	9	LTS	22.9	6.1	LTS	LTS	LTS
		10	LTS	35.7	9.2	LTS	LTS	LTS
		11	LTS	43.7	7.1	LTS	LTS	LTS
		12	LTS	25.2	18.1	LTS	15.5	LTS
4 0.05 mg/kg hu2H7 v.31	4/M	13	LTS	22.8	LTS	LTS	LTS	LTS
		14	15.8	LTS	4.2	LTS	LTS	LTS
		15	LTS	80.8	30.2	LTS	LTS	11.3
		16	LTS	89.2	24.1	LTS	43.7	8.9
5 10.0 mg/kg hu2H7 v.31	4/M	17	LTS	24.6	LTS	LTS	LTS	LTS
		18	LTS	19.6	6.2	LTS	LTS	3.6
		19	LTS	3.6	LTS	LTS	LTS	LTS
		20	LTS	15.2	LTS	LTS	LTS	LTS

LTS=Less than standard (3.12 pg/mL).
M=Male.
[a] Samples did not dilute linearly; range of results reported.

Toxicokinetics:

Table F1
Mean (SD) Pharmacokinetic Parameters for hu2H7 v.16 (Group 3) and hu2H7 v.31 (Group 5) Following Intravenous Bolus Administration to Cynomolgus Monkeys at 10 mg/kg Once Weekly for 2 Weeks using Noncompartmental Analysis

Parameter	hu2H7 v.16 (n=4)	hu2H7 v.31 (n=4)
t_{max} (hr)	169 (0)	169 (0)
t_{last} (hr)	570 (676)	588 (666)
C_{max} (μg/mL)	231 (12.3)	250 (27.6)
AUC_{0-216} (μg • hr/mL)	19700 (1330)	20900 (2500)
AUC_{last} (μg • hr/mL)	34300[a]	44000[b]
AUC_{inf} (μg • hr/mL)	47100 (6690)	46000 (10900)
$t_{1/2}$ (hr)	158 (27.2)	131 (25.5)
Slope (1/hr)	0.00447 (0.000723)	0.00547 (0.00131)
Lower timepoint (hr)	102 (108)	102 (108)
Upper timepoint (hr)	522 (708)	522 (708)
No. of points for $t_{1/2}$	3.25 (0.5)	3.25 (0.5)
R^2	0.971 (0.0444)	0.96 (0.0366)
MRT (hr)	297 (42.3)	255 (36.4)
MRT_{last} (hr)	155 (71.9)	157 (61.5)
CL (mL/hr/kg)	0.431 (0.0538)	0.460 (0.142)
V_{ss} (mL/kg)	126 (7.34)	114 (20.2)

Note: Parameters were estimated using noncompartmental methods. AUC was computed using the linear trapezoidal rule. Half-life was computed via log linear regression of the terminal concentrations between the upper and lower timepoints listed.

[a] AUC_{last} for the recovery animals (n=2) is reported in the table. AUC_{last} for the nonrecovery animals (n=2) was 20700 μg • hr/mL.

[b] AUC_{last} for the recovery animals (n=2) is reported in the table. AUC_{last} for the nonrecovery animals (n=2) was 20100 μg • hr/mL.

Immunogenicity: (Blood samples were collected prior to dosing on SD1, 10, 36, and 67.)

- By SD10, ADAs were detectable in all groups except control (incidence was approximately 50%).

Necropsy: (Animals were euthanized on SD10 and recovery animals on SD67. Full necropsy was conducted.)

Gross findings:

- No macroscopic findings considered to be related to the test articles were reported.

Organ weights:
- No consistent effects on organ weights were observed.

Histopathology (tissues with gross findings only):
- One of 4 animals in Group 5 (HD, v.31) showed follicular atrophy in the spleen.

Immunohistochemistry: (Immunohistochemistry for CD20 was conducted on lymphoid tissues and bone marrow.)
- Wide variation in CD20+ staining was observed among all animals including controls. Therefore, no conclusions could be drawn from the results.

Dosing solution analysis: All dosing samples were determined to be within the pre-established acceptance criteria for concentration.

Conclusion:
The data indicate that cynomolgus monkey is an appropriate model for testing ocrelizumab. ADA responses at the low doses may preclude use of the lower doses in toxicology studies. Both product clones appeared to be similar in pharmacodynamic and pharmacokinetic activity. (The study was conducted to evaluate products from 2 different clones. The sponsor has not made clear which clone [or if either of the clones] was selected for further development.)

Study #03-0235-0349
Title: A pharmacokinetic and pharmacodynamic study of rhuMAb 2H7 in cynomolgus monkeys with a 12-week recovery period.

Testing Facility:	(b) (4)
Date of Study Initiation:	Not specified (May, 2003)
GLP Compliance:	No (Exploratory study)
Drug, Lot #, purity:	Ocrelizumab, M3-TOX64, 99%

Methods:
Animals: Cynomolgus monkey, males only
Weight: 2.5 to 4 kg
Number per group: 4
Doses: 0.2, 0.5, or 2.0 mg/kg
Route of administration: IV
Regimen: Weekly for 2 doses on SD1and 8
Study design:
No control group was included in this exploratory study. The first day of dosing was SD1; SD11 was designated the first day of recovery.

Table 1: Group Assignments

Treatment Groups/ Color Codes	Test Article	Route	Dose Level (mg/kg)	Dose Volume (mL/kg)	Days of Dosing	Animals ID Numbers
1/white	rhuMAb 2H7	IV	0.2	1.0	1 and 8	021949, 021976, 022011, 022156
2/yellow	rhuMAb 2H7	IV	0.5	1.0	1 and 8	021338, 021952, 021961, 021999
3/green	rhuMAb 2H7	IV	2.0	1.0	1 and 8	021969, 021992, 021993, 022021

Observations/Results:

Clinical signs: (Recorded twice daily beginning on SD -3 until SD92.)

- No clinical signs related to ocrelizumab were observed.

Body weight: (Data were recorded once on the day prior to each day of dosing and weekly thereafter.)

- No ocrelizumab-related effects on body weight were observed.

Food consumption: (Recorded daily from day -3 until SD92.)

- No ocrelizumab-related effects on food consumption were observed.

Hematology: (Samples were collected on SD -12, -7, -6, SD1 and 8 prior to dosing at 1, 4, 24, 48 hours post-dose. Samples were also collected on SD15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 92.)

- A decrease in mean total lymphocytes was observed in all dose groups on SD1. The magnitude of decrease was generally dose-related. By 1 hour post-dose decline in mean total lymphocytes was -61%, -64% and -58% for the LD, MD and HD, respectively. (Three of four HD animals showed an unusual drop in total lymphocytes of nearly 50% between SD-6 and pre-dose on SD1. The reason for this decline was not given.)
- By SD8 total lymphocyte counts showed trend toward recovery for the LD and MD groups. On SD8, the HD group showed continued suppression of lymphocyte counts.
- After dosing on SD8, the reduction in mean total lymphocytes was again observed in all dose groups, but the magnitude of the effect was smaller than after the first dose.
- By the end of the recovery period, mean lymphocyte counts had recovered to baseline (SD1 pre dose) levels for all dose groups.
- Small reductions in red blood cell parameters were observed during the study. These effects were considered to be due to the frequent blood draws and were accompanied by an increase in RET.

Clinical chemistry: (Samples were collected prior to dosing on SD1 and on SD92.)

- No effects related to ocrelizumab on serum chemistry parameters were observed.

Immunophenotyping: (Samples were collected on days -12, -7, -6, SD1 and 8 prior to dosing at 1, 4, 24, 48 hours post-dose. Samples were also collected on SD15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 92.)

- B cells were depleted to almost undetectable levels in most animals by one hour after dosing in all 3 dose groups. (The data were highly variable among animals.)
- Partial recovery was observed by SD8 in the LD and MD groups. Near full depletion was again observed 1 hour after the second dose in most animals in all dose groups.
- At the end of the recovery period, near baseline B cell levels were observed in the LD and MD groups. The HD group did not show full recovery to baseline, but partial recovery was observed.
- Other cell types including T lymphocytes (CD4+ and CD8+) and NK cell showed high variability after each dose. An effect of ocrelizumab on T cells and NK cannot be ruled out under the conditions of this study.

Pharmacokinetics: (Blood samples were collected prior to dosing, then at 1, 4, 24, and 48 hours post-dose on both dosing days. Samples were also collected weekly from SD15 through the recovery period.)

Table D2

Mean±SD Noncompartmental PK Parameters of Serum rhuMAb 2H7 Following Intravenous Bolus Administration to Cynomolgus Monkeys (n=4 males/group) at 0.2, 0.5, and 2 mg/kg Once Weekly for 2 Weeks (Dosed on Elapsed Days 0 and 7)

Parameter	Dose Level (mg/kg)		
	0.2	0.5	2
C_{max} (µg/mL)	3.48±1.15	12.2±1.75	61.5±9.24
AUC_{last} (µg•day/mL)	3.56±4.66	14.5±6.66	90.2±37.9
$t_{1/2z}$ (day)	0.981±1.22	1.12±0.427	1.73±0.192
Auc_{inf} (µg•day/mL)	3.71±4.83	17.3±5.75	140±12.0
CL_{ss} (mL/day/kg)	154±122	32.2±10.6	15.4±1.46
V_{ss} (mL/kg)	88.4±42.1	49.0±8.21	39.8±6.76
R_{Cmax} (µg/mL)	1.43±0.188	1.25±0.121	1.29±0.142

AUC_{inf}=Area under the concentration–time curve from zero to infinity.

AUC_{last}=Area under the concentration–time curve from zero to last.

CL_{ss}=Drug clearance at steady state.

C_{max}=Maximal serum concentration.

R_{Cmax}=Accumulation ratio of C_{max}, calculated by comparing the C_{max} after the second dose to that after the first dose.

$t_{1/2z}$=Terminal half-life.

V_{ss}=Volume of distribution at steady state.

Elimination of ocrelizumab after the first dose was biphasic at all dose levels. After the second dose, the biphasic elimination was less clear, most likely due to generation of ADAs.

Immunogenicity: (Blood samples were collected for ADA detection prior to dosing on SD1, and on SD15, 29, 43, 57, 78, and 92.)

- ADAs were detected in all animals at all dose levels.

Necropsies were not conducted.

Dosing solution analysis:
All dosing formulations were determined to be within the pre-determined acceptance criteria for concentration.

Study #03-0387-0349

Title: In vitro binding and activity of humanized anti-CD20 2H7

Testing Facility: Genentech, Inc.
GLP Compliance: No
Date of Study Initiation: 9/5/2002
Drug, Lot #: (List taken from the study report, PRO70769 is ocrelizumab)

PRO70769, Lots: 38903-70A, 40454-42, 40711-24, 40711-29, 38903-25A, 40305-29, L80458, L81631, L81629

Rituximab, Lots: C2B81298-2, C2B8 UFB 136618T1, C2B8 UFB 135453T1, L76282, K9028A

Murine monoclonal 2H7, Lot: M06795
(b) (4)

Trastuzumab (control for FcRn binding assay), rhuMAb Her2, Lot: L9064AX134BN

Trastuzumab (negative control for ADCC activity with NK cells and apoptosis assay), rhuMAb Her2, Lot: G123AUB9817AX

Results:

Scatchard analysis: A CD20-expressing human lymphoblastoid B cell line (WIL2-S) was used to determine the K_d of CD20 binding of each product listed above. Cells were incubated in the presence of radio-labeled ocrelizumab, rituximab, and murine monoclonal 2H7 in serial dilutions for 24 hours then harvested, washed, and counted to produce values from which binding affinities could be calculated.

Table 1
Equilibrium Binding Affinity of Anti-CD20 Antibodies to WIL2-S Cells

Antibody Variant	n	K_d (nM)
Rituximab	3	0.99±0.49
Murine monoclonal 2H7	3	1.23±0.29
PRO70769	4	0.84±0.37

K_d=Dissociation constant.

FcγR binding: Fc gamma receptor (FcγR) binding was compared among test articles using standard *in vitro* methods. Binding to the following related Fc receptors was assessed: FcγRIa (CD64), FcγRIIa (CD32A), FcγRb (CD32B), and FcγRIIIa (CD16).

Assay plates coated with the various receptor types were incubated in the presence of each test or control article for 2 hours. The bound test or control antibody was detected using standard immunochemical techniques and developed with TMB.

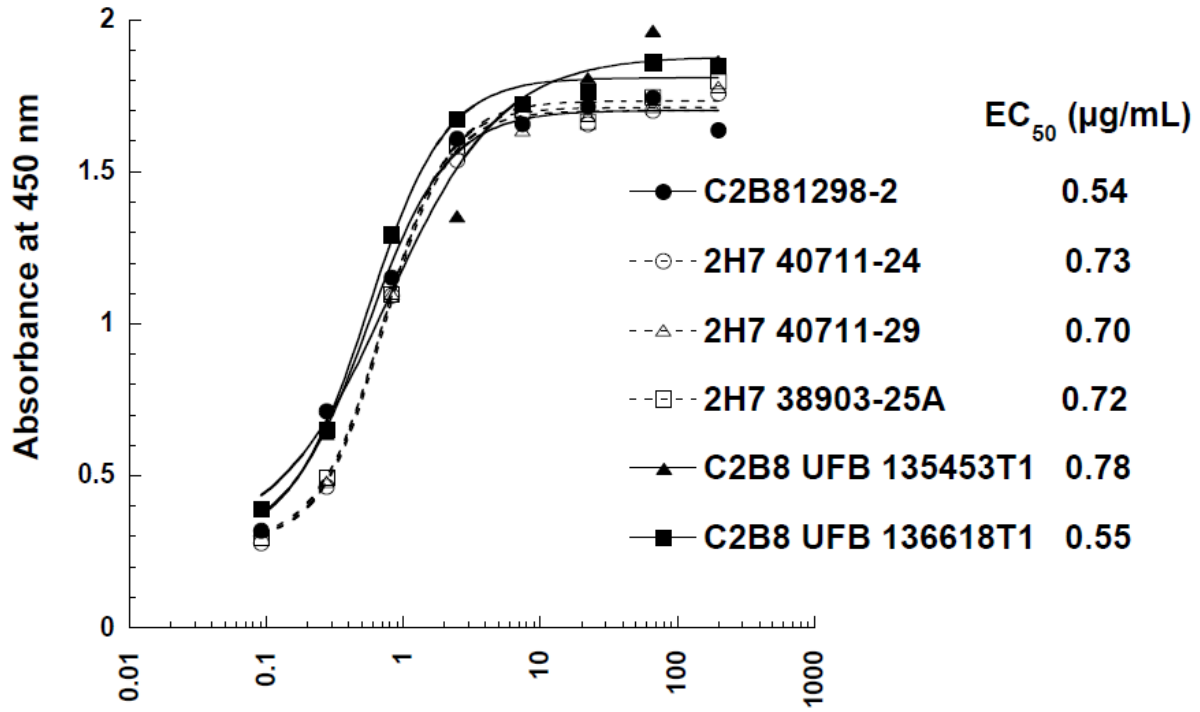
Table 2

EC_{50} Values (µg/mL) for Antibody Binding to Human Fc Gamma Receptors

Fcγ Receptor	PRO70769	Rituximab
FcγRIa	0.0036 ± 0.0005 (n=3)	0.0037 ± 0.0002 (n=3)
FcγRIIa	1.65 ± 0.76 (n=5)	1.68 ± 0.54 (n=4)
FcγRIIb	9.17 ± 1.77 (n=5)	10.9 ± 1.52 (n=4)
FcγRIIIa (F158)	3.28 ± 1.59 (n=5)	10.8 ± 2.64 (n=4)
FcγRIIIa (V158)	0.35 ± 0.14 (n=5)	0.67 ± 0.15 (n=4)

Complement C1q binding assay: Serial dilutions of test and control antibodies were coated onto culture plates and subsequently incubated with purified human C1q (2µg/mL). Bound C1q was detected using standard immunochemical methods using goat anti-human C1q followed by horseradish-conjugated donkey anti-goat IgG and developed with TMB as substrate. Absorbance was read at 450 nm. EC_{50} values for antibody binding were calculated using computer assisted nonlinear regression analysis.

Figure 6
Antibody Binding to Complement C1q
24Jan2003



FcRn binding assay: Binding of antibodies to FcRn was evaluated by coating culture plates with commercially available human FcRn and incubated in the presence of serial dilutions of control or test antibody. Bound antibody was quantified by absorbance at 450 nm after reaction with peroxidase-conjugated-goat anti-human IgG and TMB using standard immunochemical techniques. The absorbance values were plotted against concentration of antibody and analyzed via a commercially available program. Trastuzumab was used as assay control for calculating relative binding affinities. Five lots of ocrelizumab were compared.

Figure 7
Antibody Binding to FcRn
25Jul2003

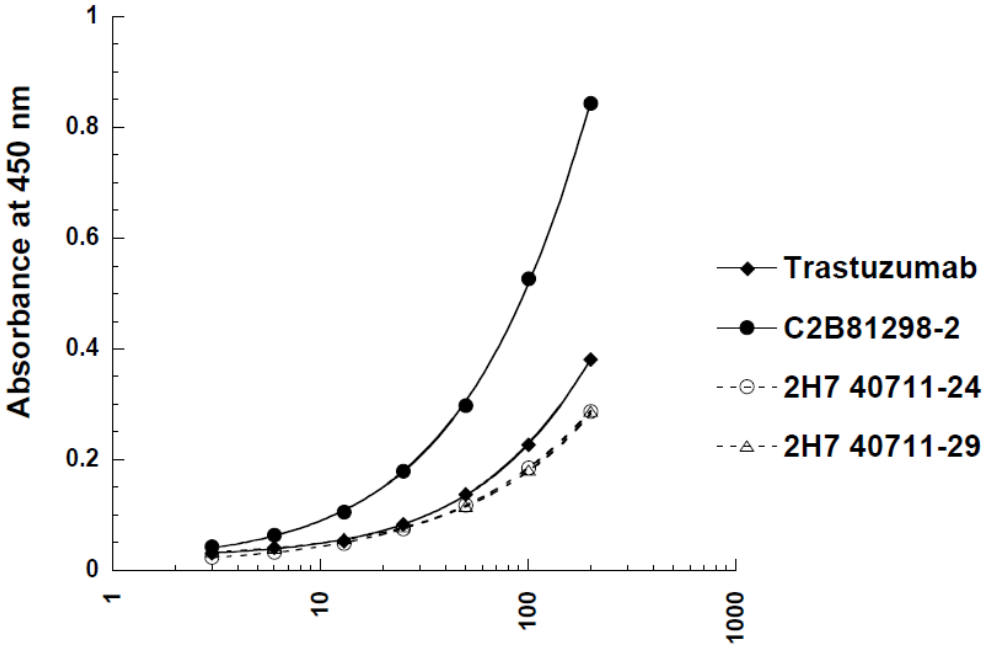


Table 3
Relative Binding to Human FcRn

Parameter Evaluated	Trastuzumab	PRO70769	Rituximab
Concentration at midpoint absorbance of Trastuzumab reference curve (ng/mL)	87 (n=2)	124±9.7 (n=6)	31 (n=1)
Midpoint concentration of test antibody divided by midpoint concentration of Trastuzumab reference	1.0 (n=2)	1.43±0.12 (n=6)	0.35 (n=1)
Aggregation (%)	0.7	0.4±0.2	3

ADCC assay: Anti-CD20 antibodies were compared for the ability to mediate NK cell lysis of WIL2-S cells. Serial dilutions of antibody were incubated in culture plates with WIL2-S cells for opsonization. NK cells, purified from human donors, were added to each plate to initiate ADCC. After the reaction was completed, the wells were developed and absorbance at 490 nm was recorded and used to calculate percent cytotoxicity. EC_{50} values were determined after plotting cytotoxicity as a function of

antibody concentration. Trastuzumab was evaluated as negative control. Spontaneous lysis was also evaluated in the absence of antibody.

Table 4
ADCC Activity of Anti-CD20 Antibodies with NK Cells as Effectors

Test Antibody	n	EC_{50} Test Antibody (pM)
PRO70769	5	5.2±2.6
Rituximab	4	23±6.2

ADCC with PBMCs: ADCC was also evaluated using similar methods as above, but using peripheral blood mononuclear cells (PBMCs) from healthy donors. WIL2-S cells were used as target cells after opsonization with serial dilutions of antibody. Percent cytotoxicity was calculated based on readings from each well at 490 nm plotted against antibody concentration to generate EC_{50} values.

Table 5
ADCC Activity of Anti-CD20 Antibodies with PBMCs as Effectors

Test Antibody	n	EC_{50} Test Antibody (nM)	Maximal % Lysis
PRO70769	10	0.12±0.08	46.5±8.8
Rituximab	12	0.31±0.23	34.1±12.5

CDC assay: WIL2-S cells were incubated in the presence of serial dilutions of antibody and commercially available human serum complement. After 1.5 to 2 hours, alamar blue dye was added to detect live cells. The fluorescence was read at 530 nm and 590 nm. EC_{50} values were then generated from the data.

Table 6

CDC Activity of Anti-CD20 Antibodies

Test Antibody	n	EC_{50} (nM)
PRO70769	26	4.04±1.87
Rituximab	11	1.09±0.32

Apoptosis assay: Ramos cells (a human CD20-expressing B cell line) were incubated in the presence of serial dilutions of antibody. After 24 hours, the cells were stained with Annexin V (stains apoptotic cells) and propidium iodide (stains dead cells). The staining patterns were analyzed by flow cytometry. Cells were counted according to staining characteristics (single stain for Annexin V, double stained with both Annexin V and propidium iodide, or non-stained live cells). Results indicate that both ocrelizumab and rituximab induced apoptosis of Ramos cells.

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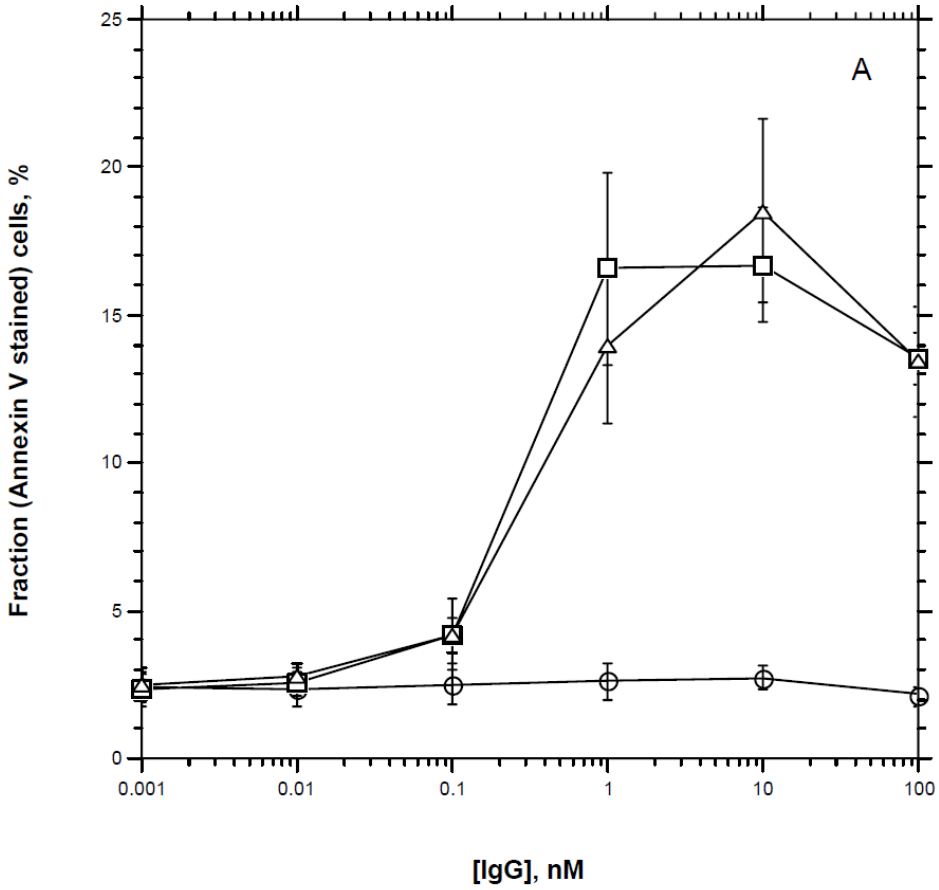
Figure 11
Apoptotic Activity: Annexin V Staining
15Aug2003



The following conclusions were drawn from the results of this study:
Ocrelizumab and rituximab bound with high affinity to CD20 on WIL2-S cells and promoted ADCC mediated by both NK cells and PBMCs. Ocrelizumab also promoted CDC activity against WIL2-S cells and apoptosis of Ramos cells after cross-linking with anti-human Fc. The data suggest that any of these mechanisms could be responsible for depletion of B cells *in vivo.*

Study # 06-1069
Title: Fc gamma receptor binding and ADCC analysis of ocrelizumab (2H7) samples produced from the 2006 process

Testing Facility:	Genentech, Inc.
GLP Compliance:	No
Date of Study Initiation:	September, 2006
Drug, Lot#:	Test article lots are listed in the table below

Table 1
Ocrelizumab Lots

Sample ID	Process Information	Concentration (mg/mL)
2H7 Reference (2H7203-1)	Original Process	11.05
2H7 (b) (4) Run 5	Harvested 031706, 2006 Process	12.16
2H7 Run 7	Harvested 042906, 2006 Process	11.35
2H7 Run 8	Harvested 050106, 2006 Process	11.86
2H7 Run 9	Harvested 050306, 2006 Process	11.71
2H7 Run 10	Harvested 050506, 2006 Process	11.55
2H7 Run 11	Harvested 050706, 2006 Process	11.51

Methods:
Binding of antibody samples to FcγR:
In vitro assay methods were used to assess the binding of the lots above to members of the Fc gamma receptor family: FcγRIa (CD64), FcγRIIa (CD32A), FcγRIIb (CD32B), and FcγRIIIa (CD16). Culture plates were coated with each FcγR. Serial dilutions of each antibody were added to the plates and incubated. The plates were washed and processed using standard immunochemical techniques and TMB as substrate for the HRP reaction. EC_{50}s were calculated for antibody binding to each FcγR.
ADCC assay with PBMCs:
ADCC was evaluated for each lot of ocrelizumab using WIL2-S cells as the target and PBMCs from healthy donors (4) as effector cells. After completion of the reactions, the plates were read for absorbance at 490 nm and the data were used to calculate percent cytotoxicity.

