CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125486Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

GAZYVA (obinutuzumab)

Date: September 17, 2013
To: File for BLA 125486
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Gazyva conducted by Drs. Ricci, Del Valle, Emami and Simpson and secondary memorandum and labeling provided by Dr. Saber. I concur with Dr. Saber's conclusion that Gazyva may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
09/17/2013

MEMORANDUM

Date: September 17, 2013
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
BLA: 125486
Drug: Gazyva (obinutuzumab), Injection for intravenous infusion
Indications: In combination with chlorambucil, for the treatment of patients with previously untreated chronic lymphocytic leukemia (CLL)
Applicant: Genentech, Inc.

Obinutuzumab is a recombinant humanized monoclonal IgG1 antibody. Obinutuzumab binds to CD20 expressed on B lymphocytes and mediates B-cell lysis through mechanisms that include antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC). Pharmacology studies to demonstrate these activities were conducted using different types of lymphoma cells, e.g. diffuse large B-cell, mantle cell, and Burkitt's lymphomas. Obinutuzumab was engineered to have less fucose molecules compared to rituximab for a higher ADCC activity. Obinutuzumab binds to FcγRIIIa/b (CD16a/b) with higher affinity than rituximab; this may explain the increased ADCC activity of obinutuzumab compared to rituximab. The pharmacologic class assigned to obinutuzumab is "CD20-directed cytolytic monoclonal antibody" to be consistent with other products in the same class; i.e. Rituxan and Arzerra.

Pharmacology, safety pharmacology (combined with toxicology), pharmacokinetic, and toxicology studies were conducted in *in vitro* systems or in animal species. Genetic toxicology studies were not conducted or needed per ICH S6 guidance. Obinutuzumab does not bind to the target in rodents. Toxicology studies were conducted in the cynomolgus monkey, a pharmacologically relevant species, using the administration route and dosing regimens that adequately addressed safety concerns in humans. Obinutuzumab-related toxicities in animals included depletion of B lymphocytes and immunogenicity/ hypersensitivity reactions. Infection seen in some animals may be secondary to lymphocyte depletion and inflammation in multiple organs may be secondary to the immunogenicity. Anti-drug-antibody was formed in animals; however, this did not interfere with the study results as adequate exposure to obinutuzumab was obtained. There were no drug-related effects in male or female reproductive organs in general toxicology studies.

An enhanced pre- and post-natal development (ePPND) study was conducted in cynomolgus monkeys. Pregnant animals received weekly IV doses of obinutuzumab during the period of organogenesis and lactation and through postpartum Day 238. Obinutuzumab was not teratogenic in animals; however, B cells were depleted in the

offspring. The B-cell counts returned to normal levels within 6 months of birth Pregnancy category C is recommended for Gazyva and is consistent with the labels for Rituxan and Arzerra, CD20-directed antibodies with similar findings in the reproductive toxicology studies.

The nonclinical studies needed to support product labeling were reviewed by Drs. Stacey Ricci, Pedro Del Valle, Armaghan Emami, and Natalie Simpson. The nonclinical findings are summarized in the "Executive Summary" of the BLA review and reflected in the product label.

Recommendation: I concur with the pharmacology/toxicology reviewers that from a nonclinical perspective, Gazyva may be approved and that no additional nonclinical studies are needed to support approval of Gazyva for the proposed indication.

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/s/

HALEH SABER
09/17/2013

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:	BLA 125486
Supporting document/s:	Original Application
Applicant's letter date:	April 22, 2013
CDER stamp date:	April 25, 2013
Product:	Gazyva™ (Obinutuzumab)
Indication:	Patients with previously untreated chronic lymphocytic leukemia (administered in combination with chlorambucil)
Applicant:	Genentech, Inc.
Review Division:	Hematology and Oncology Toxicology on behalf of the Division of Hematology Products, Office of Oncology Products, Office of New Drugs
Reviewers:	M. Stacey Ricci, M.Eng., Sc.D. Pedro L. Del Valle, Ph.D. Armaghan Emami, Ph.D. Natalie E. Simpson, Ph.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John K. Leighton, Ph.D., D.A.B.T.
Project Manager:	Beatrice Kallungal

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125486 are owned by Genentech, Inc. or are data for which Genentech, Inc. has obtained a written right of reference. Any information or data necessary for approval of BLA 125486 that Genentech, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 125486.

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1 Executive Summary

1.1 Introduction

Obinutuzumab[1] (Gazyva™) is a recombinant humanized anti-CD20 monoclonal IgG1 antibody (mAb) being developed for the treatment of various hematological malignancies including, chronic lymphocytic leukemia (CLL). This BLA is seeking approval for use of obinutuzumab in combination with chlorambucil for previously untreated patients with CLL.

Obinutuzumab binds to the extracellular loop of the CD20 transmembrane antigen expressed on the surface of pre B and mature B lymphocytes (B-cells). The CD20 antigen is present on both normal and malignant B-cells, and there are multiple anti-CD20 antibody therapies approved for treatment of B-cell malignancies.[2] The proposed mechanism of action of obinutuzumab is that upon binding to CD20, obinutuzumab mediates B-cell lysis through (1) engagement of immune effector cells that mediate B-cell cytolysis, (2) activation of the complement cascade, and/or (3) by directly activating intracellular death signaling pathways. The immune effector cell mechanisms include antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), both activities mediated through binding of the Fc region of obinutuzumab with Fc gamma receptor (i.e., FcγRIIIa) receptor positive effector cells, such as natural killer (NK) cells and macrophages/monocytes. Complement-mediated cytotoxicity (complement-dependent cytotoxicity; CDC) can also mediate obinutuzumab-induced cell death. The mechanism of directly activated cell death is still under investigation but data provided suggests that it is independent of caspase activation, involves homotypic aggregation of CD20 molecules and is dependent on actin reorganization and lysosome disruption.

The manufacturing of obinutuzumab involves the manipulation of its pattern of glycosylation (termed glycoengineering), a process that reduces fucosylation of the Fc region of the mAb and results in an increased affinity for FcγRIII receptors and subsequent activation of antibody-dependent cellular cytotoxicity.[3,4]

1.2 Brief Discussion of Nonclinical Findings

Pharmacology studies demonstrated that obinutuzumab has a high selectivity and affinity for human CD20 ($K_D \approx 4.0$ nM). Mechanistic studies identified that obinutuzumab can induce cell death in three ways: (1) autonomously, by directly

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[1] Also known as GA101 and RO5072759.

[2] FDA-approved anti-CD20 antibodies include rituximab, ofatumumab, and radiolabeled antibodies tositumomab and ibritumomab tiuxetan.

[3] Ferrara C, et al., Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcγRIII and antibodies lacking core fucose. *Proc. Natl. Acad. Sci. USA* (2011), 108:12669-12674.

[4] Mössner E, et al., Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell–mediated B-cell cytotoxicity. *Blood* (2010), 115:4393-4402.

activating internal cell death signaling pathways; (2) through Fc receptor-mediated immune effector cell activated pathways (ADCC/ADCP); and (3) antibody activation of the complement cascade (CDC).

Toxicology studies were conducted solely using cynomolgus monkeys because other species available for toxicity testing are not pharmacologically responsive to obinutuzumab. Obinutuzumab binds human and cynomolgus monkey CD20 with similar affinity. Evaluation of the genotoxicity, carcinogenicity and dedicated safety pharmacology studies were not conducted and were not needed for this application.[5]

Repeat dose toxicology studies of 13-week or 26-week duration with extended recovery periods were conducted using IV administration of obinutuzumab. A separate 4-week study that evaluated subcutaneous (SC) administration was also completed. The IV route is recommended for administration of Gazyva. Toxicities observed from repeat-dose studies were consistent with the intended pharmacology of obinutuzumab or were the apparent result of cross-species immunogenicity effects:

- Near-100 per cent decreases in circulating B lymphocytes occurred after the 1st dose of ≥1 mg/kg or SC doses of 30 mg/kg. Corresponding B-cell depletion in lymphoid tissues at IV doses was also observed at these doses at necropsy. At the end of a 37-week recovery, circulating B-cell recovery was variable (individual peak values ranged from 7% to 152% of baseline), while lymphoid tissue B cells fully recovered when compared with controls. Transient decreases in NK cells were observed which can be explained by obinutuzumab-mediated ADCC activity.

- Hypersensitivity reactions were noted at all doses (≥5 mg/kg) in the 26-week study and were attributed to cross-species reactivity to a foreign protein. Clinical observations included acute anaphylactic/anaphalactoid reactions (clinical signs consisted of excessive salivation, facial erythema that progressed to the arms, with evident pruritus). Microscopic findings included an increased prevalence of systemic inflammation and infiltrates consistent with immune-complex mediated hypersensitivity reactions including glomerulonephritis, and arteritis/periarteritis and serosal/adventitial inflammation in multiple tissues. These reactions led to the unscheduled deaths of 6 (possibly 7) monkeys during the 26-week study. Although animals treated at all obinutuzumab dose levels were affected, the incidence and severity of inflammatory changes were greater in animals given 25 or 50 mg/kg than in animals given 5 mg/kg. Immune-complex deposition in glomeruli of some animals was confirmed by detection of electron dense deposits by immunohistochemistry or transmission electron microscopy.

[5]As per recommendations provided in the ICH S6(R1), S7A and S9 Guidance documents regarding biological products intended for the treatment of cancer.

- Suspected opportunistic infections in an additional three unscheduled deaths from the shorter repeat-dose studies were considered a secondary result of obinutuzumab-mediated immunosuppression.

No effects were noted for male and female reproductive organs in repeat dose toxicology studies. In males, no effects were noted on sperm morphology, concentration or motility. In females, no effects were noted on prolactin, estrus-related hormone levels or cycle duration.

An enhanced pre- and post-natal development (ePPND) toxicity study was conducted. Administration of obinutuzumab to pregnant cynomolgus monkeys resulted in a complete depletion of B lymphocytes in infants. Obinutuzumab did not affect embryo-fetal development, parturition, postnatal survival, or the growth and development of infants. The incidence of prenatal loss was higher in treatment groups when compared to controls but were within the range of historical data provided for the testing facility. Obinutuzumab crosses the blood-placental barrier and it is secreted in the milk of pregnant monkeys. A comparison of systemic exposure estimates between the high dose group in the ePPND study and clinical AUCs measured in patients with CLL showed that the exposure to obinutuzumab in monkeys were ~5 times the exposure in patients at the recommended clinical dose. Because of the depletion of B-cells and possible opportunistic infections, use of obinutuzumab during pregnancy is not recommended.

In vitro analysis of obinutuzumab using whole blood from healthy human donors indicated that obinutuzumab can cause first-infusion cytokine release in patients. Clinical study results demonstrated the frequent occurrence of infusion-related reactions IRRs) within 24 hours following the first infusion of obinutuzumab. The most common symptoms of the IRRs were nausea, chills, hypotension and pyrexia. Protocol amendments made during clinical investigation of obinutuzumab included reducing infusion rates, splitting the dose administered over two days or premedication with anti-pyretic, anti-histamine and corticosteroid prophylaxis. Prophylactic pretreatment with analgesics, and possibly steroids or antihistamines is recommended prior to dosing (refer to Section 2.6).

Unexpected tissue cross-reactivity staining using obinutuzumab was observed localized in the membrane of human and monkey liver epithelium, salivary glands and lung endothelium (refer to Section 11). The appearance of off-target effects from binding to these tissues was not readily apparent in the studies conducted in monkeys.

1.3 Recommendations

1.3.1 Approvability

RECOMMEND APPROVAL: The submitted pharmacology and toxicology studies using obinutuzumab (Gazyva) support the safety of its use in patients with previously untreated chronic lymphocytic leukemia.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies using obinutuzumab are necessary for the proposed indication.