Results:
FcγR binding:

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Table 2
EC_{50} Values (Mean±SD) of Ocrelizumab Binding to Human Fcγ Receptors

Human FcγR	Ocrelizumab Lot						
	2H7203-1 (n=5)	2H7 12K Run 7 (n=3)	2H7 12K Run 8 (n=5)	2H7 12K Run 9 (n=5)	2H7 12K Run 10 (n=5)	2H7 12K Run 11 (n=5)	2H7 400L Run 5 (n=2)
FcγRIa (µg/mL)	0.0125±0.00234	0.0125±0.00177	0.0128±0.00212	0.0136±0.00304	0.0132±0.00188	0.0143±0.00220	0.0121; 0.0177
FcγRIIa (µg/mL) [a]	2.67±0.456	2.81±0.737	3.34±0.942	4.45±2.16	3.76±1.11	4.30±1.82	3.73; 2.55
FcγRIIb (µg/mL) [a]	4.28±0.963	4.60±0.415	6.17±1.83	8.94±3.43	12.3±6.88	19.5±18.1	3.84; 7.43
FcγRIIIa (F158) (µg/mL)	6.43±0.590	2.54±0.455	1.99±0.0353	3.08±0.731	2.59±0.854	2.73±0.721	7.10; 4.80
FcγRIIIa (V158) (µg/mL)	0.994±0.103	0.553±0.0900	0.500±0.0868	0.700±0.164	0.672±0.179	0.743±0.224	1.04; 0.951

n=number of assays.
[a] EC_{50} values were estimated based on binding curves that did not completely reach upper asymptotes. Values are mean of EC_{50} with standard deviations for the assays of n.

Table 3
Fold Increase of Ocrelizumab (2006 Process) in Binding to Human Fcγ Receptors Compared with the Reference Material (Original Process)

Human FcγR	Ocrelizumab Lot					
	2H7 12K Run 7 (n=3)	2H7 12K Run 8 (n=5)	2H7 12K Run 9 (n=5)	2H7 12K Run 10 (n=5)	2H7 12K Run 11 (n=5)	2H7 400L Run 5 (n=2)
Fcγ RIa	0.942±0.0800	0.963±0.0538	0.966±0.188	0.909±0.0785	0.865±0.0470	0.968; 0.862
Fcγ RIIa	1.03±0.0958	0.875±0.224	0.723±0.294	0.802±0.183	0.843±0.482	0.655; 0.916
Fcγ RIIb	0.921±0.166	0.778±0.289	0.591±0.381	0.634±0.558	0.577±0.622	0.825; 0.766
Fcγ RIIIa (F158)	2.54±0.512	3.32±1.03	2.42±0.712	2.88±0.947	2.71±0.927	1.01; 1.24
Fcγ RIIIa (V158)	1.85±0.274	2.26±0.350	1.67±0.376	1.83±0.438	1.74±0.540	0.837; 1.12

n=number of assays.
Note: Fold increase in binding affinity of each 2006 lot of ocrelizumab over that of the reference material (2H7203-1) in each assay was calculated using the following formula: Fold Increase=EC_{50} (2H7203-1)/EC_{50} (test antibody). Values are mean of fold increase with standard deviation for the assays of n.

ADCC activity:

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Table 4
ADCC activity in EC_{50} (ng/mL) of Ocrelizumab with PBMCs as Effectors

FcγRIIIa Allotype	Assay 1 F158/F158	Assay 2 F158/F158	Assay 3 F158/V158	Assay 4 F158/V158	Mean
2H7203-1	10.0	25.0	8.68	3.82	11.88
2H7 (b) (4) Run 7	2.62	8.79	4.12	1.86	4.35
2H7 Run 8	1.85	12.9	1.16	1.65	4.39
2H7 Run 9	2.98	12.5	4.59	2.76	5.71
2H7 Run 10	2.31	20.5	3.26	2.19	7.07
2H7 Run 11	1.43	8.02	8.58	1.78	4.95
2H7 Run 5	5.20	17.2	NA	2.34	8.25

NA=not available.

Table 5
Fold Increase of Ocrelizumab (2006 Process) in ADCC Activity in Comparison with the Reference Material (Original Process)

FcγRIIIa Allotype	Assay 1 F158/F158	Assay 2 F158/F158	Assay 3 F158/V158	Assay 4 F158/V158	Mean	SD	CV
2H7203-1	1	1	1	1	—	—	—
2H7 (b) (4) Run 7	3.82	2.84	2.11	2.05	2.71	0.82	30.5%
2H7 Run 8	5.41	1.94	7.48	2.32	4.28	2.64	61.5%
2H7 Run 9	3.36	2.00	1.89	1.38	2.16	0.84	39.0%
2H7 Run 10	4.33	1.22	2.66	1.74	2.49	1.36	54.8%
2H7 Run 11	6.99	3.12	1.01	2.15	3.32	2.60	78.3%
2H7 Run 5	1.92	1.45	NA	1.63	1.67	0.24	14.2%

NA=not available.

Note: Fold increase in ADCC activity of each 2006 lot of ocrelizumab over that of the reference material (2H7203-1) in each assay is calculated using the following formula: Fold Increase=EC_{50} (2H7203-1)/EC_{50} (test antibody).

The results show that the product produced by the 2006 process binds with higher affinity to the FcγRIIIa F158 and V158 isoforms than the reference standard (original process). The increased affinity for these receptors is reflected in the increased ADCC activity for those lots. The sponsor states that the increased effector function activity is consistent with a higher level of non-fucosylated glycan G0-F observed in the 2006 (b) (4) runs as compared to the reference standard. These data indicate that the product derived from the 2006 process may not be comparable to the product derived from the original process.

Study #14-1579

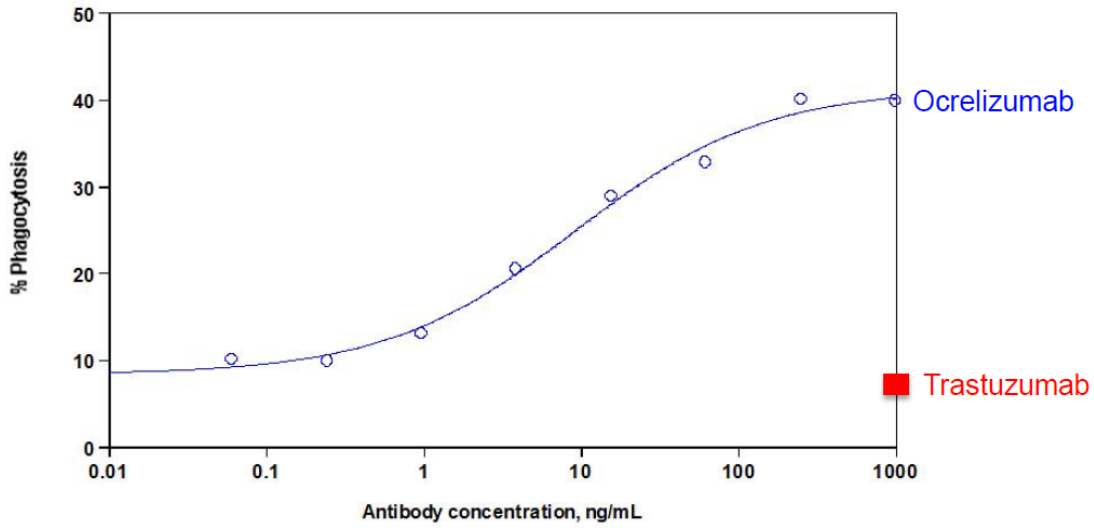
Title: In vitro evaluation of ocrelizumab for antibody-dependent cell-mediated phagocytosis activity

Testing Facility:	Genentech, Inc.
GLP Compliance:	No
Date of Study Initiation:	Not specified, 8/2014
Drug, Lot#:	Ocrelizumab, lot M96341 Trastuzumab, lot HER401-4

Methods:
Target cell lines: human B cell lines WIL2-S and Daudi
Effector cells: Human macrophages, purified from healthy donors.
Target cells were seeded in culture plates and incubated with varying concentrations of test antibody to allow opsonization. (Trastuzumab was used as negative control, at a single concentration.)

Results:

Figure 3 Dose Response Curve of Ocrelizumab-Induced Macrophage-Mediated Phagocytosis of WIL2-S Cells



The data indicate that ocrelizumab can function to eliminate B cells through ADCP.

Tissue Cross Reactivity

Study # 03-0216-0349

Title: Cross-reactivity of rhuMAb 2H7 (anti-CD20) with human and non-human primate (cynomolgus monkey) tissues *Ex Vivo*

Testing Facility: (b) (4)

GLP Compliance: Yes
Date of Study Initiation: 5/14/2003
Drug, Lot#: Ocrelizumab, M3-TOX64

Methods:
Species: Human (3 donors), cynomolgus monkey
Negative control: biotinylated non-specific human IgG
Negative control tissue: FS1 and FS2 CHO cells (CHO.DP.12)
Positive control tissue: Ramos cells (expressing the CD20 antigen)

- The activity of the biotinylated test article was assured using flow cytometry methods to compare binding of the labeled product to a neoplastic B cell line to binding activity of the un-biotinylated product.
- Preliminary methods development to optimize specific binding conditions demonstrated that maximal binding was observed was 30.0 mcg/mL. A concentration 5X the optimal concentration was also evaluated (150 mcg/mL).
- Adequacy of tissue samples was confirmed by staining all samples with an antibody directed against the CD31 antigen by immunohistochemistry. CD31 is an abundant marker on endothelial cells and positive staining for this marker was considered evidence of tissue integrity. Specific CD31 staining was detected in all human tissue samples except thyroid from one of three donors. CD31 staining was detected in all tissues from the cynomolgus monkey except tonsil and parathyroid from one of three donors.
- Final staining results for ocrelizumab were subject to peer review.

Human Tissues

Human Tissue (Normal) from Three Separate Individuals		
Adrenal	Heart	Skin
Bladder	Kidney (glomerulus)	Spinal Cord
Blood	Kidney (tubule)	Spleen
Bone Marrow	Liver	Striated Muscle
Breast	Lung	Testes
Cerebellum	Lymph Node	Thymus
Cerebral Cortex	Ovary	Thyroid
Colon	Pancreas	Tonsils
Endothelium*	Parathyroid	Ureter
Eye	Pituitary	Uterus (cervix)
Fallopian Tube	Placenta	Uterus (endometrium)
Gastrointestinal Tract	Prostate	

* The endothelium was evaluated in each section from the above multiple tissue list.

Cynomolgus Monkey Tissues

Cynomolgus Monkey Tissue from Three Separate Animals		
Adrenal	Heart	Spinal Cord
Bladder	Kidney (glomerulus)	Spleen
Blood	Kidney (tubule)	Striated Muscle
Bone Marrow	Liver	Testes
Breast	Lung	Thymus
Cerebellum	Lymph Node	Thyroid
Cerebral Cortex	Ovary	Tonsils
Colon	Pancreas	Ureter
Endothelium*	Parathyroid	Uterus (cervix)
Eye	Pituitary	Uterus (endometrium)
Fallopian Tube	Prostate	
Gastrointestinal Tract	Skin	

* The endothelium was evaluated in each section from the above multiple tissue list.

Results:

- Positive control: Nearly all Ramos cells showed mild or moderate staining for rhuMAb 2H7 at a concentration of 30.0 and 150 mcg/mL, respectively.
- Negative control: No negative control cells showed positive staining for CD20 with ocrelizumab.

Staining in human tissues:

At 30.0 mcg/mL, specific staining was observed in lymphoid follicles in a variety of tissues including GI tract, lymph node, pancreas, parathyroid, prostate, spleen, thymus and tonsil. Most intense staining was associated with the germinal centers of the follicle with more diffuse staining of the lymphocytes surrounding the germinal centers.

- Absence of staining in the GI tract, lymph node and spleen in tissue from one of three donors was attributed to freezing artifact formed during tissue preparation (ice crystal formation leading to tissue disruption).
- Minimal diffuse staining was also noted in the lens fibers from 2 of three eyes examined.

At a concentration of 150 mcg/mL, the pattern of specific binding was very similar to that at the lower concentration, but more intense. Mild positive staining of the lens in 2 of three donors was observed (the third sample did not contain lens issue).

Staining in cynomolgus monkey tissues

At 30.0 mcg/mL, specific staining was observed in lymphoid follicles, GI tract, kidney, lung, lymph node, pancreas, spleen, thyroid and tonsil. Specific staining was also noted in scattered mononuclear cells in the medullary region of the thymus. The lens tissue of one of 3 eyes showed diffuse staining. Staining of the lens was not observed with the control antibody.

At a concentration of 150.0 mcg/mL, very similar pattern of specific binding, with higher intensity observed.

4.2 Secondary Pharmacology

N/A

4.3 Safety Pharmacology

Stand-alone safety pharmacology studies were not conducted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study # 03-0235-0349

Title: Pharmacokinetic and pharmacodynamic study of rhuMAb 2H7 in cynomolgus monkeys with a 12-week recovery period (non-GLP)

Animals: Cynomolgus monkeys, 4 males /group
Doses: 0.2, 0.5, or 2.0 mg/kg
Study design: Animals were dosed by IV infusion on SD1 and 8. Treatment period was followed by 12-week recovery period for all animals. Parameters monitored included clinical observations, body weight, food consumption, hematology, flow cytometry, pharmacokinetics, immunogenicity, and serum chemistry.

Results:

No ocrelizumab-related adverse effects were noted in clinical observations, body weight, food consumption, or serum chemistry. Hematology analyses demonstrated a reduction in total lymphocytes by 1 day after dosing. The magnitude of decrease was dose-related. Immunophenotyping showed that the reduction in total lymphocytes was due to B cell depletion.

Immunophenotyping:
Dose-dependent depletion of B cells was observed one hour after ocrelizumab administration at all dose levels on both days of dosing. In the LD group, circulating B cell numbers recovered by the second dose on SD8, and partial recovery was observed for the MD group. The B cell levels of the HD group showed near complete depletion after the first dose and slower recovery by the second dose than the lower dose groups. B cells levels again dropped to near zero after the second dose and the beginning of recovery was observed at approximately SD15, for all dose groups, with the HD group again showing slower recovery. B cell numbers at the end of the recovery period were variable among animals, but most animals showed recovery to near baseline.

Pharmacokinetics:
Biphasic clearance of ocrelizumab was observed, with a rapid initial distribution phase followed by a slow elimination phase. The elimination phase was shortened after the second dose, most likely due to the presence of ADAs.

- ADAs developed in all animals in all dose groups by 2 weeks after the first dose.

- The results of pharmacokinetic analyses are summarized in the table below, provided by the sponsor. (rhuMAb 2H7 is ocrelizumab)

Table 3

Mean±SD Noncompartmental PK Parameters of Serum rhuMAb 2H7 Following Intravenous Bolus Administration to Cynomolgus Monkeys (n=4 males/group) at 0.2, 0.5, and 2 mg/kg Once Weekly for 2 Weeks (Dosed on Elapsed Days 0 and 7)

Parameters	Dose Level (mg/kg)		
	0.2	0.5	2
C_{max} (µg/mL)	3.48±1.15	12.2±1.75	61.5±9.24
AUC_{last} (µg • day/mL)	3.56±4.66	14.5±6.66	90.2±37.9
$t_{1/2z}$ (day)	0.981±1.22	1.12±0.427	1.73±0.192
AUC_{inf} (µg • day/mL)	3.71±4.83	17.3±5.75	140±12.0
CL_{ss} (mL/day/kg)	154±122	32.2±10.6	15.4±1.46
V_{ss} (mL/kg)	88.4±42.1	49.0±8.21	39.8±6.76
R_{Cmax} (µg/mL)	1.43±0.188	1.25±0.121	1.29±0.142

AUC_{inf} = Area under the concentration–time curve from zero to infinity.

AUC_{last} = Area under the concentration–time curve from zero to last.

CL_{ss} = Drug clearance at steady state.

C_{max} = Maximal serum concentration.

R_{Cmax} = Accumulation ratio of C_{max} calculated by comparing the C_{max} after the second dose to that after the first dose.

$t_{1/2z}$ = Terminal half-life.

V_{ss} = Volume of distribution at steady state.

Study # 03-0155-0349

Title: Pharmacokinetics of rhuMAb 2H7in mice

Testing Facility: Genentech, Inc.

GLP Compliance: No

Drug, lot#: Ocrelizumab (rhuMAb 2H7), lot # M3-TOX64

Methods:

Animals: CD-1 mouse, females only

Age: 7-8 weeks old

Weight: Average 27.5 g

Number/group: 18

Route: IV

Regimen: Single dose

Doses: 0.5, 5, or 50 mg/kg

Study design:

Table 1
Study Design

Group	No./Sex	Route [a]	Test Material	Dose [a] (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)
1	18/F	IV Bolus	rhuMAb 2H7	0.5	0.25	12
2	18/F	IV Bolus	rhuMAb 2H7	5	1.25	12
3	18/F	IV Bolus	rhuMAb 2H7	50	12.5	12

IV = Intravenous.
[a] Dosing solution was administered intravenously into the tail vein.

Samples were collected pre-dose followed by 5 minutes, 20 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 1, 2, 3, 7, 14, 21, 28, and 35 days post-dose.

Results:

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Table 2

Mean (±SD, n=3/timepoint) Serum rhuMAb 2H7 Concentrations and Noncompartmental Pharmacokinetic Parameters Following Single IV Dose of 0.5, 5, or 50 mg/kg in CD−1 Mice

	Serum rhuMAb 2H7 Concentration (μg/mL)		
Time (Day)	0.5 mg/kg	5 mg/kg	50 mg/kg
0.003	13.2±1.35	137±13.4	985±65.0
0.01	11.1±1.35	94.7±19.5	936±46.0
0.04	9.44±1.01	66.0±10.6	774±79.1
0.08	8.97±0.13	71.2±2.65	495±61.4
0.17	7.95±0.81	56.3±8.45	641±81.0
0.33	6.41±0.40	57.5±3.82	575±82.2
1	4.73±0.69	42.9±4.85	374±109
2	3.92±0.40	38.9±6.90	293±51.6
3	4.36±0.33	40.5±1.30	272±20.6
7	3.00±0.46	31.9±0.82	183±31.8
14	2.22±0.09	18.7±1.92	102±19.7
21	1.57±0.18	14.21 [a]	106±41.2
28	0.89±0.25	10.2±2.22	64.4±19.6
35	0.88±0.18	8.10±0.30	54.6[a]
C_{max} (μg/mL)	13.2	137	985
AUC_t (μg•day/mL)	76.0	722	4791
$t_{1/2}$ (day)	13.4	13.7	14.5
AUC_{inf} (μg•day/mL)	93.0	882	5936
CL (mL/day/kg)	5.38	5.67	8.42
V_{ss} (mL/kg)	105	111	169

[a] Positive anti-rhuMAb 2H7 antibodies were detected in 2 animals from the 5 mg/kg dose group on Day 21 and in 1 animal from the 50 mg/kg dose group on Day 35. Their values were excluded from mean and standard deviation calculations and from the PK analysis.

Conclusions:

Ocrelizumab does not bind CD20 in rodents. Therefore, the data provided by this study are not relevant to assessing safety of ocrelizumab for human use.

Study # 03-0157-0349

Title: Pharmacokinetics of rhuMAb 2H7 in rat

Testing Facility:	Genentech, Inc.
GLP Compliance:	No
Drug, lot #:	Ocrelizumab, M3-TOX64

Methods:
Animals: Male Sprague-Dawley rats, 6/group
Age/Weight: 10 weeks old/average body weight was 314 ± 20.0 g
Route: IV
Regimen: Single dose
Dose levels: 0.5, 5, or 50 mg/kg

Results:

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Table 2
Mean (±SD) Serum rhuMAb 2H7 Concentrations and Noncompartmental Pharmacokinetic Parameters [a] Following Single IV Dose of 0.5, 5, or 50 mg/kg in Rat

	Serum rhuMAb 2H7 Concentration (µg/mL)		
	Group 1	Group 2[b]	Group 3
Time (Day)	(0.5 mg/kg)	(5 mg/kg)	(50 mg/kg)
0	LTR	LTR	LTR
0.003	13.7±1.93	149±11.1	1590±185
0.04	11.0±0.95	120±10.8	1330±150
0.17	10.2±0.76	107±13.2	1150±209
0.33	6.97±0.75	77.8±4.55	703±320
1	5.03±0.45	51.6±5.68	552±57.8
2	3.62±0.53	39.8±5.31	420±49.2
3	3.18±0.46	32.6±3.99	373±36.3
7	2.58±0.47	25.9±3.71	270±23.2
14	1.85±0.26	18.3±2.80	185±28.1
21	0.94±0.19	7.71±2.48	95.3±22.3
28	0.45±0.12	5.07±1.94	56.5±16.8
35	0.06±0.13	3.21±0.80	31.0±12.6
C_{max} (µg/mL)	13.7±1.93	149±11.1	1590±185
AUC_t (µg • day/mL)	57.1±7.38	583±55.7	6350±800
$t_{½ z}$ (day)	8.88±1.01	9.17±1.46	8.85±1.53
AUC_{inf} (µg • day/mL)	62.4±8.96	621±61.6	6770±950
CL (mL/day/kg)	8.15±1.16	8.11±0.75	7.53±1.24
V_{ss} (mL/kg)	95.3±13.0	91.0±19.2	88.7±8.43

LTR=Less than reportable.

[a] For definitions of noncompartmental pharmacokinetic parameters, see Pharmacokinetic Data Analysis section.

[b] Positive anti-rhuMAb 2H7 antibodies detected on Day 28 (n=1) of Group 2 and Day 35 (n=2) of Group 2 were excluded from mean, SD, and PK analysis.

Conclusions:

Ocrelizumab does not bind CD20 in rodents. Therefore, the data produced in this study are of little value in evaluating safety of the drug for human use.

Study #034-0431-0349

Title: Tissue distribution of rituximab and rhuMAb 2H7 in normal and human CD20 transgenic mice:

Testing Facility: Genentech, Inc.
GLP Compliance: No
Date of Study Initiation: 9/10/2003
Drug, lot#:

Rituximab, Lot 132BL 12K R9100A
PRO70769 (rhuMAb 2H7), Lot M3-TOX64
^{125}I-Rituximab, Reference No. 41852-79
^{125}I-PRO70769, Reference No. 41852-77

(PRO070769 is ocrelizumab.)