1.3.3 Labeling

The proposed labeling describes obinutuzumab as [redacted] (b) (4) Using the nomenclature [redacted] (b) (4) is not appropriate for labeling because the recognition and meaning of the terminology is obscure, and likely limited to the scientific research community that [redacted] (b) (4)

A labeling review is not considered necessary at this time. A separate labeling review may be included following discussions of the proposed package insert with the Applicant.

2 Drug Information

2.1 Drug

CAS Registry Number
949142-50-1

Generic Name (also the INN and USAN/BAN Name)
Obinutuzumab

WHO Reference Number
9043

Code Name
RO5072759; GA101

Chemical Name
Recombinant humanized anti-CD20 monoclonal antibody

Structure or Biochemical Description
Obinutuzumab is a humanized monoclonal antibody based on a human IgG1 (κ) framework. It is a recombinant protein produced in Chinese hamster ovary (CHO) cells [redacted] (b) (4) The calculated molecular mass of

[6] Glennie MJ, et al., Mechanism of killing by anti-CD20 monoclonal antibodies. *Mol. Immunol.* (2007) 44:3823-37.

intact obinutuzumab is [redacted] (b) (4)).

Pharmacologic Class
CD20-Directed cytolytic antibody

2.2 Relevant INDs, NDAs, BLAs and DMFs

The IND 104405 was the original submission for RO5072759 by Genentech.

2.3 Drug Formulation

Obinutuzumab is provided as a sterile liquid, and contains no preservatives. Each single-use 50 mL vial contains 1000 mg (nominal) obinutuzumab for intravenous (IV) infusion. The Drug Product is formulated as 25 mg/mL obinutuzumab in 20 mM L-histidine / L-histidine hydrochloride [redacted] (b) (4) 240 mM trehalose, and 0.02% (w/v) poloxamer 188 at pH 6.0.

Table 1 Batches used for Toxicology Studies

Toxicology Study	Batches Used (according to CoA)	Process	API Source	Use of Batch
13 Week Monkey Study	GWT0021, GWT0022, GWT0024, GWT0025	G1	41214	Toxicological studies, clinical studies
4 Week Subcutaneous Monkey Study	GWT0024, GWT0025	G1	41214	Toxicological studies, clinical studies
6 Month Monkey Study	PDH0000001	G3	PZ08128001 (G002.03ER)	Technical batch, stability study
ePPND Monkey Study	H0002	G3	PZ1009P038 (G016.03E)	Registration batch, clinical use

2.4 Comments on Novel Excipients

The composition of the Phase 3/Commercial Drug Substance Formulations changed to include Polaxamer 188 at a concentration of [redacted] (b) (4) (b) (4) The FDA Inactive Ingredient database lists poloxamer 188 in approved drug products for intravenous use up to 0.6% potency. The amount of Poloxamer 188 is equivalent to [redacted] (b) (4)

Table 2 Obinutuzumab formulation components

(Excerpted from BLA Section 2)

Table 1 Obinutuzumab Clinical and Commercial Formulations and Configurations

Component	Phase I / Phase II[a] Ro 507-2759/F01-01	Phase III / Commercial[a] Ro 507-2759/F06-01
Active ingredient Obinutuzumab	(b) (4)	25 mg/mL
L-Histidine L-Histidine hydrochloride	20 mM	20 mM
Trehalose (b) (4)	90.8 mg/mL (240mM)	90.8 mg/mL (240mM)
(b) (4)	(b) (4)	-
Poloxamer 188	-	(b) (4)
pH	6.0	6.0
Nominal fill volume	(b) (4)	40 mL
Dose strength	(b) (4)	1000 mg
Dosage form	(b) (4)	Concentrate for solution for infusion
(b) (4)	(b) (4)	not applicable

a. (b) (4)

2.5 Comments on Impurities/Degradants of Concern

None.

2.6 Proposed Clinical Population and Dosing Regimen

Obinutuzumab is proposed for the treatment of patients with previously untreated chronic lymphocytic leukemia. Obinutuzumab drug product is to be diluted and administered as an intravenous infusion in combination with chlorambucil.

The recommended dosage of obinutuzumab is 1000 mg administered on Days 1/2 (100 mg on Day 1 and 900 mg on Day 2), 8, and 15 of the first treatment cycle followed by 1000 mg administered on Day 1 only for each subsequent treatment cycle (Cycles 2 to 6). Each treatment cycle is 28 days in duration.

Patients are to be pre-medicated with acetaminophen, plus anti-histamine if a previous ≥Grade 1 infusion-related reaction (IRR) occurred. Also, glucocorticoids are indicated if a previous ≥Grade 3 IRR occurred OR if lymphocytes counts are >25 $x10^9$/L prior to next treatment.

Table 3 Gazyva dose to be administered during 6 treatment cycles of 28 days

Day of treatment cycle		Dose of Gazyva	Rate of infusion (in the absence of infusion reactions/ hypersensitivity during previous infusions)
Cycle 1	Day 1	100 mg	Administer at 25 mg/hr over 4 hours. Do not increase the infusion rate.
	Day 2	900 mg	Administer at 50 mg/hr. The rate of the infusion can be escalated in increments of 50 mg/hr every 30 minutes to a maximum rate of 400 mg/hr.
	Day 8	1000 mg	Infusions can be started at a rate of 100 mg/hr and increased by 100 mg/hr increments every 30 minutes to a maximum of 400 mg/hr.
	Day 15	1000 mg	
Cycles 2 - 6	Day 1	1000 mg	

2.7 Regulatory Background

Genentech submitted their original application for obinutuzumab under IND 104405 on February 6, 2009. The BLA 125486 was received on April 22, 2013 and filed on June 21, 2013.

3 Studies Submitted

3.1 Studies Reviewed

	Study Report	Title
Pharmacology		
1	1025241	ADCC (Antibody Dependent Cellular Cytotoxicity) Activity of RO5072759 (GA101) in Comparison to Rituximab
2	1025340	FcγRIIIa Receptor Binding Affinity of RO5072759 (GA101) in Comparison to Rituximab
3	1053079	The Glycoengineered Therapeutic Monoclonal Antibody GA101 (RO5072759) Triggers Superior Macrophage-Mediated Phagocytosis and Cytotoxicity
4	1025131	Phosphatidylserine Exposure and Cell Death Induction of RO5072759 (GA101) in comparison to Rituximab
5	1025240	Induction of Phosphatidylserine Exposure and Homotypic Aggregation of RO5072759 (GA101) in Comparison to Rituximab
6	1025235	Evaluation of the CDC (Complement Dependent Cytotoxicity) Properties of RO5072759 (GA101) in Comparison to Rituximab
7	1025126	Cynomolgus monkey (*M. fascicularis*) as suitable species for toxicological assessment of the CD20 antibody RO5072759 (GA101)
8	1025341	Cynomolgus FcγRIIIa Receptor Binding Affinity of RO5072759 (GA101) in Comparison to Rituximab
9	1025348	Evaluation of the Affinity of RO5072759 (GA101) in Comparison to Rituximab
Toxicology		

	Study Report	Title
10	1024830	RO5072759 13-Week Intravenous Administration Toxicity Study in the Cynomolgus Monkey with a 37-Week Recovery Phase
11	1036190	6-Month Infusion Toxicity and Toxicokinetic Study with RO5072759 in Cynomolgus Monkeys with a 37-Week Recovery Period
12	1024838	RO5072759 4-Week Subcutaneous Administration Toxicity Study in the Cynomolgus Monkey with a Recovery Phase
13	1045612	RO5072759 (huMAb Anti-CD20) An Intravenous Administration Study for Effects on Embryo-Fetal and Pre- and Postnatal Development in Cynomolgus Monkeys (Enhanced Design)
14	1024158	RO5072759: Cross-Reactivity Study of RO5072759-000 (huMAb_CD20) with Normal Cynomolgus Monkey Tissues
15	1024159	RO5072759: Cross-Reactivity Study of RO5072759-000 (huMAb_CD20) with Normal Human Tissues
16	1025124	Effect of RO5072759 on Cytokine Release and Neutrophil Activation in Human Whole Blood (incl. Amendment 1)
17	1045703	Effect of RO5072759 on Cytokine in a 24-Hour Whole Blood Assay
18	1025140	RO5072759-000: In Vitro Hemolysis and Plasma Precipitation and Turbidity Tests with Human Heparinated Blood and Plasma

3.2 Studies Not Reviewed

Please refer to the Appendix for the list of 132 studies submitted to the BLA that were not reviewed.

3.3 Previous Reviews Referenced

Study 1024830: "A 13-week intravenous administration toxicity study in the cynomolgus monkey with a 37-week recovery phase," was reviewed under IND 104405 by Dr. Michael S. Orr. The review was modified to maintain format consistency for this BLA review.

4 Pharmacology

4.1 Primary Pharmacology

The mechanism of action of obinutuzumab involves a combination of (1) antibody-dependent cell-mediated cytotoxicity and phagocytosis (ADCC and ADCP), (2) caspase-independent apoptosis or direct cell death induction, and (3) complement-dependent cytotoxicity (CDC). Relevant data provided to support each proposed mechanism of action for obinutuzumab was reviewed. These data were provided in individual Study Reports listed or were published in peer-reviewed journals.[7,8] Information to support the use of the cynomolgus monkey as a relevant test species to test obinutuzumab was also reviewed. The pharmacology data reviewed is categorized into the following sections:

[7] Herter S. et al. Preclinical activity of the Type II CD20 antibody GA101 (Obinutuzumab) compared with rituximab and ofatumumab *in vitro* and in xenograft models. *Mol. Cancer Ther.* (2013), Published online July 19, 2013.

[8] Kern DJ, et al. GA101 induces NK-cell activation and antibody-dependent cellular cytotoxicity more effectively than rituximab when complement is present, *Leukemia Lymphoma* (2013*)* Published online April 16, 2013.

- **Binding to CD20**
- **ADCC/ADCP**
- **Direct Cell Death Induction**
- **CDC**
- **Cynomolgus Monkey as a Pharmacologically Responsive Species**

Much of the pharmacology data provided in the BLA are from studies that conducted side-by-side comparisons of obinutuzumab and rituximab, and in some experiments, comparator arms of ofatumumab were included.

Binding to CD20

Key findings:

- The core epitope to which GA101 binds (residues 172-176, PSEKN) was determined by Pescan technology and confirmed by X-ray crystallography and site directed mutagenesis.[9]
- Scatchard plot analysis of obinutuzumab using cultured Non-Hodgkin Lymphoma cell lines calculated a K_D of 4.0 nM for GA101. Side-by-side experiments using rituximab demonstrated a similar K_D of 4.5 nM for rituximab.[10]

ADCC/ ADCP

Genentech distinguishes antibody-dependent cell death that results from macrophage/monocyte phagocytosis as ADCP from ADCC, cytotoxicity that is mediated by other effector cells (predominantly NK cells). Selected pharmacology studies related to ADCC/ADCP activity of obinutuzumab that were reviewed included Reports 1025340, 1025241, and 1053079. Report 1043692 is a manuscript of original research that was published subsequently during the review of the BLA. [11] The data presented in the published article is referenced in the following discussion about ADCC/ADCP.