Methods:
Animals: Mouse (FVB) Wild-type; mouse huCD20.Tg
Route of administration: IV
Number/sex/group: 12 females/per group
Regimen: Single dose
Dose: 3.4 µCi

Study Design:

Table 1
Group Designation and Dosing Information

Group	No./Sex/ Mouse Type	Route	Test Material	Dose (µCi)	Dose Conc. (µg/mL)	Dose Volume (mL)
1	12/F/hu-CD20.Tg	IV	^{125}I-rituximab	3.4	4	0.1
2	12/F/hu-CD20.Tg	IV	^{125}I-PRO70769	3.4	4	0.1
3	12/F/normal FVB	IV	^{125}I-rituximab	3.4	4	0.1
4	12/F/normal FVB	IV	^{125}I-PRO70769	3.4	4	0.1

Tissue collection: Tissue samples were collected from 3 mice/group at 5 and 30 minutes, 4, and 24 hours post-dose. Tissues included liver, lungs, thymus, right kidney, spleen, pancreas, right femur, and mesenteric lymph nodes. Levels of radiolabel in each tissue were determined using standard gamma counting methods.

Blood collection: Blood samples were collected from each mouse at the time of tissue collection. Total radioactivity in each blood sample was determined. A portion of each sample was processed to separate plasma followed by determination of radioactivity in plasma.

Results:
Binding of radiolabeled test articles was confirmed *in vitro* prior to use *in vivo*. No difference in binding was observed between ocrelizumab and rituximab in the *in vitro* assays.

In normal mice the amount of radioactivity in blood exceeded the amount in tissues. However, in the huCD20 Tg mice, a majority of radioactivity was associated with the spleen.

Figure 1
Tissue Distribution of ^{125}I-PRO70769 in hu-CD20.Tg and Normal (WT) Mice Following a Single IV Bolus Dose

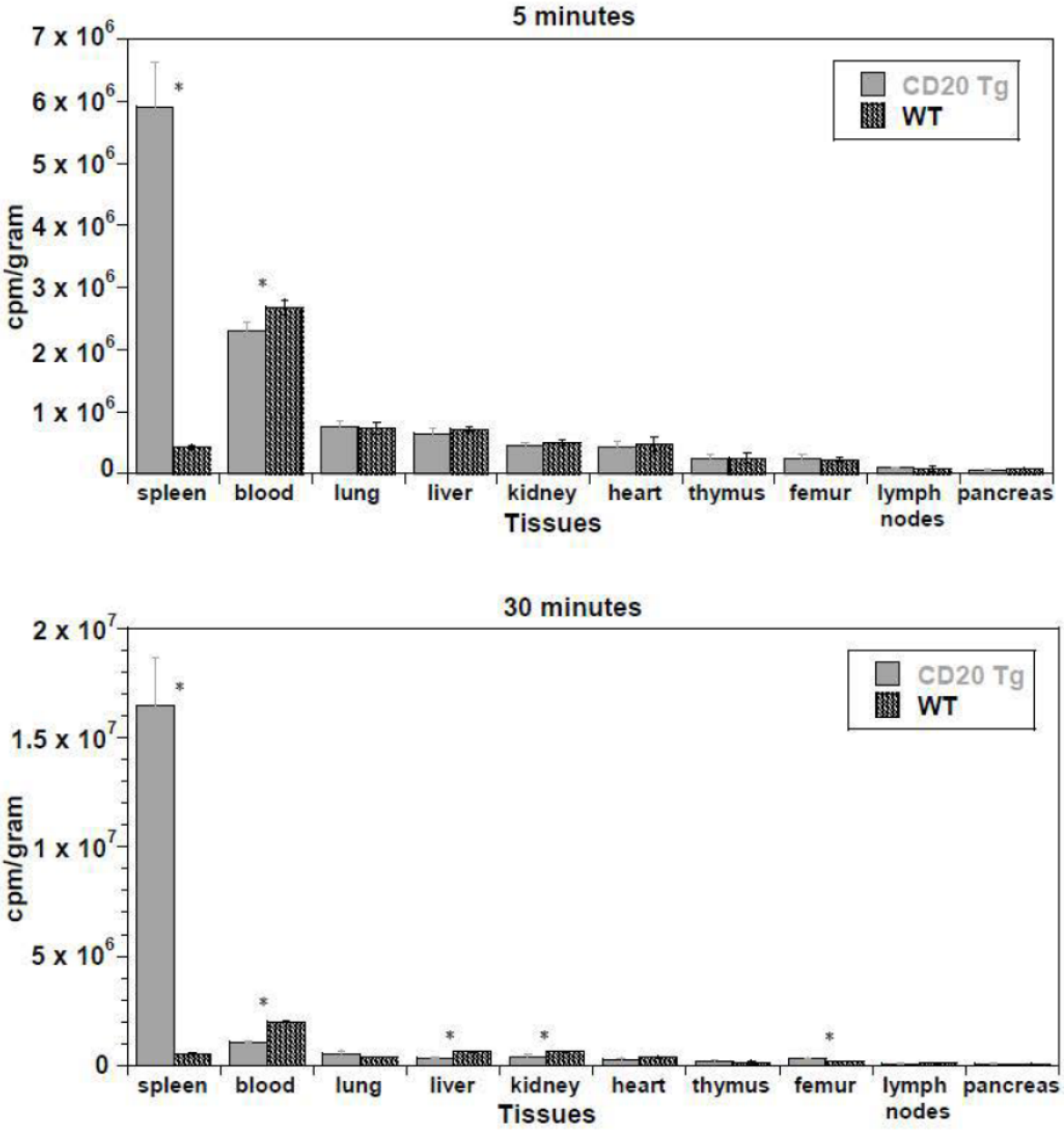
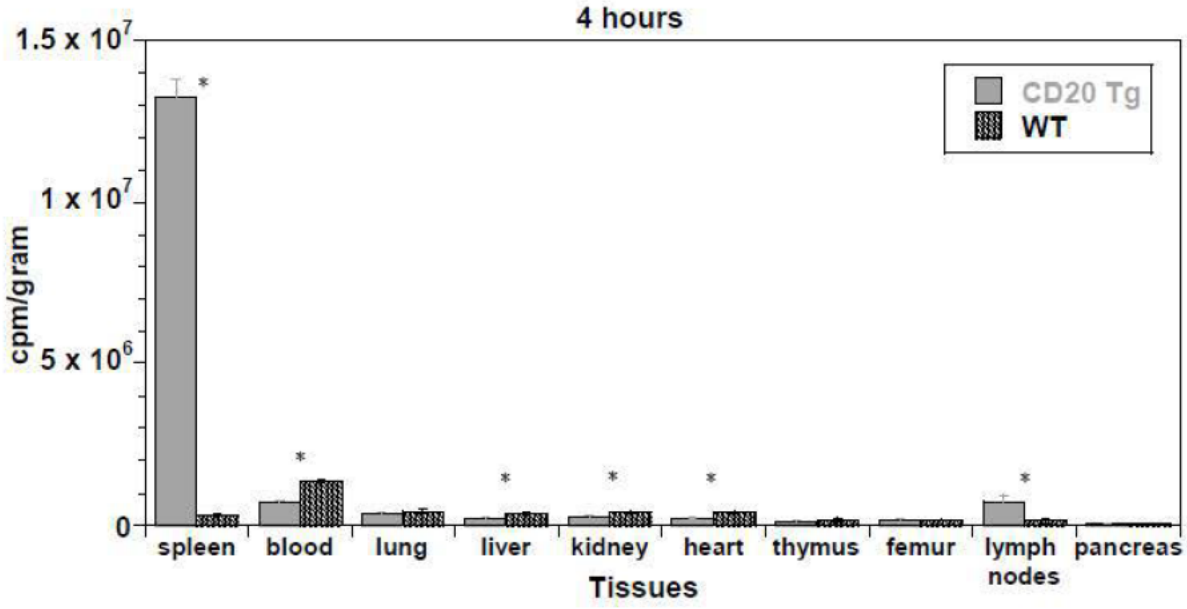


Figure 1 (cont'd)
Tissue Distribution of ^{125}I-PRO70769 in hu-CD20.Tg and Normal (WT) Mice Following a Single IV Bolus Dose



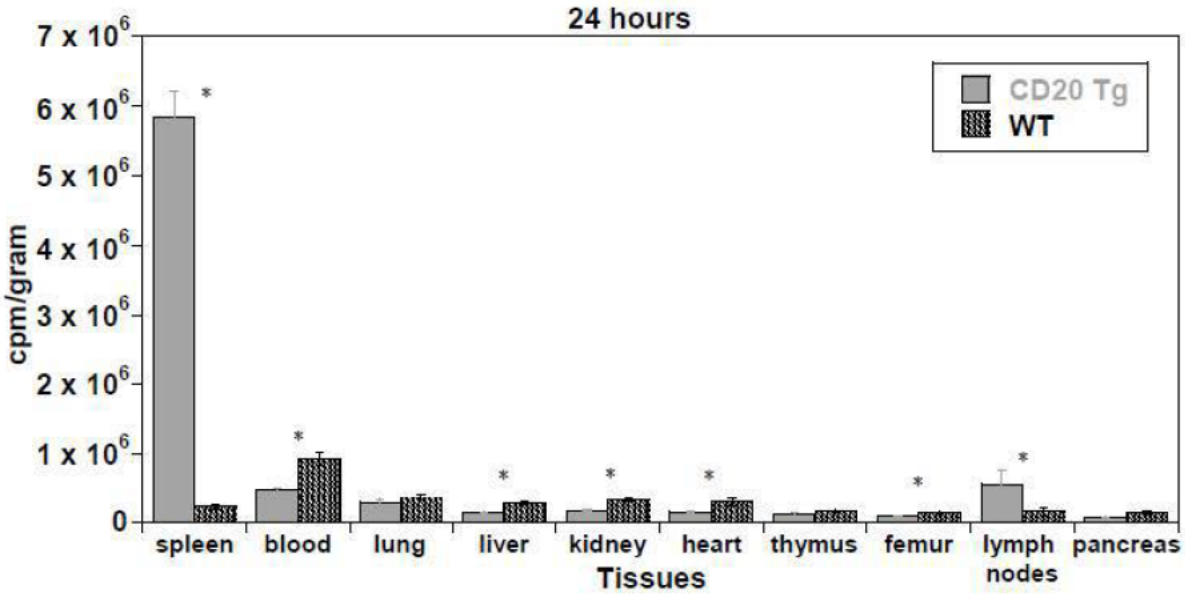
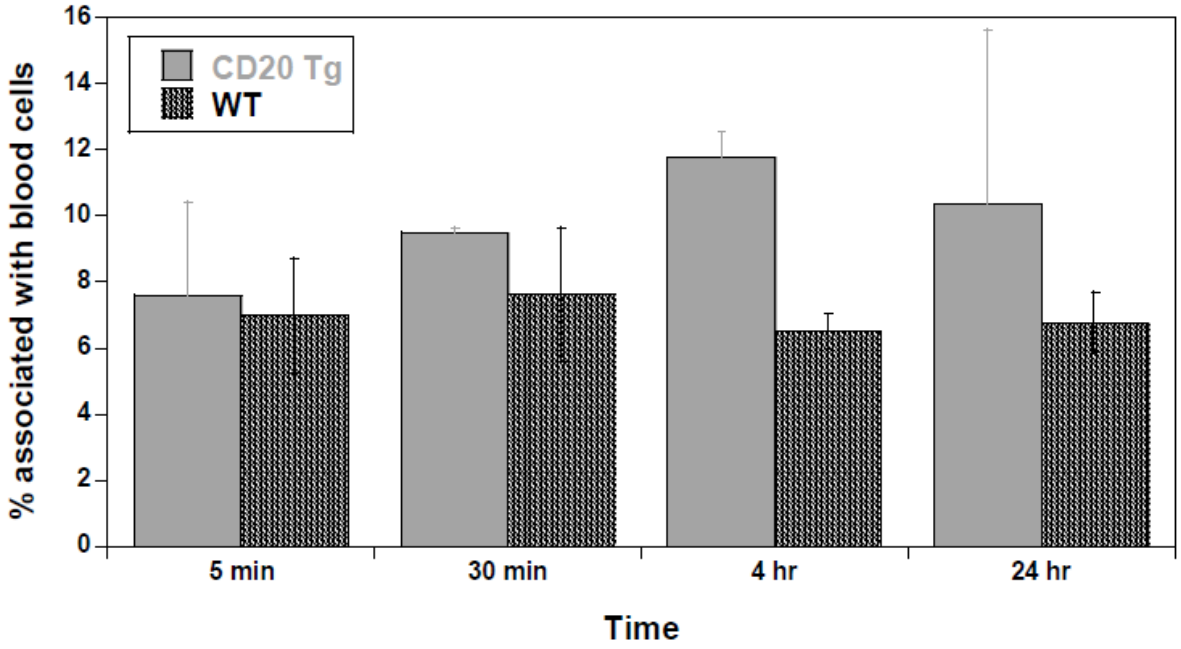


Figure 3
^{125}I-PRO70769 Associated with Peripheral Blood Cells in hu-CD20.Tg and Normal (WT) Mice Following a Single IV Bolus Dose



The data suggest that, in the huCD20 Tg mice, a significant portion of ocrelizumab was bound to B cells in lymphoid organs. Normal mouse B cells do not bind ocrelizumab. Therefore, in normal mice, more radioactivity remained in the plasma.

Study # 14-3756

Title: Whole-body imaging and radiation dosimetry in nonhuman primates of [111-In] ocrelizumab, a potential SPECT radiotracer to measure B-cell tissue depletion/repletion kinetics and antibody tissue penetration

Testing Facility:	(b) (4)
GLP Compliance:	No
Date of Study Initiation:	Not Specified
Drug, lot #:	Ocrelizumab (conjugated to indium-111, specific concentration of labeled antibody was 19 µg/mCi), lot # 792571

Methods:
Animals: Cynomolgus monkey, 2 adult males
Route of Administration: IV
Dose: 10 mg/kg unlabeled ocrelizumab was injected up to 48 hours prior to injection of the radiolabeled ocrelizumab for the first series of scans; for the second series of scans, no unlabeled loading dose was used.

Table 1 Study Design

Group	No./Sex	Route	Day of Dosing	Ocrelizumab (mg/kg)	[111-In] Ocrelizumab (μg/mCi)
1	2/M	IV	–2	10	0
			0	0	19
2	2/M	IV	0	0	19

The two sets of SPECT scans (depleted and repleted conditions) were conducted 10 months apart. Whole-body distribution over time was calculated using image analysis.

Results:
Uptake in the depleted condition was highest in liver. Peak levels in the liver, kidney, heart, brain, and spleen were 30%, 5%, 5% <1%, and <1% of the injected dose, respectively. Peak levels in all organs were observed within 72 hours post-dose. The same animals were scanned under the repleted condition 10 months later.
Scans after repletion showed high levels of radioactivity in the liver. The sponsor suggests that this finding may be due to ADAs. Relative levels in organs from scans conducted in the repleted condition are not specified in this report.

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Figure 2 Percent of Injected Dose of Radioactivity Visually Identified in Organs on Whole-Body Images

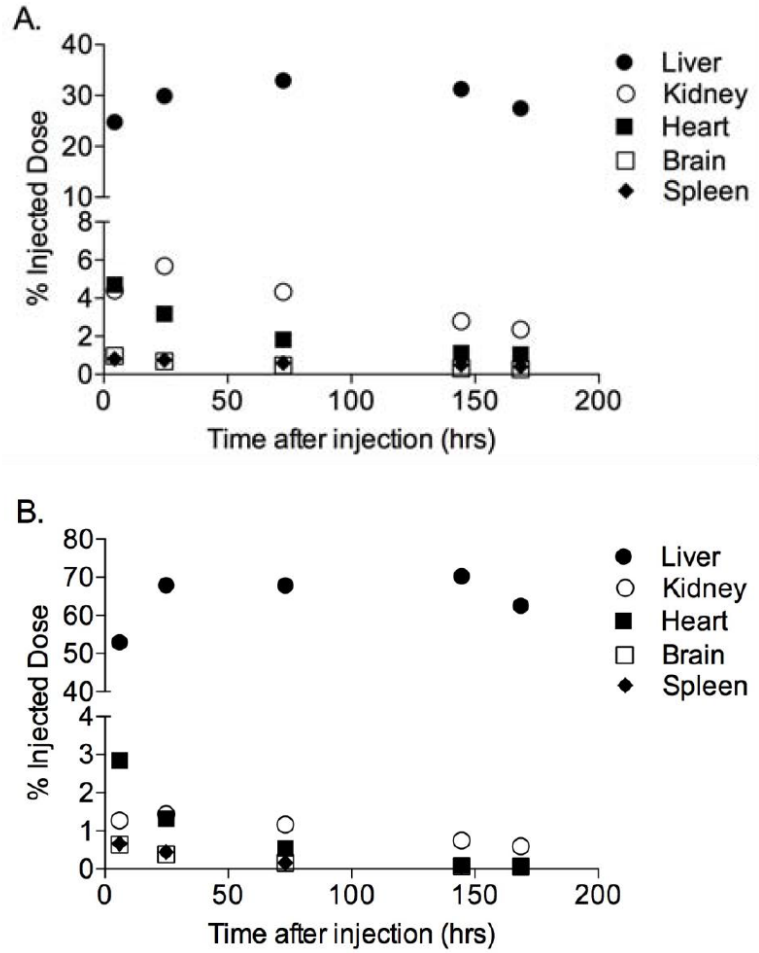

Figure A represents data from the depleted condition; B represents the repleted condition.

From the sponsor's figures above, radioactivity appears to be similar under the depleted and repleted conditions. If the product was pharmacologically active, more binding in the spleen after reconstitution of the B cell population would be expected. Studies using radiolabeled drug to demonstrated biodistribution are generally not considered useful for biological therapeutics and are not recommended. Interpretation of results is difficult since radiolabel may not remain conjugated to the full biologically active protein.

General Toxicology

Four different dosing regimens were explored in toxicology studies. The various regimens are listed below:

- Two doses given 1 week apart (0.05, 0.2, 0.5, 2, and 10 mg/kg ; Studies 02-182-0352 and 03-0235-0349)

- Four doses given 1 week apart (50 and 100 mg/kg QW; Study 03-0684-0134)
- Two doses given 2 weeks apart (10, 50, and 100 mg/kg Q2W; Studies 03-0113-0349 and 03-0114-0349)
- Study 03-0114-0349 included a retreatment design, such that after a 3-month recovery period, some animals were retreated at 50 and 100 mg/kg given twice, 2 weeks apart.
- Eight doses given 3 weeks apart (50 and 100 mg/kg Q3W; Study 04-0192-0134).

6.1 Single-Dose Toxicity

No single-dose toxicity studies were conducted with ocrelizumab.

6.2 Repeat-Dose Toxicity

Study #03-0684-0134

Title: A repeated dose (x4) toxicity and toxicokinetic evaluation of rhuMAb 2H7 antibody (PRO70769)

Testing Facility: (b) (4)

GLP Compliance: Yes
Date of Study Initiation: 11/25/2003
Drug/Lot #: Ocrelizumab/M3-TOX 64 (20 mg/kg)
Vehicle/Placebo: (b) (4) mM sodium acetate, 10 mM histidine, 6% sucrose, 0.02% polysorbate 20, pH (b) (4)

Methods:
Animals: Cynomolgus monkeys, purpose bred
Group Assignments: 2/sex for the control group (1/sex designated for recovery), 4/sex/group for the LD and HD groups (2/sex/group designated for recovery)
Age: 4 to 7 years old
Weight: 4.42 to 5.56 kg for males, 2.40 to 5.20 kg for females
Doses: 0, 50, or 100 mg/kg (doses were chosen based on proposed clinical doses and on previous nonclinical studies that demonstrated the development of high incidence of ADAs at doses less than 10 mg/kg.)
Regimen and ROA: 4 IV injections given once weekly
Study Design: The general study design is summarized in the table below. Main study animals were euthanized on SD28. The recovery period duration was 32 weeks with B cell recovery defined as greater than 25% of baseline.

Table A: Group Assignments

Group / Color Code	Test Material	Dose Level (mg/kg)	Nominal Dose Concentration (mg/mL)	Dose Volume (mL/kg)[a]	Total Number of Animals		Study Specific Numbers	
					Females	Males	Females	Males
1/white	rhuMAb 2H7 Vehicle	0	0	5	2	2	1[b], 3	2[b], 4
2/gray	rhuMAb 2H7 (PRO70769)	50	10	5	4	4	5[b], 7[b], 9, 11	6[b], 8[b], 10, 12
3/red	rhuMAb 2H7 (PRO70769)	100	20	5	4	4	13[b], 15[b], 17, 19	14[b], 16[b], 18, 20

[a] Total dose volume (mL) was calculated based on the most recent body weight. Dose volumes were rounded **(up)** to the next readable syringe increment.

[b] Recovery animals. Once dosing was complete, the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects until B cells returned to >25% of baseline for each animal, on 08/17/04.

Observations/Results:

Mortality/clinical observations: (Clinical observations were recorded twice daily.)

- All animals survived to scheduled termination.
- No clinical observations attributable to ocrelizumab were noted during the dosing or recovery phase.

Food consumption: (Estimated twice daily)

- No effects on food consumption attributable to ocrelizumab were observed.

Body weight: (Recorded twice prior to study initiation, once on SD1 and weekly thereafter.)

- No effects on body weight attributable to ocrelizumab were observed for any group during the dosing and recovery phases.

Physical examinations: (Conducted once prior to study initiation, 1-2 hours after the first and last dose, and once during weeks 20 and 36 of the recovery period.)

- No effects attributable to ocrelizumab were observed.

Blood pressure: (Recorded twice prior to study initiation, 1-2 hours after the first and last dose and once during weeks 20 and 36.)

- No ocrelizumab-related effects on blood pressure were observed.

Heart Rate: (Recorded for all animals once prior to study initiation, 1-2 hours after the first and last dose and once during weeks 20 and 36.)

- Heart and respiratory rate showed fluctuations but did not vary significantly from baseline values for any group.

ECG: (Conducted twice prior to study initiation, 1-2 hours after the first and last dose and once during weeks 20 and 36.)

- All ECG parameters were qualitatively and quantitatively within normal limits. (One female in the LD recovery group showed QTc prolongation on SD248. This

finding was accompanied by marked reduction in HR and the veterinary cardiologist considered this a normal variation and not likely to be related to ocrelizumab.)

Body temperature: (Rectal temperature was recorded for all animals twice prior to study initiation, 1-2 hours after the first and last dose, and once during the final week of recovery.)

- No effects on body temperature attributable to ocrelizumab were reported.

Ophthalmology: (Testing of all animals once prior to study initiation, once during the final dosing week, and once during the final week of recovery.)

- No effects attributable to ocrelizumab are reported. The single finding was, at Week 36, one HD male showed a unilateral posterior capsular cataract in the right eye. The pathologist considers this finding to be sporadic often seen following intraocular inflammation.

Urinalysis: (Conducted twice prior to study initiation [including once during the week preceding the first dose], and on SD9, 27, and 253.)

- No effects attributable to ocrelizumab were reported.

Hematology: (Blood samples were collected on SD-16, 1 (pre-dose), 8 (pre-dose), 22 (pre-dose), and 28 [at terminal necropsy], and SD 42, 56, 70, 84, 98, 112, 126, 140, 154, 161, 168, 182, 196, 211, 224, 238, and 252.)

- Decreases in mean lymphocyte counts were noted for LD males on SD 28 and HD males on SD 15, 22, and 28.
- For females, the LD group showed reduced mean lymphocyte counts on SD8, 15, and 28, and the HD group showed reduced lymphocyte counts on SD 15, 22, and 28
- Increased individual leukocyte and neutrophils counts were observed in 2 HD males on SD8, 15, and 22 for #14 and on SD8 for #16.
- Reductions in mean hematocrit were observed after SD1 for all groups. This was considered to be due to frequent blood draws and was accompanied by the expected elevation in RET.

Coagulation: (Blood sample collected on SD-6, 1 [pre-dose], 9, 23, 28, and 253.)

- No ocrelizumab-related effects on coagulation were observed.

Serum Chemistry: (Blood samples were collected on SD-6, 1 [pre-dose], 9, 23, and 253.)

- No significant effects on serum chemistry values attributable to ocrelizumab were reported.

Flow cytometry: (Samples collected on the same schedule as for hematology. Immunophenotyping was conducted for CD3, 4, 8, 14, 40, and 45. Samples of lymphoid tissue and bone marrow were collected at necropsy.)