The ability of obinutuzumab to activate FcγRIIIa receptor-mediated effector cell cytotoxicity is proposed to be a primary mechanism of action. Human polymorphisms of the FcγRIIIa at position 158 result in a high affinity receptor (158V) and a low affinity receptor (158F) on immune effector cells that impacts the ability of therapeutic antibodies to mediate ADCC. [12] Clinical response to rituximab can be influenced by this

[9] Niederfellner G. et al., Epitope characterization and crystal structure of GA101 provide insights into the molecular basis for type I/II distinction of CD20 antibodies *Blood* (2011), 118: 358-367.
[10] Moessner E. et al., Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood* (2010), 115: 4393-4402.
[11] Herter S. et al. (2013) *Ibid.*
[12] Koene, H.R. et al., FcgRIIIa-158V/F Polymorphism Influences the Binding of IgG by Natural Killer Cell FcgRIIIa, Independently of the FcgRIIIa-48L/R/H Phenotype (2007), *Blood*, 90: 1109-1114.

polymorphism. Genentech proposes that the reduced fucosylation of obinutuzumab offers a means to obviate the clinical effect of the FcγRIIIa 158V/F polymorphism.

Key Findings:

- GA101 activates ADCC in cultured human B-cell cancer cells using human PBMCs or an engineered cell line that expresses FcγRIIIa (Report 1025241, reviewed below). The ADCC activity is greater in side-by-side comparisons with rituximab but the magnitude of differences varies with the concentrations of mAbs used. The addition of nonspecific human IgGs reduces the ADCC activated by either GA101 or rituximab.
- The ability of GA101, rituximab and ofatumumab to activate ADCC was compared using two human cell lines (Z138 and SU-DHL4) with human PBMCs expressing the V158/V158 or the F158/F158 FcγRIIIa receptor subtype.[13] The percentage of ADCC activated by GA101 was dose dependent for both cancer cell types used (see Figure 1). Interestingly, a greater overall percentage of ADCC was achieved using GA101 in the presence of PBMCs with the V158/V158 (compare %ADCC in panels C&A and D&B). Note that GA101 WT (which is produced in cells that produce mAbs that are glycoengineered for reduced fucose in the Fc-region) displayed a much lower percentage of ADCC when compared to GA101 with reduced fucosylation.
- The ability of GA101, rituximab and ofatumumab to activate ADCP was compared using M1 and M2c macrophages generated from human monocyte-derived macrophages.[14] No significant differences were observed between the three antibodies with respect to ADCP, in the presence or absence of competing endogenous human IgGs.
- GA101 binds to either FcγRIIIa 158V/F variant with greater affinity than rituximab (Report 1025340, reviewed below).

[13] Herter S. et al. (2013) *Ibid.*
[14] Herter S. et al. (2013) *Ibid.*

Figure 1 ADCC activation by GA101, rituximab and ofatumumab using both FcγRIIIa 158F/V human variants

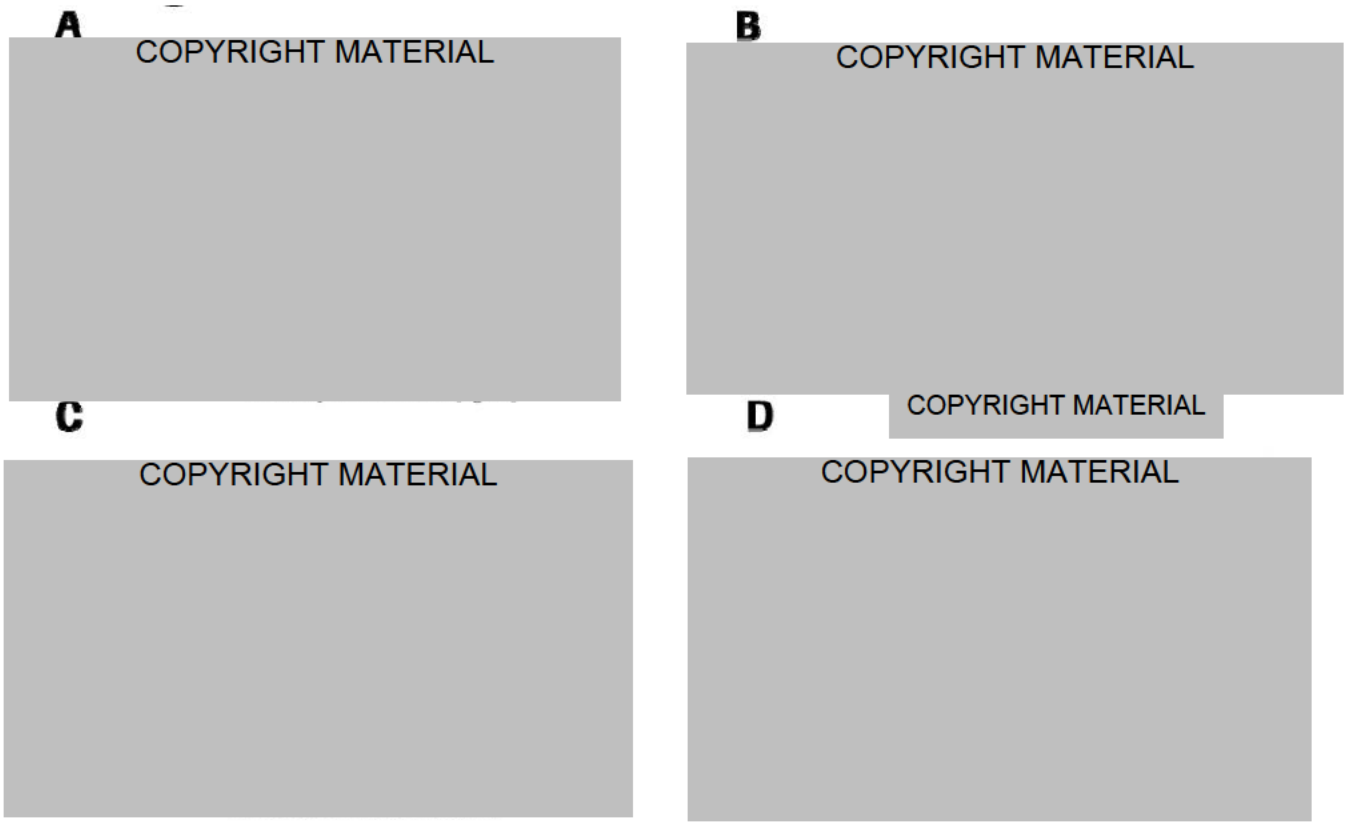


Fig. 4. (Excerpted from Herter et al.) ADCC induced by standard doses of GA101 (black squares), GA101 WT (open squares; fucosylated version of GA101), rituximab (open diamonds), and ofatumumab (open triangles). Cells were incubated for 4 h in the presence of the CD20 antibodies and human PBMCs as effectors (effector: target ratio 25:1) and percentage of ADCC was calculated by measuring lactate dehydrogenase release in cell supernatants. PBMCs expressing the V158/V158 FcγRIIIa receptor were incubated with Z138 (*A*) and SU-DHL4 (*B*) cell lines. PBMCs expressing the F158/F158 FcγRIIIa receptor were incubated with Z138 (*C*) and SU-DHL4 (*D*) cell lines.

A. Report 1025241: ADCC (antibody dependent cellular cytotoxicity) activity of RO5072759 (GA101) in comparison to Rituximab

This study compared the ADCC activity of GA101 and rituximab using human B-cell cancer cell lines and a genetically engineered NK-92 cell line that expresses human FcγRIIIa receptors or freshly isolated human PBMCs as effector cells.

<u>Methods</u>

Human lymphoma cell lines used were:

- Burkitt's Lymphoma (Raji, RAMOS, Namalwa)
- Mantle Cell Lymphoma (Z-138)

- Diffuse Large Cell NHL (SU-DHL-4)

NK-92 cells are human NHL cell line that can be engineered to express either 158V or 158F FcγRIIIa haplotypes.[15]

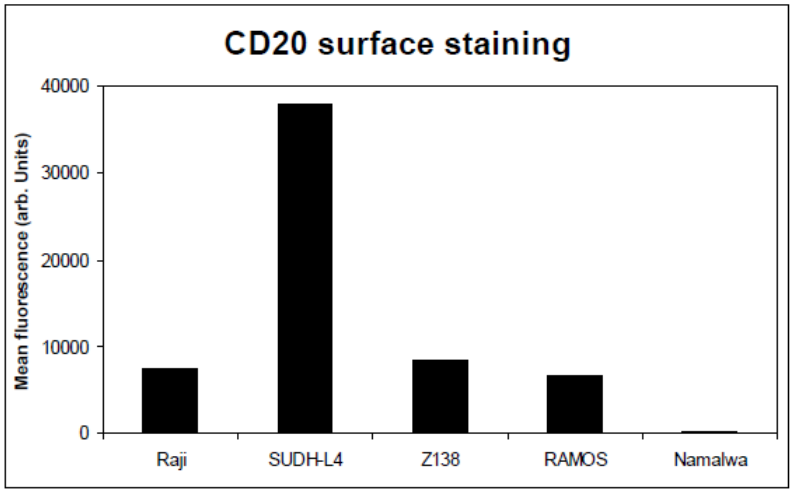
Cells stained with Calcein AM were incubated with antibodies for 10 min prior to addition of effector cells. Effector to target ratio was 3:1 for NK-92 cells and 25:1 for PBMCs. Co-incubation lasted 2 hours with NK-92 cells and 4 hours with PBMCs.

Cytolysis was measured two ways: release of LDH into supernatant or the retention of Calcein in the remaining viable cells.

Unspecified human IgG preparation (RedImmune, Behringwerke, Bern) derived from a pool of 1000 healthy donors were added to experiments as indicated to mimic physiological concentrations (range of 4, 10, and 20 mg/kg).

Results

Surface expression of CD20 was measured on the cell lines to estimate ADCC susceptibility.