- Mean T cell populations, CD3+CD40-, CD3+CD4+, CD3+ CD8+, showed slight increases in both sexes in both dose groups on SD8 through 28. Males recovered to pre-dose levels in both groups, but female values fluctuated through the recovery period.
- B cell populations decreased markedly for both LD and HD groups (both sexes) to 1% of baseline values or lower.
- A trend toward recovery of B cell populations was noted for both sexes in both dose groups relative to levels on SD28. B cell values for all animals recovered to

25% of the mean pre-dose value or higher, except in HD animals #13 and #16 (one female and one male).

- NK cell counts for both dose groups fluctuated during the dosing and recovery periods and did not show a clear relationship to ocrelizumab exposure.

Toxicokinetics: (Samples were collected pre dose on SD1, post-dose 1, 8, 24, 48, 72, and 120 hours; on SD22 pre-dose and 1, 8, 24, 48, 72, and 120 hours post-dose. Additional samples were collected during the recovery period on SD42, 56, 70, 84, 98, 112, 126, and 253.)

- Volume of distribution at steady state for both doses was approximately twice the normal serum volume of cynomolgus monkeys.
- Terminal $t_{½}$ after the final administration was approximately 15 and 20 days at the LD and HD, respectively.
- Elimination of the test article was very slow and the drug was detectable in the serum at 32 weeks after the final injection in all treated animals except LD females.

Table C1

Mean (±SD) Noncompartmental Pharmacokinetic Parameters of Serum PRO70769 after Four Intravenous Bolus Doses of 50 or 100 mg/kg PRO70769 Administered Weekly to Cynomolgus Monkeys (n=4 males and 4 females per group)

Dose (mg/kg)	No./Sex	C_{max} (µg/mL)	AUC_{last} (µg • day/mL)	$t_{1/2}$[a] (day)	AUC_{inf}[a] (µg • day/mL)	CL_{ss}[a] (mL/day/kg)	V_{ss}[a] (mL/kg)
50	4/F	2360±426	33200±4950	11.2[b]	50700[b]	4.13[b]	90.9[b]
	4/M	2490±112	34600±2690	18.4[b]	65100[b]	3.08[b]	84.2[b]
	8 (all animals)	2420±296	33900±3770	14.8±4.20[c]	57900±12200[c]	3.60±0.948[c]	87.5±14.4[c]
100	4/F	4340±538	57500±9500	20.5[b]	110000[b]	4.02[b]	106[b]
	4/M	5100±743	65900±9150	20.4[b]	127000[b]	3.15[b]	98.3[b]
	8 (all animals)	4720±727	61700±9740	20.5±6.05[c]	118000±29400[c]	3.59±1.13[c]	102±14.5[c]

AUC_{inf} = area under the concentration–time curve from time zero to infinity; AUC_{last} = area under the concentration–time curve from Time zero to Day 26; CL_{ss} = clearance at steady state; C_{max} = maximal serum concentration; $t_{1/2z}$ = terminal half-life; V_{ss} = volume of distribution at steady state.

[a] n=2, Parameters not calculated for non-recovery animals.

[b] SD was not calculated because n≤2.

[c] n=4.

Immunogenicity: (Blood samples were collected pre-dose on SD1, 15, 28, 56, 84, 112, and 253.)

- At least 2 animals from each dose group developed detectable ADAs. The total percentage of animals showing anti-drug immune response was 25%. ADAs were not detectable during the dosing period and became detectable in recovery animals only at SD253. At that time point, 50% of the recovery animals had detectable ADAs. The failure to detect ADAs at earlier time points may have been due to presence of the drug or due to the reduced presence of B cells.

Gross Pathology

- Thymic atrophy was observed grossly in several animals. This finding was reported for both males and females in all groups, including controls. Because there did not appear to be a dose relationship and was observed in control

animals as well as treated animals, the finding was considered to be sporadic and not related to test article administration.

No other gross findings were reported.

Organ weights

Table E: Organs Weighed

Adrenals*	Pituitary
Brain (cerebrum, cerebellum and brain stem)	Prostate/Seminal vesicles
Epididymides*	Spleen
Heart	Submandibular glands*
Kidneys*	Thyroids* (including parathyroids)
Liver	Testes*
Lung (including main stem of bronchi)	Thymus
Ovaries*	Uterus

* Weighed individually, combined in tables.

- Some differences in organ weights were noted at both the terminal and recovery necropsies. These differences were in absolute organ weights, but, when compared relative to body weight, were within normal limits. Therefore the organ weight differences were not considered to be a result of test article exposure.

Histopathology
Signed, dated Pathology Report: Yes

A full panel of tissues was retained for microscopic examination as listed in the table below.

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Table F: Histopathology Panel

Adrenals[b]	Large Intestine -cecum -colon -rectum	Small Intestine -duodenum -ileum -jejunum -Peyer's patches (if possible)
Aorta (thoracic)	Liver	Spinal Cord (thoracic)
Bone[a] -femur/knee joint[c] -sternum	Lung (with bronchi) [b] -left lobe -right lobe	Spleen
Bone Marrow [a] -sternum -right 7th rib		
Brain -cerebrum -diencephalon (thalamus) -brain stem (medulla oblongata) -cerebellum	Lymph Nodes -axillary[c] -inguinal[c] -mesenteric -mandibular[c]	Stomach -fundus -pylorus
Epididymides[b]	Mammary Gland[c]	Submandibular Glands[b]
Esophagus (thoracic)	Ovaries[b]	Testes[b]
Eye Balls/ Optic Nerves[b]	Pancreas	Thymus
Gall Bladder	Pituitary	Thyroid Gland[b] (with Parathyroids, if possible)
Gross Lesions	Prostate	Tongue
	Sciatic Nerve[c]	Trachea
Heart	Seminal Vesicles	Urinary Bladder
Kidneys[b]	Skeletal Muscle[c] (quadriceps femoris)	Uterus -body -cervix
Lacrimal Glands[c]	Skin -gluteal area[c] -tattooed area[d]	Vagina
Injection Sites	Rectum	

[a] Bone and bone marrow were examined as decalcified specimens.
[b] Both left and right organs were examined.
[c] Both left and right organs were collected. If there were no gross lesions, only the left was examined.
[d] Tattooed area of skin was not examined but collected for identification purposes.

- Bone marrow smears: CD3-CD40+ lymphocytes were reduced in bone marrow to 73% and 57% of control values for the LD and HD groups, respectively. (In bone marrow, a significant proportion of B cells is CD20- and would be resistant to the effects of ocrelizumab.)
- Microscopic examination showed marked splenic lymphoid follicular atrophy and slight lymphoid follicle atrophy in all LD and HD animals. Lymph node follicular atrophy appeared to be less severe and more variable than that of the spleen. The incidence and severity of lymph node follicular atrophy are summarized in the tables below.
- Microscopic examination also showed subcutaneous changes at the injection sites consistent with chronic inflammation, rated slight.
- At the recovery necropsy, lymphoid follicular atrophy was observed in 50% of the treated animals, graded very slight. The reduced incidence and severity of the

follicular atrophy in spleen and lymph nodes indicates that partial recovery had occurred after the final dose of ocrelizumab.

Table H. Lymph Node Histopathology Findings

Sex	Female				Male		
Group	2		3		2	3	
Tissue/Findings Animal No.	9	11	17	19	12	18	20
Mesenteric lymph node							
Atrophy, lymphoid follicle	±	±	±	±	±	±	±

Sex	Female				Male			
Group	2		3		2		3	
Tissue/Findings Animal No.	9	11	17	19	10	12	18	20
Mandibular lymph node (left)								
Atrophy, lymphoid follicle	±	±	±	±	±	±	±	±

(±) very slight; (+) slight

Sex	Female				Male			
Group	2		3		2		3	
Tissue/Findings Animal No.	9	11	17	19	10	12	18	20
Axillary lymph node (left)/								
Atrophy, lymphoid follicle	+	±	±	+	±	±	±	±

Sex	Female					Male		
Group	1	2		3		2	3	
Tissue/Findings Animal No.	3	9	11	17	19	10	18	20
Inguinal lymph node (left)/								
Atrophy, lymphoid follicle	±	±	±	±	±	±	±	±

(±) very slight; (+) slight

Immunohistochemistry:
(B cell immunohistochemistry: Samples from lymphoid tissues, including spleen, lymph nodes, Peyer's patches, and bone marrow, were retained for immunohistochemistry and flow cytometry to evaluate B cell depletion.)

- B cell depletion was observed in tissue sections of spleen and mandibular lymph node retrieved at the terminal necropsy. B cell depletion in spleen was equal to or greater than B cell depletion in the lymph node in all animals treated with ocrelizumab.
- Repopulation of B cells was observed in spleen and lymph node of recovery animals. Recovery was variable in both dose groups. Some animals had B cell follicles in spleen and lymph node that appeared similar to control; other animals showed continued reduction in B cell follicles that were graded mild to moderate relative to control.

- At the end of the recovery period, recovery of the B cell populations was observed in all tissue compartments. The LD group showed B cell levels of 75 to 100% of control levels. The repletion observed in the HD group was lower (34 to 74%, relative to controls).

Imunophenotyping of spleen/lymph node cells:

- At the terminal necropsy, flow cytometry showed decreased B cells in the spleen, inguinal and mandibular lymph nodes. The range of depletion in the LD group was from 95 to 98% reductions relative to control values. In the HD group, the depletion was near complete, ranging from 98 to 99%. At the end of the recovery period, B cell levels were variable, but partial recovery in all compartments was observed.

APPENDIX D

Recovery Necropsy CD40+ CD3– Percent of Control

Animal ID	Study-Specific Animal No.	Sex	Group	PRO70769 Dose Level	CD40+CD3– Fraction as % Control			
					Spleen	Inguinal LN	Submandibular LN	Bone Marrow
010446	1	F	1	Vehicle (0.0 mg/kg)	108.01	129.62	120.51	84.45
021936	2	M	1	Vehicle (0.0 mg/kg)	91.99	70.38	79.49	115.55
021517	5	F	2	50.0 mg/kg	49.17	84.16	90.46	86.72
021910	6	M	2	50.0 mg/kg	205.82	100.43	102.26	179.56
030437	7	F	2	50.0 mg/kg	151.24	101.46	128.82	103.31
021458	8	M	2	50.0 mg/kg	36.46	12.67	11.28	18.86
021519	13	F	3	100.0 mg/kg	58.79	11.73	64.72	105.08
030295	14	M	3	100.0 mg/kg	126.63	66.61	154.97	72.17
030477	15	F	3	100.0 mg/kg	47.65	46.40	53.03	81.87
022015	16	M	3	100.0 mg/kg	89.55	9.76	21.64	75.40
Group Mean			1	Vehicle (0.0 mg/kg)	100.00	100.00	100.00	100.00
			2	50.0 mg/kg	110.67	74.68	83.21	97.11
			3	100.0 mg/kg	80.66	33.63	73.59	83.63
SD			1	Vehicle (0.0 mg/kg)	11.33	41.89	29.01	21.99
			2	50.0 mg/kg	81.63	42.09	50.56	66.00
			3	100.0 mg/kg	35.40	27.69	57.22	14.86
Dunnett test p-value			2	50.0 mg/kg	0.96	0.63	0.89	1.00
			3	100.0 mg/kg	0.89	0.12	0.76	0.87

LN=lymph node.

Conclusions:

Ocrelizumab was well tolerated at both dose levels. B cell depletion is the expected result of test article pharmacologic activity. The NOAEL is the HD of 100 mg/kg.

Study title: A repeat-dose (once every 3 weeks for 8 doses) toxicity and toxicokinetic evaluation of rhuMAb 2H7 (PRO70769) by intravenous administration in cynomolgus monkeys, with a 23-week recovery period

Study no.:	**04-0192-0134**
Testing Facility and location:	(b) (4)
Date of study initiation:	3/9/2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ocrelizumab, lot #M3-TOX78, 99%
Signed, dated Pathology Report	No, the sponsor states that this study was completed before a separate signed, dated Pathology report was being required. All necessary signatures are integrated into the report.

Methods

Doses:	0, 50, or 100 mg/kg
Frequency of dosing:	Once every 3 weeks (total of 8 doses)
Route of administration:	IV bolus
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) mM sodium acetate, (b) (4) mM trehalose dihydrate, 0.02% (w/v) polysorbate 20, at pH 5.3
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	Groups 1 and 3: 6/sex, group 2: 4/sex
Age:	Males: 3.0 to 3.9 years old, Females: 2.6 to 3.6 years old
Weight:	Males: 2.3 to 3.4 kg, Females: 2.0 to 2.6 kg
Satellite groups:	2/sex in groups 1 and 3 for recovery

Group No.	No. of M/F	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Conc. (mg/mL)	No. Euthanized: Day 149	Day 315
1	6/6	0 (control)	5	0	4/4	2/2
2	4/4	50	5	10	4/4	0/0
3	6/6	100	5	20	4/4	2/2

No. = number; M/F = male/female; Conc. = concentration

Observations and Results:

Mortality: (Observations were conducted twice daily.) All animals survived to scheduled euthanasia.

Clinical Signs: (Observations were conducted twice daily. On dosing days, observations were made pre-dose and 1 hour post-dose. Physical examinations were conducted once prior to initiation of dosing, on dosing days 1-3 hours post-dose, and twice during recovery in the first and last weeks.)

- No effects attributable to ocrelizumab were observed.

Body Weights: (Data were recorded prior to initiation of dosing and weekly thereafter.)

- No effects on body weight attributable to ocrelizumab were observed.

Food Consumption: (Data were collected once daily.)

- No effects on food consumption attributable to ocrelizumab were observed.

Ophthalmoscopy: (Conducted once prior to study initiation, 1-3 hours post-dose on dosing days, and once during the recovery period. Testing included electroretinograms to be conducted once prior to initiation of dosing, on SD59 and 143, and once during the recovery period.)

- No effects on ophthalmology parameters related to ocrelizumab were reported.

ECG: (Conducted once prior to initiation of dosing and approximately 1-2 hours post-dose on each day of dosing. During recovery, testing was conducted once during the first and last weeks. Blood pressure, heart rate, respiratory rate, and body temperature were recorded once prior to initiation of dosing, 1-2 hours post-dose on dosing days, and once during the first and last weeks of recovery.)

- No effects attributable to ocrelizumab on blood pressure, heart rate, respiratory rate, or body temperature were observed.
- No effects on ECG parameters attributable to ocrelizumab were observed.

Clinical Pathology: Samples were collected according to the following schedule.

Samples Collected	Groups	Study Days
Serum Chemistry	All available	Prestudy; prior to dosing on Day 1; on Days 24, 66 and 149; and during recovery on Day 315
Hematology	All available	Prestudy; prior to dosing on Days 1, 22, 43, 64, 85, 106 and 127; on Days 8, 15, 147 and 149; and approximately every 2 to 3 weeks during recovery period on Days 176, 190, 204, 218, 232, 246, 258, 272, 286, 307, 308 and 314
Urinalysis	All available	Prestudy; prior to dosing on Day 1; on Days 23, 65 and 149 (at euthanasia or in the morning for recovery animals); and during recovery on Day 315 (at euthanasia)

Hematology:

- Slightly reduced mean WBC counts (accounted for by reduced lymphocyte counts) in LD and HD groups. Total lymphocyte counts were reduced (approximately 10%, relative to baseline, for LD males and 32% for LD females, 10% for HD males, and no change from baseline for HD females).
- Reduction in circulating erythrocyte mass (RBC, HGB) was observed in males and females in LD and HD groups (graded slight to mild, -7 to 8%, relative to baseline), with a slight to mild increase in reticulocytes. The values were variable among animals and timepoints and did not appear to be clearly dose-related. The changes were small and the biological significance is unclear. The changes may be the result of frequent blood draws.
- In both dose groups, slight to mild bone marrow hypercellularity was noted and was described as erythropoiesis on microscopic examination.
- At the end of the recovery period, hemoglobin concentration and bone marrow cellularity showed recovery.

Clinical Chemistry: No effects on clinical chemistry parameters related to ocrelizumab were observed.

Urinalysis: (Samples were collected once prior to initiation of dosing and on SD23, 65, and 149. Samples also collected at necropsy by cystocentesis.)

- No effects on urinalysis parameters attributable to ocrelizumab were observed.

Necropsy:

The animals were sacrificed according to the following schedule:

Group No.	Day 149 (Terminal) Number of Males/Females	Day 315 (Recovery) Number of Males/Females
1	4/4	2/2
2	4/4	0/0
3	4/4	2/2

No. = number

Gross pathology:
No macroscopic findings related to ocrelizumab were observed at the end of the dosing period or the recovery period.

Organ Weights:

Organs Weighed	
Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Ovaries
Pituitary (post fixation)	Spleen
Testes	Thymus
Thyroid with parathyroids	

- No ocrelizumab-related effects on organ weights were observed.

Histopathology:

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Adequate Battery: Yes. All tissues below were examined microscopically.

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymis
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine
Large Intestine	Adrenals
Cecum	Pituitary
Colon	Thyroid/Parathyroids*
Rectum	Skin/ Musculoskeletal
Pancreas	Skin/Mammary Gland
Liver	Bone (femoral head)
Gallbladder	Bone (7th rib)
Respiratory	Skeletal Muscle (psoas and diaphragm)
Trachea	Nervous/Special Sense
Lung	Eyes with Optic Nerve
Lymphoid/Hematopoietic	Sciatic Nerve
Bone Marrow (sternum)	Brain
Thymus	Spinal Cord (thoracic)
Spleen	Other
Lymph Nodes	Animal Number Tattoo
Mandibular	Gross Lesions
Inguinal	Injection Sites (saphenous vein or cephalic vein, if used)
Mesenteric	
Peyer's Patches (included in sections of the small intestines)	

* The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.

Peer Review: Yes.

Histological Findings:

- Lymphoid follicular atrophy was observed in spleen and lymph nodes of both dose groups.
- No ocrelizumab-related findings were observed at the end of the 5.5 month recovery period.

- Signs of inflammation and tissue damage were observed at the injection site in all groups including control.

Findings in lymphoid tissue at the end of the dosing period are summarized in the table below:

Frequency and Severity of Lymphoid Follicular Atrophy			
	Group 1: 0 mg/kg No. Animals [n = 8]	Group 2: 50 mg/kg No. Animals [n = 8] (Severity)	Group 3: 100 mg/kg No. Animals [n = 8] (Severity)
Inguinal Lymph Node	0	7 (2-3)	7 (3-4)
Mandibular Lymph Node	0	5 (2-3)	7 (3)
Mesenteric Lymph Node	0	6 (2-3)	8 (2-3)
Spleen	0	8 (3)	8 (3-4)
(Severity) 2 - mild (2-3 follicles with small germinal centers in section examined) 3 - moderate (single or no follicles in section examined; normal lymphoid density/area) 4 - marked (no follicles in section and a decrease in the regional lymphoid)			

Samples for TK, immunogenicity (ADA detection), and immunophenotyping were collected according to the schedule below.

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Samples Collected	Groups	Study Days
Toxicokinetic	All available	Day 1: prior to dosing and 1, 8, 24 (*Day 2*), 48 (*Day 3*), 72 (*Day 4*), 120 (*Day 6*), 168 (*Day 8*), 216 (*Day 10*), 312 (*Day 14*) and 408 (*Day 18*) hours postdose; Days 22, 43, 64, 85, 106 and 127: prior to dosing and 1 hour postdose; Day 148: prior to dosing and 1, 8 and 23 (Group 1, *Day 149*) or 24 (Groups 2 and 3, *Day 149*) hours postdose. Additionally, for the recovery animals only, 48 (*Day 150*), 72 (*Day 151*), 120 (*Day 153*), 168 (*Day 155*), 216 (*Day 157*), 312 (*Day 161*) and 408 (*Day 165*) hours postdose on Day 148; and during the recovery period on Days 176, 190, 204, 218, 232, 246 and 314* (*Dates italicized in parenthesis are the actual day of study and are listed for informational purposes.*)
Antibody	All available	Prior to dosing on Days 1, 22, 43, 64, 106; on Day 149; and during recovery period on Days 204, 232 and 314*
Flow Cytometry	All available	Prestudy; prior to dosing on Days 1, 22, 43, 64, 85, 106 and 127; on Days 8, 15, 147 and 149; and approximately every two to three weeks during the recovery period on Days 176, 190, 204, 218, 232, 246, 258, 272, 286, 308 and 314*

* The collection days during the recovery period depended on when B-cells recovered to greater than 25% of baseline values.

Toxicokinetics:

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Table H1
Mean (±SD) Non-Compartmental TK Parameter Estimates following Eight Intravenous Bolus Doses of Ocrelizumab at 50 or 100 mg/kg Administered Every 3 Weeks to Cynomolgus Monkeys

Dose/ No./Sex	C_{max} (µg/mL)	AUC_{last} (µg • day/mL)	$t_{1/2z}$ (day)	AUC_{inf} (µg • day/mL)	CL_{ss} (mL/day/kg)	V_{ss} (mL/kg)
Group 2 (50 mg/kg)						
4/M	1450±43.3	100000±7140	NA	NA	NA	NA
4/F	1540±121	115000±13100	NA	NA	NA	NA
Group Mean:	1500±98.0	107000±12500	NA	NA	NA	NA
Group 3 (100 mg/kg)						
6/M	3240±344	229000±17700	15.7 [a]	274000 [a]	4.22 [a]	92.9 [a]
6/F	3020±327	208000±21500	12.7 [a]	221000 [a]	5.57 [a]	92.3 [a]
Group Mean:	3130±341	218000±21700	14.2±1.76	2480008±35900	4.90±1.04	92.6±17.3

AUC_{inf}=area under the concentration-time curve from zero to infinity; AUC_{last}=area under the concentration-time curve from zero to Day 148; C_{max}=maximal serum concentration; CL_{ss}=clearance at steady state; NA=not applicable; $t_{1/2z}$=terminal half-life; V_{ss}=volume of distribution at steady state.

[a] SD was not calculated when n≤2.

Immunogenicity:
ADA development was detected in 1 of 8 LD animals and 1 of 12 HD animals. ADAs were first detected on SD22 for both dose groups but did not have a significant effect on exposure.

Immunophenotyping: (Testing by FACS analysis was conducted on whole blood and tissue samples from lymphoid tissues and bone marrow.)

- Circulating B-cells were depleted after the first dose in both LD and HD groups. Maximal reduction was observed by SD8. After the recovery period (SD307), B cell levels were 58% in HD animals, relative to baseline levels.
- Tissue B cell depletion and recovery data are summarized in the table below.

Group (Day Euthanized)	B-cell populations (CD3-CD40+), expressed as group mean of percent of B-cells in organ relative to the mean of the control group			
	Spleen	Mandibular Lymph Node	Inguinal Lymph Node	Bone Marrow
Day 149				
Group 2 (50 mg/kg)	0.4	2.8	1.4	20.3
Group 3 (100 mg/kg)	0.1	1.6	1.1	23.9
Day 315				
Group 3 (100 mg/kg)	135.6	73.0	44.5	103.2

Dosing Solution Analysis: All dosing solutions were determined to be within the pre-determined acceptance criteria for concentration.

Conclusion:
The depletion of B cells observed in this study is the result of the known pharmacological activity of ocrelizumab. The NOAEL is the HD of 100 mg/kg. Full recovery was observed in bone marrow and spleen. Lymph nodes and circulating B cells showed partial recovery.

Study title: A toxicity and toxicokinetic evaluation of a humanized anti-CD20 antibody (rhuMAb 2H7) by intravenous administration in cynomolgus monkeys

Study no.:	**03-0113-0349**
Testing Facility and location:	(b) (4)
Date of study initiation:	3/24/2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ocrelizumab, M3-TOX64, 99%

Methods

Doses:	0, 10, 50, or 100 mg/kg
Frequency of dosing:	Every 2 weeks, 2 doses administered on SD1 and 15
Route of administration:	IV
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) mM sodium acetate, (b) (4) mM trehalose dihydrate, 0.02% (w/v) polysorbate 20, at pH 5.3
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	4/sex/group, 2/sex/control and HD for recovery
Age:	Males: 3.2 to 4.2 years old, Females: 2.8 to 3.8 years old
Weight:	Males: 2.4 to 3.5 kg, Females: 2.1 to 3.1 kg

Study Design:

Group No.	Number of Males/Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number Euthanized: Day 29	Day 113
1	6/6	0 (control)	5	4/4	2/2
2	4/4	10	5	4/4	--
3	4/4	50	5	4/4	--
4	6/6	100	5.1	4/4	2/2

Observations and Results:

Mortality: (Observations were recorded twice daily. On dosing days, each animals was observed pre-dose and within 2 hours of dosing.)

- All animals survived to scheduled euthanasia.

Clinical Signs: (Observations were recorded as stated above for Mortality.)

- No effects on clinical signs related to ocrelizumab were observed.

Body Weights: (Recorded once prior to initiation of dosing and weekly thereafter.)

- No effects on body weight or body weight change related to ocrelizumab were observed.

Food Consumption: (Data were recorded once daily.)

- No ocrelizumab-related effects on food consumption were observed.

Ophthalmoscopy: (Conducted twice prior to initiation of dosing, on SD1, 24, and 111.)

- No ocrelizumab-related effects on ophthalmology parameters were observed.

ECG and vital signs: (Testing was conducted twice prior to initiation of dosing, on SD1 within 2 hours post-dose, SD24, and SD111. Vital signs included heart rate, blood pressure, body temperature and respiratory rate.)

- No effects on ECGs or vital signs related to ocrelizumab were observed.

Physical examinations: (Detailed physical examinations were conducted while animals were sedated for ECG and vital signs.)

- No findings in physical examinations related to ocrelizumab were reported.

Clinical pathology: (Sample collection was conducted according to the schedule below.)

Sample Collection

Samples Collected	Groups	Study Days
Serum Chemistry	All available	Once within 2 weeks prior to study start (prestudy); on Days 1(pre-dose), 16, and 29 During Recovery: Day 113
Hematology	All available	Twice prestudy; on Days 1 (pre-dose), 2, 8, 15 (pre-dose), 16, 22, and 29 During Recovery: Days 43, 57, 71, 84, 99, and 113
Urinalysis	All available	Once within 2 weeks prior to study start (prestudy); on Days 1 (pre-dose), 16, and 29 During Recovery: Day 113

The animals were fasted overnight prior to blood collection for serum chemistry.

Hematology:

- Decreased absolute lymphocyte counts were observed in all ocrelizumab-dosed groups. By SD2, lymphocyte counts were reduced to 60-70% of baseline. Trends toward recovery were observed prior to the 2nd dose on SD15. On SD16, total lymphocyte counts were again reduced to 60-70% of baseline. At the end of the 12-week recovery period, total lymphocyte counts in the HD group were similar to baseline. (Total lymphocyte counts in the control group rose steadily throughout the study. At the end of the recovery period, levels were nearly 100% greater in controls than the HD group. Continued B cell depletion may be at least partially responsible for this difference.)

Clinical Chemistry:

- No changes related to ocrelizumab were observed.

Urinalysis:

- No ocrelizumab-related effects on urinalysis parameters were observed.

Gross Pathology

- No macroscopic observations related to ocrelizumab were reported.

Organ Weights:

Organs Weighed	
Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Ovaries
Pituitary (post fixation)	Spleen
Testes	Thymus
Thyroid with parathyroids	

- No ocrelizumab-related effects on organ weights were observed.

Histopathology:

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymis
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine
Large Intestine	Adrenals
Cecum	Pituitary
Colon	Thyroid/Parathyroids[a]
Rectum	Skin/Musculoskeletal
Pancreas	Skin/Mammary Gland
Liver	Bone (femoral head)
Gallbladder	Bone (7th rib)
Respiratory	Skeletal Muscle (psoas and diaphragm)
Trachea	Nervous/Special Sense
Lung	Eyes with optic nerve
Lymphoid/Hematopoietic	Sciatic Nerve
Bone Marrow (sternum)	Brain
Thymus	Spinal Cord (thoracic)
Spleen	Other
Lymph Nodes	Animal Number Tattoo
Mandibular	Gross Lesions
Mesenteric	Injection Sites - saphenous vein or cephalic vein, if used

[a] The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.

Adequate Battery: *Yes.*

Peer Review: *Yes.*

Histological Findings:

- Spleen and lymph nodes: At the end of the dosing period (SD29) lymphoid follicular atrophy was observed in all ocrelizumab-dosed groups. Lymphoid depletion was described as decreased size and number of lymphoid germinal centers in spleen and lymph nodes. Mucosa-associated lymphoid tissue (MALT) germinal centers were not affected. Frequency and severity were similar in all ocrelizumab-dosed groups.
- Protein deposition (described as homogeneous, eosinophilic material) was observed in lymph node germinal centers (those that were discernable) in the MD and HD groups. The biological significance is not clear.
- Injection site reactions: Fibroplasia, graded minimal to mild, was observed at the injection site in LD, MD, and HD animals (dose-related in incidence). The

sponsor related these findings to mechanical injury to tissues due to the injection. There is no explanation as to why, if this is the case, no control animals show the reaction to injection.

At the end of the recovery period, no microscopic findings related to ocrelizumab were observed.

Immunohistochemistry of lymphoid tissues (spleen, lymph nodes, and Peyer's patches):

- Animals euthanized on SD29 showed B cell depletion in lymphoid tissues. Depletion of B cells appeared more severe in the spleen relative to lymph nodes and Peyer's patches. The severity of depletion was dose-related, with nearly 100% depletion in spleens of HD animals.
- At the end of recovery, the spleens from HD animals showed the reduction in B cell levels to be minimal, demonstrating partial restoration of the B cell population.

Special Evaluations: (Samples were collected according to the schedule below for TK, immunogenicity, and immunophenotyping.)

Samples Collected	Groups	Study Days
Toxicokinetic	All available	Prior to dosing and 1, 8, 24, 48, 72, and 120 hrs post-dose on Days 1 and 15; on Day 22 at approximately the same time as when dose was administered; and on Day 29 (pre-euthanasia) During Recovery: Days 43, 57, 71, 84, 99, at approximately the same time as when dose was administered and Day 113 (pre-euthanasia)
Antibody	All available	Once within 2 weeks of study start (prestudy), and on Days 15 (pre-dose), and 29. During Recovery: Days 57, 84, 99 and 113
Flow Cytometry	All available	Twice within 2 weeks of study start (prestudy)[1], and Days 1 (pre-dose), 2, 8, 15 (pre-dose), 16, 22, and 29 During Recovery: Days 43, 57, 71, 84, 99, and 113
Samples for Research (Serum)	All available	Once within 2 weeks of study start; 1, 3, and 24 hrs post-dose on Day 1; and pre-dose and 1, 3, and 24 hrs post-dose on Day 15
Samples for Research (Whole Blood)	All available	Twice prestudy (all groups); 1, 3 and 24 hrs post-dose on Day 1 (Groups 1 and 2 only); and pre-dose and 1 and 3 hrs post-dose on Day 15 (Groups 1 and 2 only)
Samples for Research (Plasma)	All available	Once within 2 weeks of study start

[1] Due to sample quality, only values from one prestudy sample (Day –4) was reported.

Toxicokinetics:

Table 4
Mean (±SD) Noncompartmental Pharmacokinetic Parameters of Serum rhuMAb 2H7 After Two Intravenous Bolus Doses of 10, 50, or 100 mg/kg rhuMAb 2H7 Were Given 2 Weeks Apart to Cynomolgus Monkeys (n=4–6/sex/group)

Dose	Sex	C_{max} (µg/mL)	AUC_{last} (µg • day/mL)	$t_{1/2}$ (day)	AUC_{inf} (µg • day/mL)	AUC_{inf} [a] (µg • day/mL)	CL_{ss} (mL/day/kg)	V_{ss} (mL/kg)
10 mg/kg	F	262±56.1	550±507	1.79±1.62	594±565	601±565	27.3±15.3	45.5±4.67
10 mg/kg	M	303±96.1	791±505	2.87±1.73	963±660	1050±640	29.7±40.9	47.0±12.1
50 mg/kg	F	1670±404	5280±2120	4.45±2.46	6320±2960	6350±2990	10.9±4.96	54.4±21.9
50 mg/kg	M	1520±110	6010±1310	5.24±1.22	7320±2080	7340±2080	8.62±1.84	60.4±9.26
100 mg/kg	F	3360±258	15800±3650	6.87±1.97	18400±4630	18500±4670	7.50±1.77	64.5±12.8
100 mg/kg	M	2970±202	15000±4300	7.18±2.81	17000±3670	17000±3670	7.85±0.97	69.5±21.5

AUC_{inf}=Area under the concentration-time curve from zero to infinity.
AUC_{last}=Area under the concentration-time curve from zero to last.
C_{max}=Maximal serum concentration.
CL_{ss}=Clearance at steady state.
$t_{1/2z}$=Terminal half-life.
V_{ss}=Volume of distribution at steady state.
[a] AUC_{inf} includes data testing positive for anti-rhuMAb 2H7 antibodies.

Immunogenicity:

- ADAs were detected in 75% of LD animals, 13% of MD animals, and 17% of HD animals. The ADA response was detected by SD15 in the LD, SD29 in the MD group, and SD113 in the HD group. The ADAs were most likely masked by presence of high concentrations of ocrelizumab in the MD and HD groups. Based on the TK data, the ADAs did not have a significant effect on ocrelizumab exposure.

Immunophenotyping:

- Significant decreases in circulating B lymphocytes were observed by SD2 (24 hours post-dose). By SD8 (prior to the 2nd dose), B cells remained nearly totally depleted. A trend toward recovery was observed at the end of the recovery period, but B cell levels at that time were less than 20% of baseline.

Group	Percent of gated B-cell populations in whole blood relative to the baseline for each respective group[1]							
	Day 2	Day 8	Day 15 (pre)	Day 16	Day 22	Day 29	Day 99 (Rec)	Day 113 (Rec)
Group 1 (control)	103.8	84.4	83.8	97.9	88.9	80.4	83.6	68.5
Group 2 (10 mg/kg/dose)	18.8	0.5	0.5	0.8	3.9	5.1	--	--
Group 3 (50 mg/kg/dose)	23.4	0.7	0.9	0.7	2.6	2.6	--	--
Group 4 (100 mg/kg/dose)	13.7	0.5	0.5	0.6	2.6	1.8	8.9	19.4

-- Not applicable
Pre Prior to dosing on Day 15
Rec Recovery Phase
[1] Baseline is an average of Day –4 and Day 1 predose samples

Dosing Solution Analysis: The concentration of test article in all formulation samples was determined to be within the pre-established acceptance criteria.

Conclusions:
B cell populations remained depressed in HD animals through the 12-week recovery period, with only partial recovery observed. The NOAEL is 100 mg/kg since B cell depletion is the expected pharmacologic effect of ocrelizumab.

Study title: A toxicity and toxicokinetic evaluation of re-treatment with a humanized anti-CD20 antibody (rhuMAb 2H7) by intravenous administration in cynomolgus monkeys

Study no.:	**03-0114-0349**
Testing Facility and location:	(b) (4)
Date of study initiation:	4/11/2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ocrelizumab, M3-TOX64, 99%

Methods

Doses:	0, 10, 50, or 100 mg/kg
Frequency of dosing:	2 cycles of 2 doses each administered 2 weeks apart, followed by 14 week recovery period between cycles and after the second cycle
Route of administration:	IV
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) mM sodium acetate, (b) (4) mM trehalose dihydrate, 0.02% (w/v) polysorbate 20, at pH 5.3,
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	4/sex group for groups 1 and 4, 2/sex/group for groups 2, 3, and 5
Age:	3.2 to 4.2 years old
Weight:	2.9 to 3.9 kg for males, 2.4 to 3.0 kg for females

Study design:

Cycle 1: Groups 1, 3, and 4 received IV injections 2 weeks apart followed by a 14 week recovery period. Cycle 2: the same animals received 2 injections 2 weeks apart. Two animals/sex/group were euthanized 2 weeks after the 2nd dose of the 2nd cycle. The remaining 2/sex in control and HD group (group 4) and group 5 were monitored until B cells had recovered to greater than 50% of baseline. Recovery animals were

euthanized in week 43 (SD297/296). (Group 5 received only a single dose cycle. The LD group was released from the study after one dosing cycle due to generation of ADAs. ADAs were also detected in 100% of animals in the MD group but not until later in the study [SD57]. For the HD group, ADAs were detected late in the study, but ADAs may have been masked due to the high concentration of ocrelizumab.)

Group No.	Number of M/F	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Treatment Cycles	Number Released from Study M/F (SDay)[b]	Number Euthanized: M/F	
						Day 141	Day 297/296
1	4/4	0 (control)	5	2	-	2/2	2/2
2	2/2	10	5	1[a]	2/2 (108)[b]	-	-
3	2/2	50	5	2	-	2/2	-
4	4/4	100	5.1	2	-	2/2	2/2
5	2/2	100	5.1	1[c]	-	-	2/2

M/F = male/female; SDay = study day

[a] The animals were not administered a re-treatment of another two doses of rhuMAb 2H7 (Treatment Cycle 2) because more than 50% of the animals within this group were positive for anti-drug antibodies.

[b] Evaluation of the development of anti-drug antibodies and the date (noted in parenthesis) the animals were released from study and returned to the SBi animal colony were determined by the Sponsor.

[c] Only one treatment cycle was administered to Group 5 based on the original study design.

Observations and Results:

Mortality: (Observations were recorded twice daily.)

- All animals survived to scheduled euthanasia. (Group 2 animals, 2/sex, were released back into the colony due to development of ADAs. These animals did not receive the second cycle of dosing.)

Clinical Signs: (Observations were recorded twice daily. On dosing days, each animal was observed prior to dosing and approximately 1 hour post-dose.)

- No clinical signs related to ocrelizumab were observed.

Physical examinations: (Conducted twice prior to initiation of dosing during weeks -3 and -2, on SD1 at 1-3 hours post-dose, SD24, SD113 at 1-3 hours post-dose, SD136, and once during week 32.)

- No ocrelizumab-related observations during physical examinations were reported.

Body Weights: (Data will be recorded once prior to initiation of dosing and weekly thereafter.

- No effects on body weight related to ocrelizumab were observed.

Food Consumption: (Data was recorded once daily.)

- No ocrelizumab-related effects on food consumption were observed.

Ophthalmoscopy: (Conducted once prior to initiation of dosing, SD1 at 1-3 hour post-dose, SD24, SD113 at 1-3 hours post-dose, SD136, and once during week 32.)

- No ocrelizumab-related effects on ophthalmology parameters were observed.

ECG and vital signs: (Testing was conducted twice prior to initiation of dosing, on SD1 at approximately 1-2 hours post-dose, SD24, SD113 at 1-2 hours post-dose, SD136, and once during week 32.)

- No effects on ECG or vital signs related to ocrelizumab were observed.

Clinical pathology sample collection:

Samples Collected	Groups	Study Days
Serum Chemistry	All available	Once within 2 weeks prior to study start (prestudy), and on Days 1 (pre-dose), 16, 29, 113 (pre-dose), 128, and 141. During Recovery*: Approximately Day 225**(pre-euthanasia)
Hematology	All available	Once within 2 weeks prior to study start (prestudy), and on Days 1 (pre-dose), 2, 8, 15 (pre-dose), 16, 22, 29, 43, 57, 71, 84, 99, 113 (pre-dose), 114, 120, 127 (pre-dose), 128, 134, and 141. During Recovery*: Approximately Days 155, 169, 183, 197, 211, and 225** (pre-euthanasia).
IgG and IgM	All available	Days 1 (pre-dose), 29, 71, 113 (predose), and 141. During Recovery : Approximately Days 183 and 225** (pre-euthanasia).
Urinalysis	All available	Once within 2 weeks prior to study start (prestudy) and on, and on Days 1 (pre-dose), 16, 29, 113 (pre-dose), 128, and 141. During Recovery*: Approximately Day 225**(pre-euthanasia).

* The collection Days during the recovery period will depend on when B-cells recover to greater than 50% of prestudy sample. If B-cells recover to greater than 50% baseline prior to Week 32, the study may end prior to Week 32 and less samples will be collected. If necessary, the alternated study end date will be determined by the Sponsor who will notify the Study Director to arrange for the alternate study end date (to be changed by amendment).

** The actual day of necropsy for recovery animals will depend on when B-cells recover to greater than 50% of prestudy sample.

Hematology:

- Reduction in total lymphocyte counts was observed by 24 hours post-dose in the LD, MD, and HD groups. (Reductions were approximately 48 to 66% of baseline values and were not clearly dose-related.) A trend toward recovery was observed up to SD15, prior to the second dose, but was variable among animals. Reduction in total lymphocyte counts was observed in all ocrelizumab groups on SD16, non-dose-related in magnitude, approximately 49 to 63% of SD15 counts (pre dose).

- Trends toward recovery were observed after the second dose of the first cycle. Recovery was variable among animals of both MD and HD groups.
- Similar reductions in total lymphocyte counts were again observed during and after the second dosing cycle (SD113 and 127). The MD and HD groups demonstrated a reduction in total lymphocytes that was not clearly dose-dependent in magnitude (approximately 32 to 50% of pre dose values on SD113).
- Absolute lymphocyte counts were highly variable after the second dose cycle. No dose-relationship was observed. However, the total lymphocyte counts appeared to recover to near baseline levels by SD169.
- Group 5 (HD, one cycle only) showed recovery to greater than 50% by SD85.

Clinical Chemistry:

- No ocrelizumab-related effects were observed.

Urinalysis:

- No ocrelizumab-related effects were observed.

Gross Pathology:

No test article related findings were observed at the end of the dosing period or at the end of the recovery period.

Organ Weights:

No ocrelizumab-related effects on organ weights were observed at the end of the dosing period or at the end of the recovery period.

Histopathology

Adequate Battery: *Yes*

Peer Review: *Yes*

Histological Findings:

- Lymphoid follicular atrophy in spleen and lymph nodes was observed in all ocrelizumab groups. Data are summarized in the tables below:

  Males at the end of the dosing period:

GROUP:		1M (1)	3M (2)	4M (3)
NUMBER OF ANIMALS:		2	2	2
		#	#	#
SPLEEN	# EX	2	2	2
LYMPHOID FOLLICULAR ATROPHY				
minimal		0	1	0
moderate		0	1	2
MESENTERIC LYMPH NODE	# EX	2	2	2
LYMPHOID FOLLICULAR ATROPHY				
mild		0	0	1
moderate		0	1	1
marked		0	1	0
MANDIBULAR LYMPH NODE	# EX	2	2	2
LYMPHOID FOLLICULAR ATROPHY				
minimal		0	1	0
mild		0	0	1
moderate		0	1	0

Females at the end of the dosing period:

GROUP:		1F (1)	3F (2)	4F (3)
NUMBER OF ANIMALS:		2	2	2
		#	#	#
SPLEEN	# EX	2	2	2
LYMPHOID FOLLICULAR ATROPHY				
minimal		0	1	0
marked		0	1	1
MESENTERIC LYMPH NODE	# EX	2	2	2
LYMPHOID FOLLICULAR ATROPHY				
moderate		0	2	2
MANDIBULAR LYMPH NODE	# EX	2	2	2
LYMPHOID FOLLICULAR ATROPHY				
moderate		0	1	1

At the end of the recovery period, no lymphoid follicular atrophy was observed.

Immunohistochemistry of lymphoid tissues:
Doses of 50 mg/kg or 100 mg/kg (groups 3 and 4) resulted in a dose-related reduction of B cells in spleen, lymph nodes, and Peyer's patches (at the end of 2 cycles of ocrelizumab). In recovery animals (SD297), immunostaining of lymphoid tissues in ocrelizumab-dosed animals was similar to that of tissues from control animals.

Serum immunoglobulin levels: No ocrelizumab effects on serum immunoglobulin levels were observed.

Toxicokinetics:

Table E1
Partial AUC Following One or Two Cycles Treatment

Group	Dose	Partial Area (mg day/mL)	Mean	SD
2 (n=4)	10 mg/kg	$AUC_{Day\ 0–112}$	2.16	0.591
		$AUC_{Day\ 112–296}$	NA	NA
3 (n=4)	50 mg/kg	$AUC_{Day\ 0–112}$	18.5	2.33
		$AUC_{Day\ 112–296}$	17.5	3.47
4 (n=8)	100 mg/kg [a]	$AUC_{Day\ 0–112}$	39.6	7.82
		$AUC_{Day\ 112–296}$	38.8	10.0
5 (n=4)	100 mg/kg [a]	$AUC_{Day\ 0–112}$	38.7	4.67
		$AUC_{Day\ 112–296}$	NA	NA

$AUC_{Day\ 0–112}$ = area under the curve from Nominal Days 0 to 112.
$AUC_{Day\ 112–296}$ = area under the curve from Nominal Day 112 to 296.
NA = Received only one cycle treatment per protocol.
[a] Group 4 received two cycles of treatment and Group 5 received one cycle of treatment.

- t½ for the LD was approximately 2 days
- t½ for the MD and HD: 8.5 and 11.9 days, respectively.

Immunogenicity:

- LD animals showed the strongest anti-drug response. ADAs in the higher dose groups may have been masked by the presence of high ocrelizumab levels.

The table, below, contains a summary of the incidence of ADAs in each dose group.

Group	Percent of Animals with Detectable Anti-rhuMAb 2H7 Antibodies	Study Day Anti-rhuMAb 2H7 Antibodies First Detected
Group 1 (0 mg/kg/dose, 2 treatment cycles)	0	NA
Group 2 (10 mg/kg/dose, 1 treatment cycle)	100	29
Group 3 (50 mg/kg/dose, 2 treatment cycles)	100	57
Group 4 (100 mg/kg/dose, 2 treatment cycles)	75	85
Group 5 (100 mg/kg/dose, 1 treatment cycle)	50	127

NA = Not applicable

Immunophenotyping:

- No consistent effects on T cell or NK cell levels were observed.
- B cells were reduced to undetectable levels up to 4 weeks after the first dosing cycle. B cell levels in the LD group began to recover by SD43. In the MD and HD groups, recovery was not observed until SD99 (12 weeks after the second dose of the first dosing cycle).
- After the second dosing cycle, B cell levels in the MD and HD groups remained undetectable up to the day of euthanasia (SD141). On SD 297, 3 of 4 HD (Group 4) animals showed B cell recovery to baseline, while the 4th animal had recovered to 25% of baseline.
- All HD, 1-cycle (group 5) animals showed complete recovery of B cells to baseline by SD297.