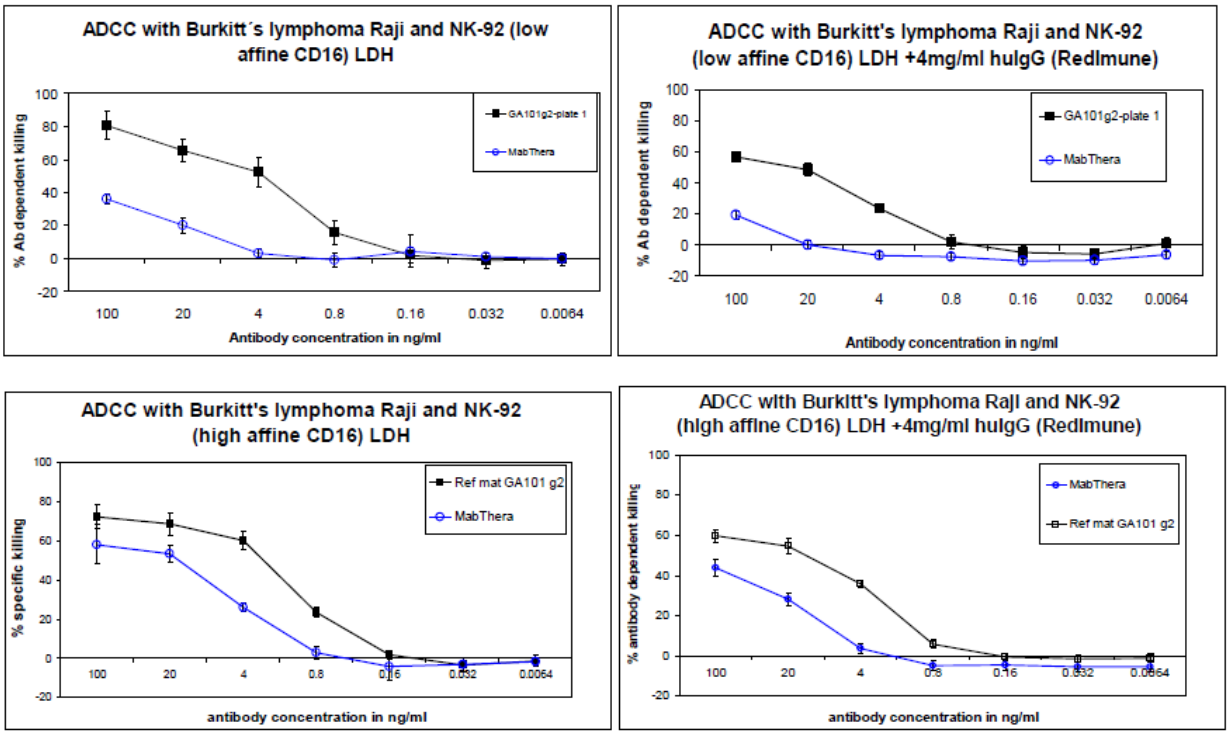


All cell lines were susceptible to ADCC except for Namalwa cells, which were not included for further investigation. The ability of the mAbs to activate ADCC in Raji cells is presented in Figure 2.

[15] Gong JH et al. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. *Leukemia.(1994)* 8(4):652-8

Figure 2 Effect of unspecific human IgGs on ADCC activity by GA101 or rituximab

(Figure excerpted from Report 1025241)



The left hand side panels of the figure show that GA101 elicits similar levels of ADCC in the presence of high affinity or low affinity CD16 (FcγRIIIa) receptors expressed on NK-92 effector cells. The maximal effect observed with rituximab is approximately 20% lower for the lower affinity CD16 (upper left panel) in comparison to the high affinity CD16 (lower left panel). The addition of nonspecific human IgG lowers the %ADCC for either antibody used by similar magnitudes (right side panels).

Reviewer's comment: Results from additional analyses are tabulated in Table 4 (table copied from the BLA). Only data derived from Raji cells using either NK-92 158V and 158F effector cells were provided in the Report, therefore the conclusion that GA101-mediated ADCC is not affected by this human polymorphism is based on this one experiment.

Table 4 GA101 and rituximab ADCC activity in lymphoma cell lines

Effector cell type	Method	IgG	Target cell line	EC50 GA101	% spec. lysis GA101	EC50 Rtx	% spec. lysis Rtx	ratio EC50	ratio depletion
NK-92 low affinity (158 F)	LDH		SUDHL4	0.514	71.9	8.181	55.7	15.91	1.2908
	Calcein		SUDHL4	0.030	85	0.868	77.1	29.26	1.1025
NK-92 low affinity (158 F)	LDH		NAMALWA	n.d.	22.4	n.d.	2		11.2
NK-92	LDH		RAMOS	0.738	92	26.18	46.8	35.46	1.9658
NK-92 low affinity (158 F)	LDH		Z-138	1.772	100	21.99	68.6	12.41	1.4577
	LDH	4	Z-138	2.049	100	28.09	78.8	13.71	1.2690
NK-92 low affinity (158 F)	LDH		Raji	2.355	78.48	19.39	37.69	8.23	2.0822
	LDH	4	Raji	4.882	57.43	89.77	43.74	18.39	1.3130
NK-92 high affinity (158 V)	LDH		Raji	1.222	71.4	4.785	57.6	3.92	1.2396
	LDH	4	Raji	4.393	57.5	19.02	43.9	4.33	1.3098
PBMC F/F	LDH		Raji	1.215	66.3	22.37	45	18.41	1.4733
	LDH	10	Raji	n.d.		n.d.			
PBMC F/V	LDH		Raji	20	69.7	28.8	48.4	14.55	1.4401
	LDH	20	Raji	n.d.	48.6	n.d.	3.2		15.1875
PBMC F/V	Calcein		Raji	3.481	n.d.	11.5	64.7	3.31	
	Calcein	20	Raji	798.9	20.6	n.d.	1		20.6

The columns from left to right give values or data on: effector cell type, assay read out format, concentration of human IgG included in the assay, the target cell line, the EC50 value of RO5072759 (GA101) in ng/ml (calculated with the Graphpad PRIZM 4 software package), maximal specific lysis in % for GA101, the EC50 value of rituximab (Rtx), maximal specific lysis in % for rituximab, the ratio of EC50 values of rituximab in ng/ml and RO5072759 (GA101) and the ratio of maximal achieved specific lysis of target cells. EC50 values were calculated where meaningful; the results for the NAMALWA cell line *e.g.* do not reach saturation and thus give no basis for calculation of non-linear regression and resulting semi-sigmoid curve fits.

Reviewer comment: For a given concentration, GA101 activates greater ADCC activity than rituximab under the in vitro conditions used. The addition of competing human IgGs resulted in diminished ADCC activity but the IgG effect did not appear preferential for the mAb used.

B. Report 1025340: FcγRIIIa receptor binding affinity of RO5072759 (GA101) in comparison to Rituximab

This study measured the binding affinities of GA101 and rituximab to variants of human FcγRIIIa.

<u>Methods</u>

Recombinant variants of the FcγRIIIa proteins (V/F158) were purified. Surface plasmon resonance (Biacore) experiments were performed using the variants and either GA101 or rituximab.

Results

Like rituximab, GA101 binds to the 158V variant with a higher affinity than the 158F variant. GA101 binds to either variant with higher affinity than rituximab. The affinity of GA101 binding to V158 (K_D = 55.5 ± 0.67 nM) was up to 12-fold higher than that observed with rituximab (K_D = 666 ± 55 nM). GA101 also bound with 4.5 higher affinity to the F158 variant than rituximab (K_D = 457 ± 14.1 nM vs. K_D = 2070 ± 360 nM).

Table 5 FcγRIII Binding Affinities

(Table excerpted from Pharmacology Written Summary)

Binding Constants	FcγRIIIa Low Affinity (158F)	FcγRIIIa High Affinity (158V)	FcγRIIIb NA2
Obinutuzumab	270 nM	55 nM	930 nM
Rituximab	2000 nM	660 nM	7400 nM
Increase	7-fold	12-fold	8-fold

Note: There is an inconsistency in the affinity reported for GA101 binding to 158 F in the Results section of the Study Report (457 ± 14.1 nM) from what is presented in the Table 2 of the Study Report, and also reported in the Pharmacology Written Summary (270 ± 3 nM).