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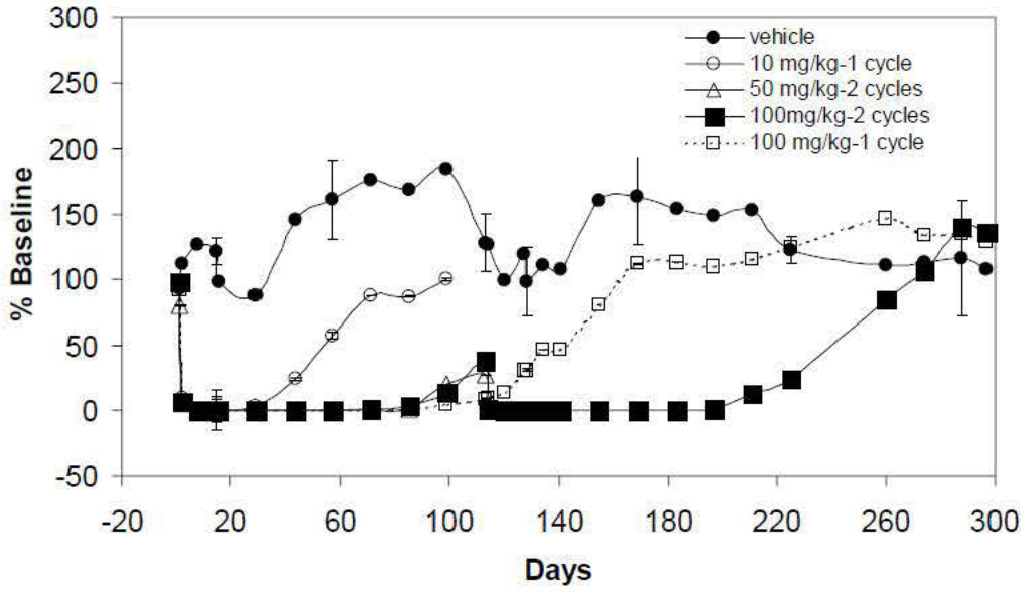
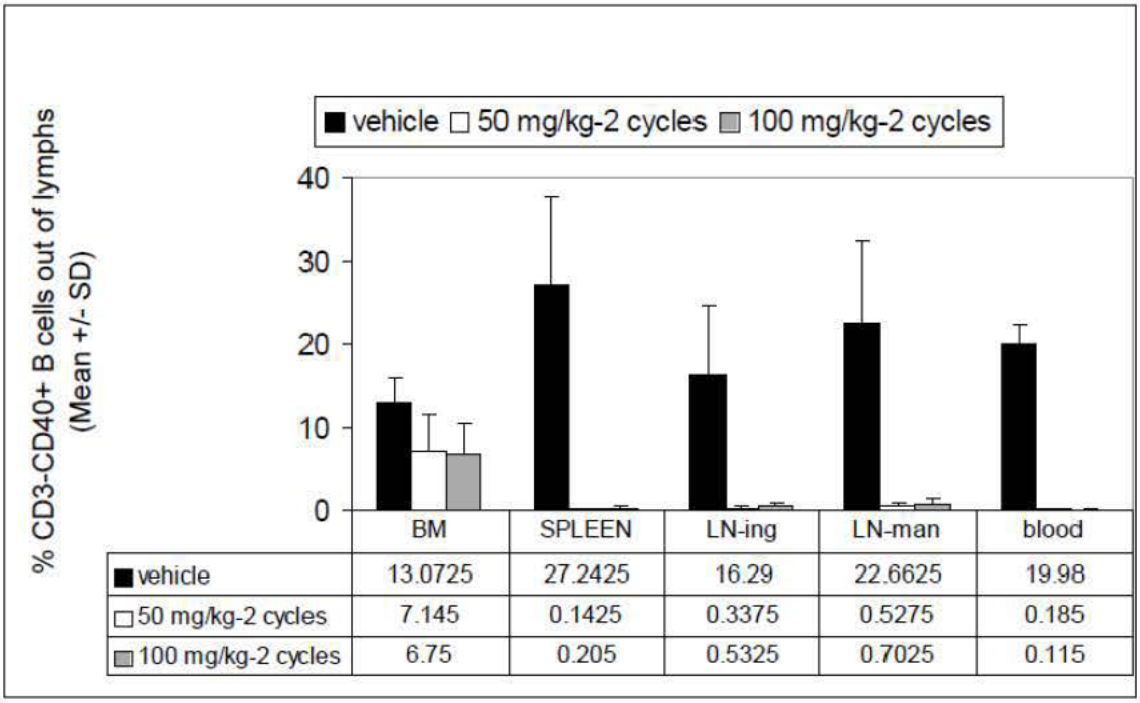
APPENDIX C

Peripheral Blood CD3-CD40+ B-Cell Count, % Baseline

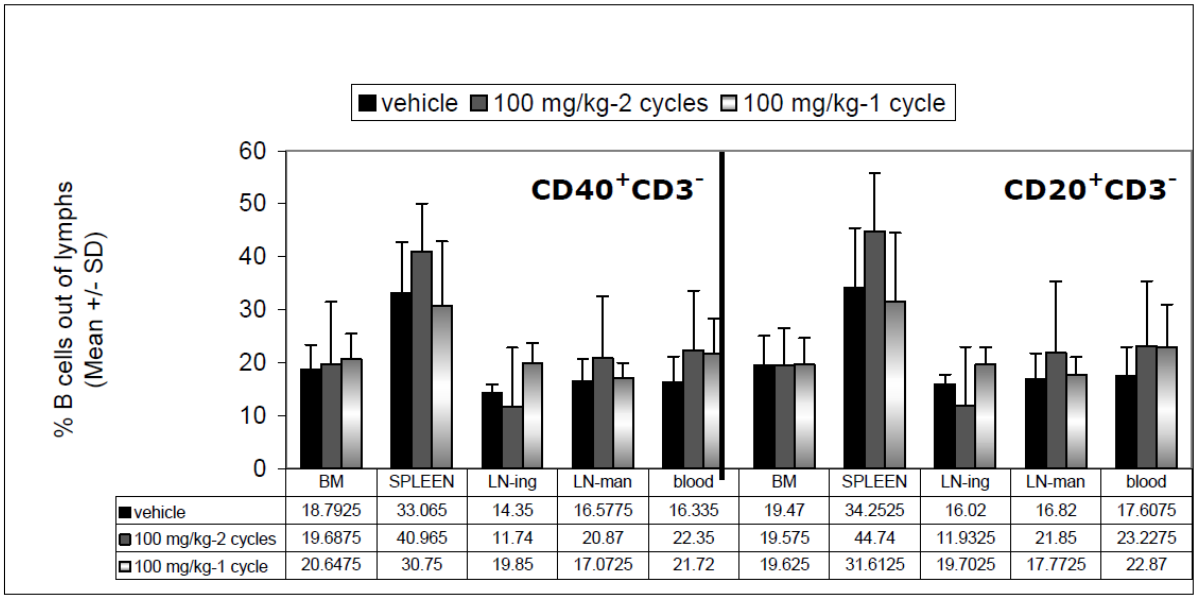


B Cells in Lymphoid Tissues at Day 141



	BM	SPLEEN	LN-ing	LN-man	blood
vehicle	13.0725	27.2425	16.29	22.6625	19.98
50 mg/kg-2 cycles	7.145	0.1425	0.3375	0.5275	0.185
100 mg/kg-2 cycles	6.75	0.205	0.5325	0.7025	0.115

B Cells in Lymphoid Tissues at Day 297



	BM	SPLEEN	LN-ing	LN-man	blood	BM	SPLEEN	LN-ing	LN-man	blood
vehicle	18.7925	33.065	14.35	16.5775	16.335	19.47	34.2525	16.02	16.82	17.6075
100 mg/kg-2 cycles	19.6875	40.965	11.74	20.87	22.35	19.575	44.74	11.9325	21.85	23.2275
100 mg/kg-1 cycle	20.6475	30.75	19.85	17.0725	21.72	19.625	31.6125	19.7025	17.7725	22.87

Dosing Solution Analysis: Duplicate samples of the dosing solutions were analyzed for concentration.

All dosing solutions were determined to be within the pre-established acceptance criteria for concentration.

Conclusions:

Two dosing cycles of 50 mg/kg were sufficient to fully deplete B cell populations. Repopulation of B cells was demonstrated at all dose levels. NOAEL is100 mg/kg.

Study title: Ocrelizumab clone (b) (4) vs. clone (b) (4): A four week toxicity and PK/PD comparison by intravenous administration in cynomolgus monkeys (GLP)

Study no.:	**07-0171**
Testing Facility and location:	(b) (4)
Date of study initiation:	5/24/2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ocrelizumab clone (b) (4) Lot#M95200, 100% (original process) Ocrelizumab clone (b) (4) Lot# L01653, 99% (2006 process)

Methods

Doses:	0 or 50 mg/kg
Frequency of dosing:	2 Doses on SD1 and SD15
Route of administration:	IV bolus injection
Formulation/Vehicle:	(b) (4) mM sodium acetate, (b) (4) mM trehalose dihydrate, 0.02% (w/v) polysorbate 20, at pH 5.3,
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	Males only, 5/group
Age:	3 to 7 years old
Weight:	2.6 to 5.7 kg
Deviation from study protocol:	None significant

Study design:
Animals (3/group) were euthanized on SD29; 2/group were euthanized on SD162 after a 134-day recovery period.

Group Assignments

Treatment Group	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals	Study Specific Animal Numbers (or Animal ID)
1	0	0	1.7	$3^a + 2^b$	$2^a,4^a,6^a,8^b,10^b$
2 clone (b) (4)	50	20	2.5	$3^a + 2^b$	$12^a,14^a,16^a,18^b,20^b$
3 clone (b) (4)	50	30	1.7	$3^a + 2^b$	$22^a,24^a,26^a,28^b,30^b$

Note: Total dose volumes (mL) were calculated based on the most recent body weight.
[a] Terminal Necropsy: D29
[b] Recovery Necropsy: D162 (R134)

Observations and Results:

This study was conducted to compare toxicity of product produced by the 2006 process to product from the original manufacturing process. Full toxicology parameters were monitored. The results indicated that the PD and toxicity of the two clones were similar. However, there appeared to be a consistent reduction in exposure in the group that received the Clone (b) (4) (2006 process). It is not clear from the information provided, if any of the general toxicity studies were conducted using the product from the 2006 (new) process. If the product derived from the 2006 process is to be marketed, the sponsor should clarify which nonclinical studies were conducted using product from each process. If the CMC team determines that the products from the 2 processes are not comparable, the nonclinical studies could be invalidated.

Flow cytometry (Immunophenotyping):

- On SD2, NK cell levels were reduced 30% relative to control and baseline for both ocrelizumab groups. NK cell levels recovered by SD8.
- B cell levels were rapidly depleted after the first dose for both ocrelizumab groups and remained depleted through the end of the dosing period.
- B cell levels in both ocrelizumab groups recovered to at least 25% of baseline by the end of the recovery period.

Toxicokinetics:

Toxicokinetic parameters

		Clone (b) (4)				Clone (b) (4)			
Parameter	**Unit**	**Day 1**		**Day 15**		**Day 1**		**Day 15**	
C_{max}	µg/mL	1440	(358)	1830	(499)	1300	(196)	1530	(213)
$AUC_{(0\text{-}168\ h)}$	µg•day/mL	4710	(883)	6170	(1710)	4290	(667)	5000	(1210)

AUC values were calculated by the linear trapezoidal rule.

Dosing Solution Analysis:

All dosing solution samples were demonstrated to be within the pre-established acceptance criteria for concentration.

7 Genetic Toxicology

Genetic toxicology studies were not conducted.

8 Carcinogenicity

No carcinogenicity evaluation was conducted. This requirement was waived (email dated May 2, 2016).

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Ocrelizumab: 9-week intravenous administration male fertility study in the cynomolgus monkey with a 16 week recovery phase

Study no.:	**12-0524**
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/20/2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ocrelizumab, lot #472259, 99%

Methods

Doses:	0, 20, or 100 mg/kg
Frequency of dosing:	Weekly for 8 weeks (total of 9 doses)
Dose volume:	5 mL/kg
Route of administration:	Intravenous injection
Formulation/Vehicle:	Ocrelizumab vehicle: (b) (4) mM sodium acetate, (b) (4) mM trehalose dihydrate, 0.02% (w/v) polysorbate 20, at pH 5.3
Species/Strain:	Cynomolgus monkey, males only, 6-8 years old, 5.5 to 13.4 kg at initiation of dosing
Number/Sex/Group:	5/group for the main study 3/group for recovery
Study design:	

Loading doses: 0, 15, or 75 mg/kg were administered on 3 consecutive days (SD1-3). Beginning on SD10, the study doses (0, 20, or 100 mg/kg) were administered weekly for 8 weeks.

Group number	Group description	Dose Level (mg/kg)		Dose Concentration 2H7 (mg/mL)		Dose Volume (mL/kg)	No. of animals (males)	Necropsy after ...	
		LD[a]	SD[b]	LD[a]	SD[b]			9 weeks	26 weeks
1	Control	0	0	0	0	5	8	5 M	3 M
2	Low	15	20	3	4	5	8	5 M	3 M
3	High	75	100	15	20	5	8	5 M	3 M

* Based on most recent individual body weight
[a] Loading dose
[b] Study dose

Observations and Results:

Dosing Solution Analysis:

All formulation samples were determined to be within the pre-determined acceptance criteria for concentration.

Formulation sample	Day 1	Day 10	Day 24	Day 38	Day 52
Group control	< LOD n.a.	< LOD n.a.	< LOD n.a.	< LOD n.a.	< LOD n.a.
Group low loading dose	103.9%; CV = 0.2%	-	-	-	-
Group low study dose	n.a.	103.0%; CV = 0.5%	103.5%; CV = 0.1%	103.9%; CV = 0.3%	102.8%; CV = 0.4%
Group high loading dose	103.2%; CV = 0.2%	-	-	-	-
Group high study dose	n.a.	103.1%; CV = 0.2%	103.4%; CV = 0.0%	104.1%; CV = 0.2%	103.1%; CV = 0.2%

CV = Coefficient of Variation

Mortality: (Observations were recorded twice daily.)

- No unscheduled deaths occurred.

Clinical Signs: (Observations were recorded twice daily. On dosing days, observations were recorded 2-4 hours post-dose.)

- No clinical observations related to ocrelizumab were reported.

Physical and neurological examinations: (Conducted once prior to initiation of dosing, 1 hour and 4 hours post-dose on SD1 and SD31 [week 5])

- No adverse effects attributable to the test article were observed.

Body Weight: (Data were recorded prior to initiation of dosing and weekly during the dosing and recovery periods.)

- No effects on body weight attributable to the test article were observed.

Food Consumption*:* (Estimated qualitatively once daily.)

- No effect on food consumption attributable to the test article was observed.

Clinical pathology: (Blood samples were collected once prior to initiation of dosing, during week 9 of the dosing period, and during the last week of the recovery period. A full panel of hematology, coagulation, and clinical chemistry parameters was monitored.)

Hematology:

- No significant effects attributable to the test article were observed on hematology parameters. A small decrease in total lymphocytes in the LD and HD groups was noted but was not statistically significant. The small decrease was most likely due to the specific depletion of B-lymphocytes demonstrated by immunophenotyping. (See below.)

Clinical chemistry:

- No significant effects attributable to ocrelizumab were observed.

Hormone analysis: (Samples were collected twice prior to initiation of dosing between 7 and 10 am, on SD24, 45, 101, and 143.) Due to the lack of effect on sperm and male reproductive tissues, no hormone analysis was conducted.

Immunophenotyping: Samples were collected prior to initiation of dosing including on SD1 prior to dosing, during weeks 4 and 9 of dosing, and weeks 5, 8, and 12 of recovery.)

The following immune-cell phenotypes were monitored.

Antibody	Cell type
Isotype controls	Background
CD3, CD4, CD8, CD45	Total T-cells, T helper-cells, cytotoxic cells
CD3, CD16, CD40, CD45	B-cells, NK-cells

Cell type	Units	Description
T-cells	10E9/L/%	CD3+ T-cells
Cytotoxic T-cells	10E9/L/%	CD3+CD8+ cytotoxic T-cells
T helper-cells	10E9/L/%	CD3+CD4+ T helper-cells
B-cells	10E9/L/%	CD40+ B-cells
Natural killer-cells	10E9/L/%	CD16+ NK-cells

- From SD28 until SD59, a dose-related reduction in B lymphocytes (CD40+) was observed. In the LD group, by the end of the recovery period, low levels of B-cells were observed. In the HD group, near total depletion was observed beginning on SD28 and persisting through the recovery period.
- No ocrelizumab-related effects on T cell populations were observed.

Toxicokinetics:

Dose Group	Dose Level (mg/kg)	Sex		Toxicokinetic Parameters					
				$C_{max\ first}$ (µg/mL)	$C_{max\ second}$ (µg/mL)	$C_{max\ third}$ (µg/mL)	AUC_{2-9} (µg·day/mL)	$C_{max\ eleventh}$ (µg/mL)	AUC_{58-t} (µg·day/mL)
2	15	M	Mean	502	828	1020	5120	1540	13485
			SD	68.9	124	72.1	676	398	11350
			N	8	8	8	8	8	3
3	75	M	Mean	2690	4090	5180	24600	5890	83255
			SD	411	409	520	3060	737	10044
			N	8	8	8	8	8	3

Semen ocrelizumab analysis:
At the end of the dosing period, ocrelizumab was measured in semen in the LD and HD groups. The mean concentration of ocrelizumab in semen was approximately dose proportional, although individual levels showed large variability (22.1 ± 29.5 mcg/mL and 113 ± 131 mcg/mL for LD and HD groups, respectively). The concentration dropped rapidly during the recovery period. By week 8 of recovery, the mean level of

ocrelizumab in semen for the LD group was 0.81 mcg/mL and 3.37 mcg/mL in the HD group.

Immunogenicity: Samples for analysis of immunogenicity were collected according to the following schedule:

Groups (animals)	Study Day	Study Week	Days post last dose [d]	ATA
All	-D8 (PRED15)	-W2		X
All	-D4 (PRED19)	-W1		X
All	D1	W1		Predose
All	D29	W5	5	X
All	D57	W9	5	X

Groups (animals)	Study Day	Study Week	Days post last dose [d]	ATA
All Recovery	D85	W13	26	X
All Recovery	D113	W17	54	X
All Recovery	D141	W21	82	X
All Recovery	D169	W25	110	X

W (week); D (day)

One animal in the LD group tested positive for ADAs (1 of 8, 13%). No animals in the HD group tested positive for ADAs. In total, 1 of 16 animals tested positive for ADAs (6%).

Sperm analysis: (Conducted twice prior to initiation of dosing, during week 8 or 9 of the dosing period, and during week 8 and 16 of the recovery period. Analysis of sperm in a stage-aware manner was conducted on testes tissue after necropsy.)

- In life sperm analysis: Evaluation included ejaculate weight, sperm count, motility, and morphology. No adverse effects on sperm parameters were observed.
- No effects on spermatogenic stages (conducted on testis sections from tissue collected at necropsy) were observed. All spermatogenic stages were present in all animals.

Testicular size: (Data recorded twice prior to initiation of dosing, during week 8 or 9 of the dosing period, and during week 8 and 16 of the recovery period.)

- No effects of ocrelizumab on testicular size or echogenicity (ultrasound analysis) were observed.

Testicular and epididymal examination: (Conducted on tissues collected at necropsy).

- No effects of ocrelizumab were observed.

Necropsy: Five animals/group were euthanized on SD63. Three animals/group were euthanized on SD172 (recovery day 113). All macroscopic findings and lesions were recorded.

11.6 Organ/tissue list

Organ / tissue	PF	SC	OW	PE	SP	Additional information
Adrenals	F		X	X		Weighed separately
Epididymides	MD		X	X	X	Weighed separately; PAS staining
Gross lesions	F			X		
Injection sites (i.v.)	F			X		
Mandibular lymph nodes	F			X		
Pituitary	F		X	X		
Prostate	F		X	X		
Seminal vesicles	F		X	X		
Spleen	F		X	X		
Testes	MD		X	X	X	Weighed separately; PAS staining
Bone marrow smears (sternum)	M					See section 11.2

PF	Tissue preservation and fixation	F	10% neutral buffered formalin
SC	Special sample collection	PE	Histologic processing and examination
OW	Organ weights	MD	Modified Davidson's fluid
SP	Special histology processing	M	Methanol
i.v.	Intravenous		

Organ weights: No effects on organ weight attributable to ocrelizumab were observed.

Macroscopic examination: No ocrelizumab-related effects were observed.

Histopathology: (External and internal peer review was conducted.)

- At the end of the dosing period, hypocellularity of lymphoid follicles in spleen and lymph nodes was observed in both ocrelizumab dose groups, dose-related in incidence and severity.
- No ocrelizumab-related findings were reported in male reproductive organs.

Lymph Node, Mandibular	Number Examined:	5	5	5
	Unremarkable:	3	0	2
Hematopoiesis, extramedullary		2	3	2
Hypocellularity, lymphoid follicles		0	5	2
Spleen	Number Examined:	5	5	5
	Unremarkable:	5	0	0
Hypocellularity, lymphoid follicles		0	5	5

Recovery: Hypocellularity in spleen and lymph node persisted through the recovery period.

Lymph Node, Mandibular	Number Examined:	3	3	3
	Unremarkable:	3	2	2
Hematopoiesis, extramedullary		0	1	0
Hypocellularity, lymphoid follicles		0	0	1
Spleen	Number Examined:	3	3	3
	Unremarkable:	3	1	1
Hypocellularity, lymphoid follicles		0	2	2

Conclusion:
NOAEL is the HD of 100 mg/kg.

Study title: A study of the effect of Ocrelizumab, administered by intravenous injection, on female fertility in cynomolgus monkeys (Seg 1)

Study no.:	**12-0525**
Study report location:	EDR
Testing Facility and location:	(b) (4)
Date of study initiation:	7/18/2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ocrelizumab/484840/99%

Methods

Doses:	0, 20, or 100 mg/kg
Frequency of dosing:	Weekly (over 3 menstrual cycles or 105 days)
Dose volume:	5 mL/kg
Route of administration:	Intravenous injection
Formulation/Vehicle:	(b) (4) mM sodium acetate, (b) (4) mM trehalose dihydrate, 0.02% (w/v) polysorbate 20, at pH 5.3
Species/Strain:	Cynomolgus monkey, females only At least 6 years old at initiation of dosing
Number/Sex/Group:	8/group
Deviation from study protocol:	None significant

Study design:

Groups 2 and 3 were given loading doses of 15 and 75 mg/kg, respectively on SD1 through 3, followed by the study doses of 20 and 100 mg/kg, respectively, beginning on SD10 and continuing weekly over 3 menstrual periods. The recovery period consisted of 3 menstrual cycles.

Group number	Group description	Color code	Dose level (mg/kg)		Dose concentration 2H7 (mg/mL)		Dose volume (mL/kg)	No. of animals (females)
			LD	SD	LD	SD		
1	Control	White	0	0	0	0	5	8
2	Low	Blue	15	20	3	4	5	8
3	High	Red	75	100	15	20	5	8

LD = loading dose

SD = study dose

Observations and Results:

Mortality: (Observations recorded twice daily.)

- All animals survived to scheduled euthanasia.

Clinical Signs: (Data recorded at least twice daily. Detailed observations recorded on SD6, 10, and weekly thereafter. Post-dose observations recorded 2-4 hours after dosing.)

- No clinical signs attributable to the test article were observed.

Body Weight: (Data recorded at least once prior to initiation of dosing, once per weekly during the dosing and recovery periods, and prior to necropsy.)

- No effects on body weight attributable to the test article were observed.

Food Consumption: (Qualitatively assessed once daily.)

- No effects on food consumption were observed.

Menstrual cycling: (Observations recorded daily beginning on day 1 of the menstrual cycle and continuing to the end of the study.)

- No effects on menstrual cycling were observed (regularity, length, or bleeding intensity).

Hormones: Samples were collected but not analyzed because there was no apparent effect of ocrelizumab on menstrual cycling.

Clinical pathology: (Samples were collected according to the schedule below. A standard battery of hematology, clinical chemistry, and coagulation parameters was conducted.)

Schedule	
Time points	Once during observation cycles 2 (menstrual cycle 2) Treatment cycle 3 (menstrual cycle 5) Recovery cycle 3 (menstrual cycle 8)

Blood sampling*

Occasion	Animals fasted	Sample type	Anticoagulant	Sample volume (mL)
All	Not required	Hematology	EDTA	0.5
All	Not required	Coagulation	Trisodium citrate	1.0
All	Yes	Clinical chemistry	None	1.5

* Sample site: *vena cephalica antebrachii* or *vena femoralis*, exceptional use of *vena femoralis* to be documented in the raw data

Hematology:
No effects on hematology parameters were observed.

Clinical chemistry:
No effects on clinical chemistry parameters were observed.

Coagulation:
No effects on coagulations parameters were observed.

Toxicokinetics:

The sponsor reported that steady state was achieved by SD65.

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Table 1 Summary of the Mean Toxicokinetic Parameters for Ocrelizumab in Female Monkey Serum

Dose Group	Dose level (mg/kg)		C_{max} (ng/mL)	T_{max} (day)	AUC_{2-9} (ng·day/mL)
			Post loading dose		
2	15	Mean	779000	3.00	4530000
		SD	64000	0	349000
		N	8	8	8
3	75	Mean	4100000	3.00	21300000
		SD	641000	0	3430000
		N	8	8	8
			Post study dose 11		
2	20	Mean	905000	80.0	4950000
		SD	197000	0	1160000
		N	8	8	8
3	100	Mean	4100000	80.0	22800000
		SD	856000	0	5100000
		N	8	8	8
			Post study dose 12		
2	20	Mean	848000	87.0	4860000
		SD	164000	0	1030000
		N	6	6	6
3	100	Mean	3750000	87.0	20700000
		SD	678000	0	4390000
		N	6	6	6
			Post study dose 13		
2	20	Mean	790000	94.0	4520000
		SD	236000	0	1420000
		N	3	3	3
3	100	Mean	3460000	94.0	19700000
		SD	480000	0	3160000
		N	4	4	4
			Post study dose 14		
2	20	Mean[1]	863000	101	5000000
		N	1	1	1
3	100	Mean[1]	3960000	101	23000000
		N	1	1	1

1 Not a true mean as N=1.

Immunogenicity*:*

- No animals tested positive for ADAs after exposure to ocrelizumab.

Immunophenotyping: Blood samples were processed to determine the levels of the individual lymphocyte phenotypes indicated in the tables below (from the study report.)

Cell Type	Units	Description
T-cells	10E9/L/%	CD3+ T-cells
Cytotoxic T-cells	10E9/L/%	CD3+CD8+ cytotoxic T-cells
T helper-cells	10E9/L/%	CD3+CD4+ T-helper-cells
B-cells	10E9/L/%	CD40+ B-cells
Natural killer-cells	10E9/L/%	CD16+ NK-cells

General procedures		
Combination of antibodies	**Antibody**	**Cell Type**
	Isotype controls	Background
	CD3, CD4, CD8, CD45	Total T-cells, T helper-cells, cytotoxic cells
	CD3, CD16, CD40, CD45	B-cells, NK-cells

- Beginning on SD2, a significant reduction in CD40+ B cells was observed in both the LD and HD groups. The magnitude of reduction was to nearly undetectable levels in both dose groups. Recovery was not observed. The sponsor stated that the lack of recovery was due to the long $t_{1/2}$ of ocrelizumab. The recovery period lasted 3 menstrual cycles (approximately 90 days).
- On SD2, a reduction in CD16+ NK cells was observed. The magnitude (approximately 30% of control) of the reduction was greater in the LD group. The NK cell levels appeared to recover somewhat over the following 2 weeks but remained slightly reduced (variable and not statistically significant) until the end of the study.
- No detectable ocrelizumab-related effects on T cells were observed.

Dosing Solution Analysis:

Dosing formulations were prepared on each day of dosing. Samples from each dose formulation were collected for analysis. All formulation samples were determined to be within the pre-established acceptance criteria for concentration.

Necropsy: Each animal was euthanized within 3 working days of completion of recovery cycle 3 of the last female per cage. (Animals were housed 2/cage because single housing requires justification.)

Selected organs/tissues were collected as listed below.

11.5 Organ/tissue list

Organ / tissue	PF	SC	OW	PE	SP	Additional information
Adrenals	F		X	X		Weighed separately and in total
Bone marrow smear (sternum)	M				X	See terminal procedures
Glands, mammary	F			X		
Gross lesions	F			X		
Implanted transponder chip	X					

Organ / tissue	PF	SC	OW	PE	SP	Additional information
Injection sites (i.v.)	F			X		
Mandibular lymph nodes	F			X		
Mesenteric lymph nodes	F			X		
Ovaries	F		X	X		Weighed separately and in total
Oviducts	F			X		
Pituitary	F		X	X		
Skin/animal identification	F			X		
Spleen	F		X	X		
Uterus/cervix	F		X	X		
Vagina	F					

PF	Tissue preservation and fixation	F	10% neutral buffered formalin
SC	Special sample collection	OW	Organ weights
PE	Histologic processing and examination	SP	Special histology processing
i.v.	Intravenous	X	Action required

Organ weight:
No effects attributable to ocrelizumab were observed on organ weights.

Histopathology: Peer review: Yes

Ocrelizumab-related microscopic findings included:

- Hypocellularity of lymphoid follicles in spleen and lymph nodes (graded minimal to moderate).
- A malignant carcinoma of the right nasal cavity was observed in one LD animal. This finding was considered incidental.
- One LD animal showed increased germinal centers in the spleen (graded moderate). The finding correlated with increased spleen weight for this single animal.

No effects attributable to ocrelizumab were observed in reproductive organs.

Conclusion:
The NOAEL is the HD of 100 mg/kg. The reduction of NK cell levels is of unclear toxicological significance since this finding was not observed in any other nonclinical studies conducted with ocrelizumab.

9.2 Embryofetal Development

Study title: An assessment of the effects of PRO70769 (rhuMAb 2H7) on embryo-fetal development when administered weekly by intravenous injection to pregnant cynomolgus monkeys

Study no.:	**04-1272-1342**
Study report location:	EDR
Testing Facility and location:	(b) (4)
Date of study initiation:	3/21/2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ocrelizumab, M3-TOX108, 99%

Methods

Doses:	0, 15/20, or 75/100 mg/kg Group 2 and 3 received loading doses of 15 or 75 mg/kg, respectively, on GD20, 21, and 22, followed by doses of 20 or 100 mg/kg.
Frequency of dosing:	3 loading doses, followed by weekly dosing (GD29, 36, 43, and 50.)
Dose volume:	5 mL/kg
Route of administration:	IV
Formulation/Vehicle:	(b) (4) mM sodium acetate, (b) (4) mM trehalose dihydrate, 0.02% (w/v) polysorbate 20, at pH 5.3,
Species/Strain:	Cynomolgus monkey, pregnant (3 to 9 years old)
Number/Sex/Group:	12/group
Deviation from study protocol:	None significant

Study design: Mating was conducted by cohabitation for 3 days; the middle day was designated as gestation day 0 (GD0). Pregnancy was confirmed by ultrasound between GD16 and GD19. Cesarean section was conducted between GD100 and 103.

Animal Assignment

Order of pregnancy	Group	Animal number	Order of pregnancy	Group	Animal number
1	1	101	19	1	107
2	2	201	20	2	207
3	3	301	21	3	307
4	1	102	22	1	108
5	2	202	23	2	208
6	3	302	24	3	308
7	1	103	25	1	109
8	2	203	26	2	209
9	3	303	27	3	309
10	1	104	28	1	110
11	2	204	29	2	210
12	3	304	30	3	310
13	1	105	31	1	111
14	2	205	32	2	211
15	3	305	33	3	311
16	1	106	34	1	112
17	2	206	35	2	212
18	3	306	36	3	312

Group Assignments

Group	Test or Control Article	Dose Level (mg/kg)		Dose Concentration (mg/mL)		Dose Volume (mL/kg)	Number of Pregnant Animals (Animal Number)
		LD [a]	SD [b]	LD [a]	SD [b]		
1	PRO70769 Vehicle	0	0	0	0	5	12 (101 – 112)
2	PRO70769	15	20	3	4	5	12 (201 – 212)
3	PRO70769	75	100	15	20	5	12 (301 – 312)

Note: Total dose volume (mL) was calculated based on the most recent body weight. Dose volumes was rounded up to the next highest readable syringe increment.
[a] Loading dose (LD): daily intravenous dose on Days 20, 21, and 22 of gestation
[b] Study dose (SD): weekly intravenous dose on Gays 29, 36, 43, and 50 of gestation

Observations and Results

Mortality: (Data were collected twice daily.) No unscheduled deaths occurred.

Clinical Signs:

Dams: (Observations were recorded twice daily from GD20 through the day of Cesarean section. On dosing days, observations were also conducted approximately 1-2 hours post-dose.)

No adverse clinical signs attributable to ocrelizumab were observed.

Pregnancy monitoring: Embryofetal viability was monitored by ultrasound or fetal doppler under sedation on GD25, 30, 37, 44, 51, 60, 70, 80, and 90.

- No effects related to ocrelizumab on maintenance of pregnancy were reported. Two of 12 control dams aborted, as confirmed by ultrasound, as well as one of 12 LD dams.
- One fetal death occurred in the HD group and was found at Cesarean section. The fetus was alive on GD90. The frequency of fetal death and abortion for each group was 16.7% for control, 8.3% for the LD group, and 8.3% for the HD group.

Body Weight: (Weights of dams were recorded on GD1, 19, 26, 33, 40, 47, 54, 61, 68, 75, 82, 89, and 100.)

- No effect on body weight attributable to ocrelizumab was observed.

Food Consumption: (Food consumption of each animal was estimated daily from GD20 until the day prior to Cesarean section.)

- No effect on food consumption related to ocrelizumab was observed.

Hematology (Dams and fetuses): Samples were collected from all dams according to the following schedule: once between GD18 and 20 (pre-dose), on GD51 (24 hours after GD50 dose), on GD75, and on the day of Cesarean section. Hematology testing was also conducted on animals that aborted, as summarized below.

Dose	Animal No.	Study Day
0 mg/kg	101	26
	110	26
15 / 20 mg/kg	211	45

On the day following the confirmation of abortion

Dams:

Total leukocyte, neutrophil and lymphocyte counts decreased slightly in all groups, including control, as pregnancies progressed. The magnitude of decrease was greater in the LD and HD groups relative to control, from initiation of dosing to GD51. By GD75, the values were similar among groups.

Changes in total leukocyte, neutrophil, and lymphocyte counts in dams

	Pre-dose ($x10^3/\mu L$)	GD51 ($x10^3/\mu L$)	GD75 ($x10^3/\mu L$)	% to pre-dose		% to the control	
				GD51	GD75	GD51	GD75
Parameters	**Total leukocyte count**						
0 mg/kg	12.101	11.328	9.453	94	78		
15/20 mg/kg	12.533	10.482	10.745	84	86	93	114
75/100 mg/kg	12.466	9.865	9.988	79	80	87	106
Parameters	**Neutrophil count**						
0 mg/kg	5.528	5.535	4.642	100	84		
15/20 mg/kg	4.995	4.145	4.517	83	90	75	97
75/100 mg/kg	5.418	4.441	4.488	82	83	80	97
Parameters	**Lymphocyte count**						
0 mg/kg	5.710	4.993	4.000	87	70		
15/20 mg/kg	6.757	5.413	5.289	80	78	108	132
75/100 mg/kg	6.337	4.518	4.634	71	73	90	116

Fetuses: (Samples were collected from all fetuses at Cesarean section.)

- No effects on hematology parameters related to ocrelizumab were observed.

Clinical chemistry (Dams and fetuses): (Samples were collected on the same schedule as for hematology.)

- No effects on clinical chemistry related to ocrelizumab were observed.

FACS analysis (immunophenotyping): Samples were collected on the same schedule as for hematology.

Antibody panel

Tube No.	Antigen Marker (Fluorochrome)	Cell Types Identified
1	CD3-FITC (20 μL), mIgG1-PE (20 μL), CD40-APC (20 μL)	T- and B-lymphocytes
2	CD3-FITC (20 μL), CD4-PE (20 μL) and CD8-APC (20 μL)	T-lymphocyte subsets ($CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$ cells)
3	CD3-FITC (20 μL), CD16-PE (20 μL), CD8-APC (20 μL)	NK-cells ($CD3^-CD16^+$ cells)
4	CD14-FITC (20 μL), CD45-PE (20 μL), CD40-APC (20 μL)	Lymphocyte gate purity

A total of 20000 events in the lymphocyte gate were collected for each tube.

- Marked decreases in B cells (CD3- CD40+) were observed in all dams in ocrelizumab dose groups on GD51. B cells remained low through the day of Cesarean section.

- In fetal blood, control group offspring had B cell levels similar to the dams (15.85%, 0.45 X 10^3/µL). B cell levels in LD and HD fetuses were 1.21 to 2.33% and0.03 to 0.07 x $10^{3/}$µL, respectively.
- Absolute T cell counts in dams remained similar to baseline in both dose groups.
- No effects related to ocrelizumab on T cell counts were observed in fetuses from either dose group.
- No effects on NK cells related to ocrelizumab were observed in either dams or fetuses.

Toxicokinetics (dams, fetuses, amniotic fluid): Samples were collected on GD20, 22, 24, 26, 29, 36, 43, 50, 52, 54, 57, 70, 80, and 100. Samples were also collected from dams that aborted. Fetal blood and amniotic fluid samples were collected at Cesarean section.

Table 7 PK Parameters (mean ± SD) of Ocrelizumab in Pregnant Cynomolgus Monkeys (Study 04-1272-1342)

Group (LD/SDL [mg/kg])	No./Sex	C_{max} (µg/mL)	AUC_{last} (µg • day/mL)	$t_{1/2, \lambda}$ (day)	AUC_{inf} (µg • day/mL)	CL_{ss} (mL/day/kg)	V_{ss} (mL/kg)
2 (15/20)	12/F	1310 ± 356	28900 ± 7720	8.93 ± 1.41	29100 ± 7830	6.08 ± 2.10	82.7 ± 48.2
3 (75/100)	12/F	5860 ± 777	136000 ± 19100	9.97 ± 1.35	137000 ± 19600	5.58 ± 0.794	64.8 ± 7.72

AUC_{inf} = area under the concentration–time curve from Time = 0 extrapolated to infinity; AUC_{20-102} = area under the concentration–time curve from GD 20 to 102; CL_{ss} = clearance at steady state; C_{max} = maximum serum concentration; GD = gestation day; IV = intravenous; LD = loading dose; SDL = study dose level; $t_{1/2, \lambda}$ = terminal half-life; V_{ss} = volume of distribution at steady state.

Note: n = 12 animals/group; LD was given by IV on GD 20, 21, and 22; SDL was given by IV once weekly for four doses starting on GD 29.

Ocrelizumab levels were generally greater in dams than in fetuses. The fetal to maternal serum concentration ratio was 1.29 ± 1.41 (n=7) and 0.605 ± 0.106 (n=7) for the LD and HD groups, respectively. Amniotic fluid levels were low but detectable in all animals. For the LD and HD groups, the mean ocrelizumab amniotic fluid to maternal serum ratio was 0.0658 ± 0.0247 (n=7) and 0.0421 ± 0.0226 (n=7), respectively.

Immunogenicity: (Blood samples and amniotic fluid were sampled for ADAs. Samples were collected once between GD18 and 20 (pre-dose), on GD50 (pre-dose), and on the day of Cesarean section. Samples were also collected from dams that aborted and from fetuses and amniotic fluid at Cesarean section.)

- ADAs were not detectable in fetal serum.
- One LD dam tested positive for ADAs but exposure was not affected. One control animal also tested positive for ADAs, possibly an assay artifact.
- ADAs were not detected in serum or amniotic fluid of HD dams.

Dosing Solution Analysis: Samples were collected from the first and last dosing solutions (loading and study doses) for each dose group and analyzed for concentration.

- All dosing formulations met the pre-established acceptance criterion for concentration.

Necropsy: No necropsies were conducted because there were no maternal deaths or dams euthanized moribund. After Cesarean section, all dams were returned to the (b) (4) stock colony.

Fetal findings (reviewer table):

Dose Group	# Live fetuses	Males	females	# Dead fetuses	Fetal Weight
Control	10	6	4	0	119.3
LD	11	8	3	0	116.5
HD	12	7	5	1	115.8

- No effects on external, visceral, or skeletal examinations related to ocrelizumab were observed.
- No effects related to ocrelizumab on placental dimensions, amniotic fluid volumes, or umbilical cord length were observed.

Fetal organ weights and histopathology:

Organs weighed and preserved

Adrenal glands*	Kidneys*	Mesenteric lymph nodes	Spleen
Brain	Liver	Ovaries* / Testes*	Thymus
Heart	Lungs	Pancreas	Uterus

*: Paired organs were weighed separately, and the combined weight was calculated.

Organ weights:

Organs preserved without weighing

Bone marrow (left femur and sternum)	Large intestines	Mandibular lymph node
Ears (auricles)	Mainstem bronchi including bronchial-associated lymphoid tissue	The skin of the head
Epididymides	Small intestines	Tongue
Esophagus	Stomach	Trachea (with thyroids)
Eyes		

- No ocrelizumab-related effects on fetal organ weights were observed.

Histopathology:

- In fetal spleen, reduced white pulp area (graded slight or very slight) was observed in both LD and HD groups.
- Immunohistochemistry conduced on spleen mandibular and mesenteric lymph nodes from fetuses showed a reduced number of B cell and B cell follicles. The

finding was observed in both the LD and HD groups and was dose-related in incidence.

Conclusions:
The NOAwas the HD of 100 mg/kg. No significant fetal deformities or effects on pregnancy parameters related to ocrelizumab were observed. The finding of reduced B cell populations in the fetuses is due to the known pharmacological activity of ocrelizumab and demonstrates that the developing fetus was exposed to the test article.

9.3 Prenatal and Postnatal Development

Study title: An assessment of the effects of ocrelizumab (rhuMAb 2H7) on pre- and postnatal development when administered weekly by intravenous injection to pregnant cynomolgus monkeys

Study no.:	**06-1260**
Testing Facility:	(b) (4)
Date of study initiation:	3/9/2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Ocrelizumab, L01653, 99%

Methods

Doses:	0, 20, or 100 mg/kg (loading doses: 15 or 75 mg/kg)
Frequency of dosing:	Weekly after 3 loading doses on GD20, 21, and 22.
Dose volume:	5 mL/kg
Route of administration:	IV
Formulation/Vehicle:	(b) (4) mM sodium acetate, (b) (4) mM trehalose dihydrate, 0.02% (w/v) polysorbate 20, at pH 5.3
Species/Strain:	Cynomolgus monkey, pregnant, 4.4 to 9.3 years old, 2.35 to 5.82 kg
Number/Sex/Group:	15/group
Deviation from study protocol:	None significant

Study design:
Dams were dosed from GD20 through LD28. Study design is summarized in the tables below.

Study design

Group	Test or Control Article (vehicle)	Dose Level (mg/kg)		Dose Concentration (mg/mL)		Dose Volume (mL/kg)	Number of Pregnant Animals[c]	Number of Neonates[d]
		PD[a]	SD[b]	PD[a]	SD[b]			
1	Vehicle	0	0	0	0	5	15	13
2	Ocrelizumab	15	20	3	4	5	15	13
3	Ocrelizumab	75	100	15	20	5	15	11

Note: Total dose volume (mL) was calculated based on the most recent body weight. Dose volumes were incremented as needed to the next highest readable syringe graduation.
[a] Priming dose (PD): daily IV dose on GD20, 21, and 22
[b] Study dose (SD): weekly IV dose starting on GD29 and continued to at least LD28 (5 doses during the lactation period)
[c] Number of maternal animals at the initiation of dosing (GD20)
[d] Number of born neonate at DB0 including stillbirth (i.e., excluding abortion and embryo-fetal deaths, maternal death during gestation)

Number of doses for each maternal animal

Group 1 (0 mg/kg)		Group 2 (15/20 mg/kg)		Group 3 (75/100 mg/kg)	
SSAN	Number of Doses (Last Dose Day)	SSAN	Number of Doses (Last Dose Day)	SSAN	Number of Doses (Last Dose Day)
101	30 (LD32)	201	23 (GD162)	301	28 (LD31)
102	27 (LD29)	202	28 (LD34)	302	28 (LD33)
103	28 (LD34)	203	23 (GD162)	303	21 (LD2)
104	27 (LD31)	204	28 (LD30)	304	27 (LD30)
105	27 (LD28)	205	6 (GD43)	305	3 (GD22)
106	28 (LD34)	206	27 (LD32)	306	28 (LD31)
107	3 (GD22)	207	27 (LD29)	307	27 (LD30)
108	31 (LD29)	208	28 (LD29)	308	22 (GD155)
109	29 (LD34)	209	28 (LD31)	309	25 (LD29)
110	27 (LD28)	210	24 (GD169)	310	4 (GD29)
111	27 (LD29)	211	28 (LD30)	311	15 (GD106)
112	27 (LD29)	212	26 (LD33)	312	26 (LD32)
113	27 (LD31)	213	28 (LD29)	313	28 (LD34)
114	4 (GD29)	214	28 (LD33)	314	17 (GD120)
115	27 (LD29)	215	29 (LD34)	315	27 (LD28)

Note: number of doses includes both priming and study doses.

Observations and Results

F_0 Dams

Survival: Observations made twice daily.
One dam from each group was euthanized early in moribund condition. Control #108 was euthanized on LD128 in poor condition, but no cause of death was determined. LD #205 was euthanized on GD44, the day after the 3rd dose. The fetus was alive at the time of the maternal euthanasia and was also euthanized. The cause of maternal death was not definitively established, but high ADAs on the day prior to euthanasia and clinical pathology results on GD44 suggest, according to the sponsor, a possible adverse immune reaction to ocrelizumab.

HD #311 was euthanized on GD111. The animal began showing signs of illness on GD109 including hunched posture and reduced activity. Reduced food consumption was observed from GD20 to 60 and again beginning on GD107. Body weight during the week prior to necropsy was reduced from 4.94 kg to 4.47 kg. Clinical pathology showed severely reduced RBC, HGB, and HCT without increased RET. No cause of death was determined after necropsy and histopathology. The fetus was alive at the time of maternal euthanasia, with no external, visceral, or placental abnormalities. The fetus was euthanized.

Since early maternal deaths occurred in each group, they were not considered related to ocrelizumab.

Clinical signs: Data were collected twice daily from GD20 through the day of necropsy of the neonate.

- No adverse clinical signs related to ocrelizumab were observed in the dams during gestation or lactation periods.

Body weight: Data were collected on GD1 and 19, then every 6 to 8 days until delivery, once between LD1 and 4, and weekly thereafter.

Food consumption: (Estimated twice daily for each maternal animal from GD20 to LD43)

- No effects on food consumption attributable to ocrelizumab were observed.

Dosing Solution Analysis: All formulation samples were determined to be within the pre-established acceptance criteria for concentration. Ocrelizumab was not detected in vehicle samples.

Pregnancy monitoring: (Embryofetal viability was monitored by ultrasound under sedation on GD25, 30, 37, 44, 51, 60, 780, 80, 90, 100, then every 9-11 days until delivery.)

- Fetal loss is summarized in the table below. Based on the data from this study the incidence of embryofetal loss in the HD

group appears to be increased relative to the control and LD groups.

Summary of abortion and embryo-fetal loss

Dose (mg/kg)	Initial Number of Pregnant Animals	Number of Embryo-Fetal Loss	SSAN and GD	Embryo-Fetal Loss Ratio
0/0	15	2	SSAN 107 / GD25 (EL)	13.3%
			SSAN 114 / GD30 (EL)	
15/20	15	1	SSAN 210 / GD171 (FD)	6.7%
75/100	15	3	SSAN 305 / GD25 (EL)	20.0%
			SSAN 310 / GD30 (EL), reconfirmed on GD32	
			SSAN 314 / GD121 (aborted)	

EL: embryo loss (*in utero* embryo death or resorption [no evidence of expulsion])
FD: *in-utero* fetal death (no fetal heartbeat)

- Gestation length: Mean gestation length was 165.4 days for control (range of 159 to 189 days). Three births in the HD group (N#303: 146 days, N #309: 147 days, and N #312: 151 days) were considered pre-term (defined in the published literature as birth between GD141 and 155). (The sponsor stated that the gestational lengths of these 3 were within published "normal" gestation lengths of 132 to 189 days.) However, no pre-term births were observed in any other group.

Stillbirths: Stillbirths occurred in 2 LD animals and one HD animal. The sponsor concluded that, due to the lack of an increase in incidence with dose, the stillbirths were not related to the test article. However, no stillbirths occurred in the control group. A relationship to the test article cannot be ruled out since all stillbirths occurred only in dams that were dosed with ocrelizumab.

Summary of stillbirth

Dose (mg/kg)	Number of maternal Animals*	Number of Stillbirth	SSAN and GD	Stillbirth Ratio
0/0	13	0	Not applicable	0.0%
15/20	13	2	SSAN 201 / GD167	15.4%
			SSAN 203 / GD168	
75/100	11	1	SSAN 308 / GD161	9.1%

* Maternal animals having embryo/fetal loss or had unscheduled necropsy prior to parturition were excluded.

Birth observations: No observations were considered related to ocrelizumab.

F_1 Generation

Survival: As described above, six embryofetal deaths occurred during the gestation period. Two HD neonates died early:

- 303N: Born preterm on GD146 with low birth weight (271g) and was removed from the mother on PND3 due to poor condition and hand-raised until it was found dead on PND 6. Histopathology showed signs of inflammation and infection as well as decreased cellularity of lymphoid tissues. Cause of death was opportunistic infection, weakness due to immaturity complicated by B-lymphocyte depletion.
- 315N: Born on GD162 with normal birth weight (379g). No adverse signs were reported until PND 136. Signs prior to euthanasia on PND138 included tremor and reduced activity/lethargy. Histopathology showed severe infection of the cerebellum and surrounding meninges, pleural fibrosis, interstitial mononuclear infiltration of the lung, and alveolar hemorrhage. Decreased lymphoid follicle cellularity of the spleen was also observed. Cause of death was meningoencephalitis involving the cerebellum and meninges. The response to the infection was most likely complicated by reduced B-lymphocytes due to exposure to ocrelizumab.

Clinical signs: (Observations were recorded twice daily beginning on PND1.) Other than the signs described above that led to early death and euthanasia of 2 neonates, no clinical signs related to ocrelizumab were observed.

Body weight: (All neonates were weighed between PND1 and 4, then weekly thereafter.)
A dose-related effect of reduced birth weight was observed as summarized in the table below. Body weight gain after birth was similar among groups.

Summary body weight of neonates

Dose (mg/kg)		Body Weight (g)				Body Weight Gain (%)			
		Birth	Week 4	Week 13	Week 26	Birth to Week 4	Week 4 to 13	Week 13 to 26	Birth to Week 26
0/0	Mean	372.9	477.3	719.2	1011.0	28.0	50.7	40.6	171.1
15/20	Mean	352.1	438.6	686.1	997.2	24.6	56.4	45.3	183.2
	% to G1	94.4	91.9	95.4	98.6	87.9	111.2	111.6	107.1
75/100	Mean	330.2	422.1	658.8	956.2	27.8	56.1	45.1	189.6
	% to G1	88.5	88.4	91.6	94.6	99.3	110.7	111.1	110.8

Birth: DB1 – 4, Week 4: DB27 – 30, Week 13: DB90, Week 26: DB180
% to G1: Percentage relative to the mean value of the control group

Physical (Measurements of head width and circumference, inter-eye distance,

development: crown-rump length, tail length, chest circumference, paw and foot length, and ano-genital distance were recorded once between PND7 and 10, PND25 and 30, and on PND90. Similar measurements were also recorded for the single stillborn neonate.)

- No effects attributable to ocrelizumab on growth were observed.

Neurological assessment: (Pupil reflex, Preyer reflex, pain response, and grip strength were evaluated in all neonates once between PND25 and 30, and on PND90)

- No consistent adverse effects on neurologic development related to ocrelizumab were observed.

Reproduction: Not assessed.

Hand-raised neonates:

- 102N (control): Hand-raised on PND0 only.
- 108N (control): Hand-raised from PND0, rejected by the dam.
- 202N (LD): Hand-raised from PND131. Mother developed staph infection in the breast during lactation. *Staphylococcus* was detected in the milk of this dam.
- 303N (HD): Hand-raised from PND3, died on PND6. Rejected by the dam
- 315N (HD): Hand-raised from PND98 (euthanized on PND138) after dam developed infection of the right breast. *Staphylococcus* was detected in the milk of this dam.

Blood samples were collected from all dams and offspring for clinical pathology, toxicokinetics, immunogenicity, and immunophenotyping according to the schedule below. Milk samples were collected from all dams.

APPEARS THIS WAY ON ORIGINAL

Sample collection schedule (maternal animals)

GD or LD	H/Co/Ch/F*	TK	ATA
Blood			
Once between GD18 – 20	1X (pre-dose)	Pre-dose and 0.25 hours postdose on GD20	1X (pre-dose)
GD22	-	Pre-dose and 0.25 hours postdose	-
GD24	-	1X	-
GD26	-	1X	-
GD29	-	Pre-dose and 0.25 hours postdose	-
GD41 – 43	1X (pre-dose)	Pre-dose and 0.25 hours postdose	1X (pre-dose)
GD71	-	Pre-dose and 0.25 hours postdose	1X (pre-dose)
GD83 – 85	1X (pre-dose)	-	-
GD125 – 127	1X (pre-dose)	-	-
GD139 – 141	1X (pre-dose)	-	-
Last LD dose (LD28 – 34)	1X (pre-dose)	Pre-dose and 0.25 hours postdose	1X (pre-dose)
LD60	1X	-	1X
LD90	1X	1X	1X
LD180	1X	1X	1X

* Data from recollection samples (e.g., due to insufficient volume or clotted sample) were included in the scheduled time point.
-: Not applicable

APPEARS THIS WAY ON ORIGINAL

Sample collection schedule (maternal animals, continued)

GD or LD	H/Co/Ch/F*	TK	ATA
Breast Milk			
Once between LD25 – 28	-	1X	1X

* Data from recollection samples (e.g., due to insufficient volume or clotted sample) were included in the scheduled time point.
-: Not applicable

Sample collection schedule (neonates)

DB	H/Ch/F*	Co	TK	ATA	KLH/TT
Maternal last LD dose (DB28 – 34)	-	-	1X	1X	-
28 – 37	1X	-	-	-	-
60 – 64	1X	-	-	1X	-
90	1X	1X	1X	1X	1X (pre-KLH/TT)
97	-	-	-	-	1X
104	-	-	-	-	1X
111	-	-	-	-	1X
118	-	-	-	-	1X
125	-	-	-	-	1X
180 – 187	1X	1X	1X	1X	1X (pre-KLH/TT)
187	-	-	-	-	1X
194	-	-	-	-	1X
201	-	-	-	-	1X
208	-	-	-	-	1X
Pre-necropsy	-	-	-	-	1X

* Data from recollection samples (e.g., due to insufficient volume or clotted sample) were included in the scheduled time point.
-: Not applicable

Clinical pathology:

Hematology:

- Dams showed dose-related reduction of WBCs in the LD and HD groups (-21% and -30% on GD43, respectively) relative to control, as expected. The dose-related reduction continued through the dosing period. The reduction in WBCs was accounted for by reduction in total lymphocytes (25% and 29% for the LD and HD groups, respectively, relative to control).
- A trend toward reduction in WBCs and total lymphocytes was also observed in neonates in both the LD and HD groups indicating exposure to ocrelizumab. However, the reduction was less consistent for the neonates than for the dams and was apparently of greater magnitude in the LD group at most time points. (The greatest magnitude of reduction in

lymphocytes in neonates was approximately 31%, relative to control for the LD group on PND60.) Results in neonates were highly variable.

Clinical Chemistry: A trend toward reduced globulin levels was observed in both dams and neonates, which persisted through the study. This finding was considered a result of reduced B lymphocyte population.

Coagulation: No effects on coagulation attributable to ocrelizumab were observed in in either dams or neonates

Toxicokinetics:

Summary of the mean toxicokinetic parameters for ocrelizumab in pregnant cynomolgus monkey serum: GD22

Dose Level (mg/kg)		C_{max} (ng/mL)	Dose Normalized C_{max}	T_{max} (day)	AUC_{22-29} (ng•day/mL)	Dose Normalized AUC_{22-29}	C_T (ng/mL)
15/20	Mean	959000	63900	22.0104	3970000	265000	521000
	SD	113000	NA	0	350000	NA	56400
	N	15	NA	15	15	NA	15
75/100	Mean	4490000	59900	22.1	17700000	236000	2790000
	SD	714000	NA	0.514	3100000	NA	454000
	N	15	NA	15	14	NA	15

NA = Not applicable
Dose Normalized C_{max} = (ng/mL)/(mg/kg)
Dose Normalized AUC_{22-29} = (ng•day/mL)/(mg/kg)

Dose proportionality ratios of ocrelizumab in pregnant cynomolgus monkey serum

Interval	Dose Level Increase	C_{max} Ratio	AUC_{22-29} Ratio
GD22	1.0 : 5.0-fold	1.0 :4.7-fold	1.0 : 4.5-fold

Immunogenicity:
ADAs were detected in one of 30 dams and one of 20 neonates, both in the LD group. The ADA-positive neonate was not from the ADA-positive dam. Ocrelizumab exposure was not limited by ADA development in the dam.

Immunophenotyping:

- Dams: Near complete depletion of B-lymphocytes (CD3-CD40+) was observed, which was maintained until the end of the study.
- Neonates: Near complete depletion of CD-CD40+ lymphocytes was observed in the LD and HD group on PND28 and remained near 0 through PND90. By PND180, the CD3-CD40+ (B cells) subset levels were similar to control levels.
- No effects on T cell populations or NK cells were observed in dams or neonates.

Breast milk analysis: The table below, from the study report provides a summary of the ocrelizumab levels detected in milk.

Ocrelizumab concentration in cynomolgus monkey breast milk (LD25 – 28)

Dose Level (mg/kg)	Number of Samples Analyzed	Number of Samples with Measurable Ocrelizumab	Mean Concentration Range (ng/mL)
15/20	11	8	428 – 1020
75/100	9	9	1200 – 3970

Necropsy: (Offspring)

Day of scheduled necropsy of neonates

Group 1 (0/0 mg/kg)		Group 2 (15/20 mg/kg)		Group 3 (75/100 mg/kg)	
SSAN	Day (DB)	SSAN	Day (DB)	SSAN	Day (DB)
101N	217	202N	215	301N	221
102N	222	204N	217	302N	220
103N	215	206N	220	304N	222
104N	215	207N	218	306N	216
105N	219	208N	218	307N	219
106N	216	209N	216	309N	220
108N	215	211N	217	312N	218
109N	215	212N	220	313N	217
110N	217	213N	221		
111N	218	214N	216		
112N	217	215N	216		
113N	216				
115N	218				

N=neonate

List of animals with an unscheduled necropsy

Group (Dose)	SSAN	Day of Necropsy	Reason for Unscheduled Necropsy
Maternal Animals			
1 (0/0 mg/kg)	108	LD128	Moribund
2 (15/20 mg/kg)	205	GD44	Moribund
3 (75/100 mg/kg)	311	GD111	Moribund

List of animals with an unscheduled necropsy (continued)

Group (Dose)	SSAN	Day of Necropsy	Reason for Unscheduled Necropsy
Neonates			
2 (15/20 mg/kg)	201N	DB0 (GD167)	Stillbirth
	203N	DB0 (GD168)	Stillbirth
3 (75/100 mg/kg)	303N	DB6	Found dead
	308N	DB0 (GD161)	Stillbirth
	315N	DB138	Moribund

Organ weights: (Neonates and dams at unscheduled necropsy)

Organs weighed

Adrenals	Pituitary
Brain (cerebrum, cerebellum and brain stem)	Prostate/Seminal vesicles
Epididymides	Spleen
Heart	Submandibular salivary glands
Kidneys	Thyroids (including parathyroids)
Liver	Testes
Lungs (including bronchi)	Thymus
Ovaries	Uterus (body and cervix)

Note: Paired organs were examined grossly and weighed together.

Histopathology/immunohistochemistry (lymphoid tissue samples were immunostained for CD3 and CD20 antigen):
Peer review was conducted.

Tissues collected for histopathology

Adrenals*	Optic nerves*
Aorta (thoracic)	Ovaries*
Bone, sternum (with marrow)	Pancreas
Brain	Pituitary
Cecum	Prostate
Colon	Rectum
Duodenum	Sciatic nerves*
Epididymides*	Seminal vesicles*
Esophagus (thoracic)	Skeletal muscle (quadriceps femoris)
Eyes*	Skin (mammary)
Gallbladder	Spinal cord (thoracic)
Gross lesions	Spleen
Heart	Stomach
Ileum (with Payer's patches if possible)	Salivary glands*
Injection site(s), maternal animals only	Testes*
Jejunum	Thymus
Joints (knee, includes proximal tibia, distal femur [with marrow], articular surface and growth plate [epiphysis])*	Thyroid, both lobes (with parathyroids if possible)
Kidneys*	Tonsil
Lacrimal glands	Tongue
Liver	Trachea
Lung (with bronchi)	Urinary bladder
Lymph node (axillary*,inguinal*, mandibular*, mesenteric)	Uterus
Mammary gland	Vagina

* Paired organs

Histopathology Findings: (Neonates)

- Kidney:
  - Dose-related incidence of glomerulopathy (characterized as a "spectrum of glomerular changes ranging from small immature glomeruli with concentric fibrosis of Bowman's capsule to severely contracted sclerotic glomeruli"

- Incidence of glomerulopathy: 0 in control group, LD group: 3 of 13 neonates, HD group: 4 of 11 neonates.
- Lymphoblastic inflammation: Infiltrating cells formed "nodular aggregates" in the kidney interstitium. Incidence of this finding was 2 of 11 neonates in the HD group.

Kidney using PAS staining: PAS staining demonstrated that immaturity was "Rarely" present in both the LD and HD groups. Global glomerulosclerosis was observed "rarely" in both the LD and HD groups. Sclerotic changes cannot be explained by glomerular infiltrates since there were no visible lesions in arterioles or arteries. No tubulitis was observed and no dysplastic features of cells or destructive vascular injuries to explain the interstitial cellular infiltrates. The sponsor concluded that no specific mechanism could be identified for the kidney changes, but there is no evidence for immune complex proliferative or necrotizing glomerulosclerosis. One mechanism suggested by the sponsor is "possible ischemic injury to developing glomeruli."

- Bone marrow: Lymphoid follicle formation was observed in the sternal bone marrow. Incidence was dose-related: 4 of 13 in the LD group, 5 of 11 in the HD group.

Immunohistochemistry for CD3 (required for T-cell activation) and CD20:

- Ocrelizumab-related changes in CD3+ and CD20+ cell populations were observed in femoral bone marrow (described as lymphoid follicle formation). CD20+ population changes were observed in 1 of 13 neonates in the control group, 7 of 11 LD neonates, and 8 of 8 HD neonates. CD3+ aggregates were observed in 0 control neonates, 2 of 10 LD neonates, and 3 of 6 HD neonates.

Conclusion: No NOAEL was identified for the kidney pathology observed in neonates. . Detectable levels of ocrelizumab were identified in milk. There appears to be a dose-related increase in incidence of stillbirth and pre-term birth.

10 Special Toxicology Studies

Study #03-0509-0349

Title: Rituximab and PRO70769 (rhuMAb2H7) stability in PBS and human blood, serum and plasma over 72 hours

Findings: Neither rituxumab nor rhuMAb 2H7 show detectable aggregation or degradation during a period of 72 hours in PBS and human blood, serum, and plasma at 37°C.

Study # 03-0236-0349

Title: Hemolytic potential and blood compatibility of rhuMAb 2H7

Summary: The purpose of this study was to assess the hemolytic potential of ocrelizumab in vehicle solution for human and cynomolgus monkey whole blood. Neither ocrelizumab at concentrations of 1.0, 5.0, or 10.0 mg/mL nor the rhuMAb 2H7 vehicle control caused hemolysis when mixed with an equal volume of human or cynomolgus monkey whole blood. Neither ocrelizumab at concentrations of 1.0, 5.0, or 10.0 mg/mL nor the ocrelizumab control caused precipitation or coagulation when mixed with equal volumes of human or cynomolgus monkey serum or plasma.

11 Integrated Summary and Safety Evaluation

Ocrelizumab is a humanized IgG1 monoclonal antibody that selectively targets the CD20 antigen expressed on B lymphocytes and is under development for treatment of multiple sclerosis ((b) (4) and PPMS). CD20 is expressed on the surface of pre-B cells, mature B cells and memory cells but is not found on lymphoid stem cells or plasma cells. (Recent published data indicate that CD20 may also be expressed on a small population of CD4+ and CD8+ T lymphocytes in humans.) Binding of ocrelizumab to CD20 results in rapid depletion of CD20+ B cells but does not affect the capacity for B cell reconstitution.

The pharmacology of ocrelizumab was investigated in a series of *in vitro* and *in vivo* studies. Because ocrelizumab binds only to human and non-human primate CD20, *in vitro* studies were conducted using human-derived cell lines and *in vivo* studies were conducted in cynomolgus monkey.

Primary pharmacology of ocrelizumab was investigated in cynomolgus monkeys using IV bolus doses at levels ranging from 0.2 mg/kg to 10 mg/kg. Results demonstrated that circulating B lymphocytes were reduced to near undetectable levels by 1 hour post-dose. No effects on T cell or NK cell phenotypes were observed in these early, non-GLP studies.

The mechanism through which ocrelizumab acts to deplete CD20+ B cells is believed to be via antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), and/or apoptosis. Each mechanism was investigated *in vitro*:

1.) Affinity of ocrelizumab for CD20 was evaluated using a CD20-expressing human cell line. The results demonstrated that ocrelizumab bound CD20 with high affinity (k_d=0.84 ± 0.37 nM).

2.) Binding of ocrelizumab to a series of Fcγ receptor subtypes was evaluated. Results demonstrated that ocrelizumab binds to 5 sub-types of the Fc gamma receptor with varying EC_{50} values in the low µg/mL, within a range similar to rituximab.

3.) Ocrelizumab binding to complement C1q was investigated with results indicating that ocrelizumab binds complement C1q with an EC_{50} value of approximately 0.70 µg/mL.

4.) Binding of ocrelizumab to the human FcRn was demonstrated with slightly lower potency than rituxumab. Binding to FcRn would allow transfer of ocrelizumab across epithelial membranes.

5.) Activity of ocrelizumab in the ADCC assay using either purified NK cells or human PBMCs as effector cells was investigated., Results indicated that ocrelizumab does mediate ADCC in the presence of purified NK cells or human PBMCs, with EC_{50} values of 5.2 ± 2.6 pM and 0.12 ± 0.08 nM, respectively.

6.) Activity of ocrelizumab in the CDC assay demonstrated that ocrelizumab mediates CDC activity, with an EC_{50} of 4.04 ± 1.87 nM.

7.) The activity of ocrelizumab in mediating apoptosis in cultured CD-20+ Ramos cells was demonstrated. The data indicate that ocrelizumab can mediate apoptosis in the presence of CD20 expressing cells. The *in vitro* data indicate that the activity of ocrelizumab in eliminating B cells *in vivo* could be via any of these processes.

Tissue cross-reactivity:
Tissue samples from 3 humans and 3 cynomolgus monkeys were stained using immunohistochemical methods. Staining results showed specific binding in organized lymphoid tissue including lymph nodes, spleen GI-associated lymphoid tissue, tonsil, and pancreas of both species with both concentrations of antibody. Scattered mononuclear cells were specifically stained in the monkey thymus but not human thymus. Diffuse specific staining in the lens of the eye was exhibited in both human and monkey. The specific positive staining was sufficiently similar between human and cynomolgus monkey tissues to confirm the cynomolgus monkey as a relevant species for nonclinical investigations.

Pharmacokinetics
Pharmacokinetic studies were conducted in monkey, rat, and mouse. Because ocrelizumab does not bind to rodent CD20, the studies in rat and mouse were not considered relevant. In monkeys, 2 doses were administered by IV bolus one week apart at dose levels of 0.2, 0.5 or 2.0 mg/kg. The results showed that the exposure increased in proportion with dose. Clearance was biphasic, with a rapid initial distribution phase followed by a slower elimination phase. The elimination phase was faster after the second dose, most likely due to development of anti-drug antibodies (ADAs). ADAs were detected in all animals by 2 weeks after the first dose. Results of TK analyses confirmed the immunogenicity of ocrelizumab at all doses levels, but ADAs were most evident at doses of 10 mg/kg or lower. Therefore, the lower doses were not used in toxicology studies. At the higher dose levels used in the toxicology studies ADAs were detected generally in all dose groups, but the immunogenicity did not appear to have a significant effect on exposure.

Toxicology:
A series of repeat-dose toxicology studies were conducted in cynomolgus monkeys in which various dosing regimens were used. In all studies, cynomolgus monkeys received ocrelizumab at doses of up to 100 mg/kg IV. No unexpected toxicities were observed. All studies showed reduced total lymphocytes in all dose groups, reflecting the rapid and near total depletion of B cells from blood and lymphoid tissues.

Histopathology typically showed follicular atrophy in spleen and lymph nodes. The atrophy of B cell follicles in lymph nodes was generally less severe than in spleen. Recovery of B cell populations was variable and dose related. Full recovery to baseline after repeat dosing required more than 6 months. In the longest duration study (Study #04-0192-0134), monkeys received IV doses every three weeks for 24 weeks (total of 8 total doses) at doses of 50 or 100 mg/kg followed by a 23-week recovery period. The results showed the expected effects of the pharmacological activity of ocrelizumab, including reduction in total lymphocytes for both dose groups that was not dose-related in magnitude. Histopathology demonstrated follicular atrophy in spleen and lymph nodes of both dose groups, dose-related in incidence and severity. Spleen appeared to be affected slightly more than the lymph nodes. At the end of the 23-week recovery period, spleen and lymph nodes appeared similar to control.

Immunophenotyping of whole blood, lymphoid tissues, and bone marrow using FACS analysis was conducted for each toxicology study. Marked depletion of B lymphocytes was consistently demonstrated in all compartments (tissue, blood and bone marrow). Recovery was variable, but at least partial recovery was demonstrated in all studies, depending on the length of the recovery period. In study #04-0192-0134 (discussed above), B cell populations recovered to 135.6%, 73.0%, 44.5%, and 103.2%, relative to control, in spleen, mandibular lymph node, inguinal lymph node, and bone marrow, respectively. No dose-related effects on T cell populations were observed in any study. In one study (#07-0171) the HD group showed 30% reduction in NK cells. This effect was not observed in other studies and no explanation for the effect was offered. Because it was observed in only a single study, the biological/toxicological significance is not clear.

TK at the higher doses used in the general toxicology studies showed that the $t_{1/2}$ was approximately 15 to 20 hours, which is similar to that observed in humans.

Reproductive toxicology:
A full battery of reproductive and developmental toxicology studies was conducted in cynomolgus monkey. In all studies, the pharmacological activity of ocrelizumab was confirmed by immunophenotyping by FACS analysis, and exposure was confirmed through toxicokinetic analysis. Fertility was assessed in separate studies in males and females. Males received 9 weekly IV doses (20 or 100 mg/kg preceded by initial loading doses of 15 or 75 mg/kg for 3 consecutive days). Females received weekly dosing over 3 menstrual cycles (including the initial loading doses). No adverse effects on fertility parameters were observed in either sex.

In an embryofetal development study, pregnant cynomolgus monkeys received IV doses of ocrelizumab with loading doses of 15 or 75 mg/kg for 3 days beginning on GD20, followed by weekly doses of 20 or 100 mg/kg through GD50. Cesarean section was conducted on GD100-103. One fetal death in the HD group was identified at Cesarean section. The frequency of fetal death and abortion was 16.7% for control, 8.3% for the LD group, and 8.3% for the HD group. Therefore, due to the lack of a dose-relationship, the death in the HD group appears to be unrelated to ocrelizumab.

No adverse effects on embryofetal development related to ocrelizumab were observed. However, in both the LD and HD groups, the fetuses showed results of the pharmacodynamic effect of ocrelizumab, indicating trans-placental exposure. Spleen and lymph nodes of fetuses from both dose groups showed reduced numbers of B cell follicles, dose-related in incidence. Reduced white pulp area was also observed in fetal spleen from both dose groups (graded slight or very slight).

In a pre-and postnatal development study, pregnant monkeys received IV doses of ocrelizumab (loading doses of 15 or 75 mg/kg for 3 days beginning on GD20, followed by weekly doses of 20 or 100 mg/kg through 28 days of lactation). Embryofetal viability was monitored with regular ultrasound evaluations. Results indicated that embryofetal loss was greater in the HD group (13.3% in control, 6.7% in LD, and 20.0% in the HD groups). The sponsor stated that the rate of embryofetal death was within the historical background rate at the testing facility. However, due to the apparent dose-related incidence, a relationship to ocrelizumab cannot be ruled out. Two stillbirths (of 13) occurred in the LD group and 1 (of 11) occurred in the HD group. Although not clearly dose-related, since none occurred in the control group, a relationship to ocrelizumab is difficult to rule out.

Three pre-term births (defined as birth GD141 to 155) occurred in the HD group compared to none in either the control or LD groups. In addition, the offspring of 2 HD dams died due to opportunistic infection which could have been related to ocrelizumab via the immune suppression induced by the B cell depletion. Immunophenotyping demonstrated that B cells were nearly completely depleted at PND28 and remained at near undetectable level through PND 90. By PND 180, B cell levels in offspring from both dose groups were similar to control. Ocrelizumab was detected in milk in both dose groups (range of 428-3970 ng/mL).

No ocrelizumab-related adverse postnatal developmental effects were observed. Physical developmental parameters and neurological parameters were monitored on a regular basis, but a definitive assessment of learning and memory was not conducted. Due to the length of postnatal development in the monkey, full neurobehavioral assessment cannot be conducted.

At necropsy, due to the fetal and neonatal deaths, the number of offspring available in each group was 13 in control, 11 in the LD and 9 in the HD groups. There were no visible developmental abnormalities (visceral or skeletal) in offspring from either dose group. However, histopathology demonstrated an increased incidence of glomerulopathy (graded minimal to mild) in the LD and HD group. The incidence of glomerular changes was 3 of 13 in the LD group and 4 of 11 (number includes the 2 neonatal deaths) in the HD group. Mild lymphoplasmacytic inflammation of the kidneys was also noted in 2 of 11 HD neonates. The mechanism for these findings was not identified, but the data suggest a dose-related injury to the developing kidney.

A significant concern regarding manufacturing was raised by the CMC team that could influence the validity of the nonclinical studies. Over the course of drug development,

significant changes were made in the ocrelizumab manufacturing process. According to the CMC team, the assays used to determine potency (ADCC and CDC) have shown significant variation among the processes (v0.1 – v0.4). At the time of this review, comparability (as determined by the CMC team) between the product intended for market, the product used in the pivotal clinical studies, and the product used for reproductive toxicology studies is in question. The sponsor should be asked to confirm that the product used in the pivotal clinical studies is comparable to the lots used in the reproductive toxicology studies and the product intended for market. If comparability cannot be demonstrated, some nonclinical studies may need to be repeated.

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