C. Report 1053079: The glycoengineered therapeutic monoclonal antibody GA101 (RO5072759) triggers superior macrophage-mediated phagocytosis and cytotoxicity

This study evaluated the role fucosylation of GA101 plays in mediating FcγRIIIa - dependent phagocytosis and compared the ADCP activity of rituximab and ofatumumab.

Methods

- Raji and WIL2S cells were used.
- Monocytes were derived from freshly isolated human PBMCs, and made into macrophage subtypes using a variety of different commercially available cytokines.
- Antibodies used were fluorescently labeled by Roche Glycart AG (GA101 g2 [reduced fucosylation; representative of the clinical candidate]; GA101 wt (without reduced fucosylation); rituximab; ofatumumab).

The ability of different CD20 antibodies (GA101 g2, GA101 wt, rituximab, ofatumumab) to bind macrophages was assessed by flow cytometry.

- GA101 g2 refers to the glycoengineered version of GA101 that contains reduced fucosylation;
- GA101 wt refers to the mAb without glycoengineering to reduce fucosylation.

Binding was challenged by the addition of human IgGs (Redimune) in an attempt to mimic physiological conditions.

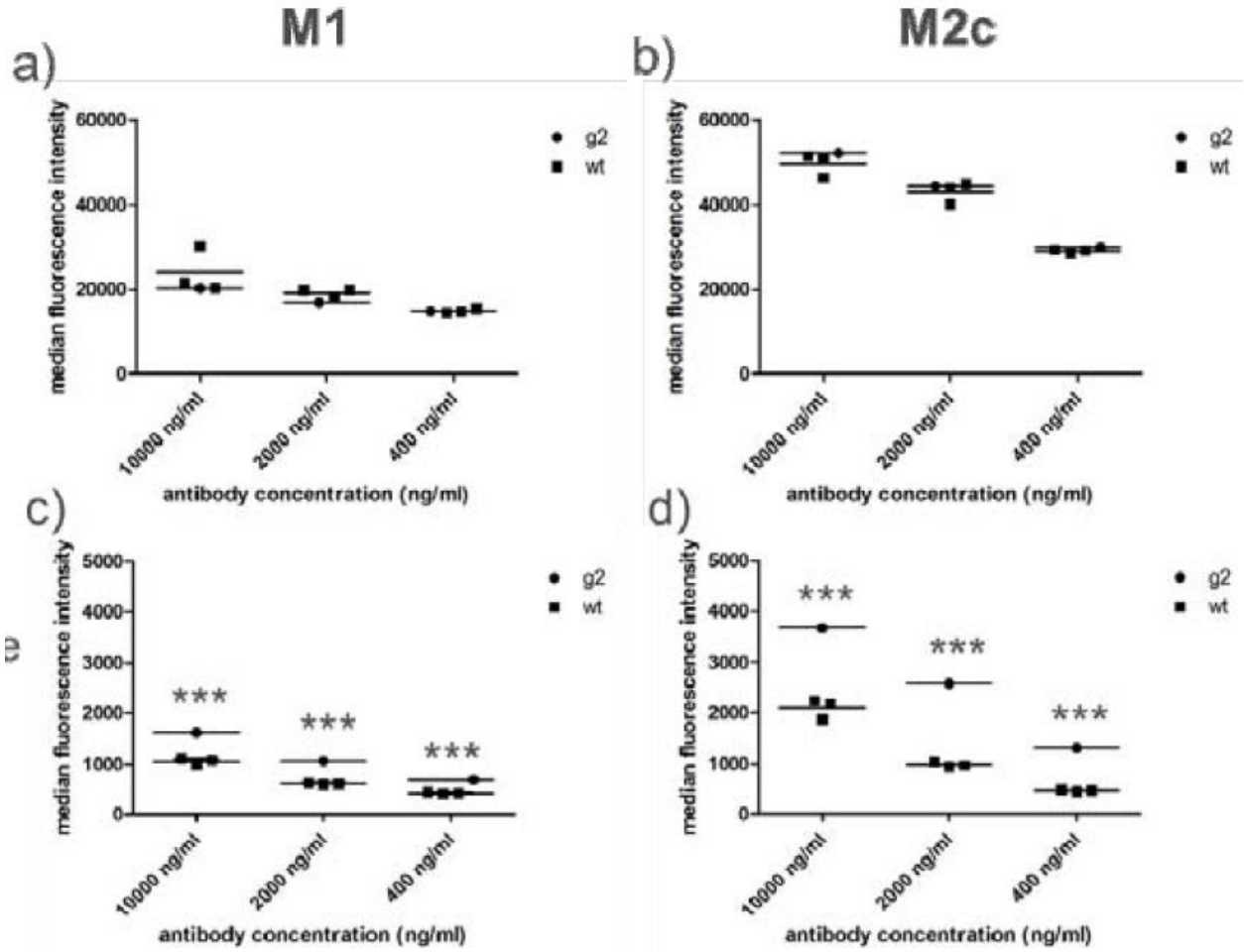
ADCP was measured by co-culturing M1 and M2c macrophage subsets with tumor cells and CD20 antibody. The percentages of phagocytosed target cells were determined by flow cytometry (Raji cells that stained positive for CD206 and negative for CD22).

Results

GA101 g2 (circles, in Figure 3 below) displayed similar binding pattern to macrophage subsets M1 and M2c as GA101 wt, rituximab and ofatumumab (squares) without the addition of IgGs (upper panels, a&b). However, in the presence of competing IgGs, GA101 g2 displayed increased binding when compared to the other mAbs in the presence of IgGs (lower panels, c &d).

Figure 3 Binding of different CD20-binding mAbs to FcγRIIIa on macrophages

(Figure excerpted from Study Report 1053079)

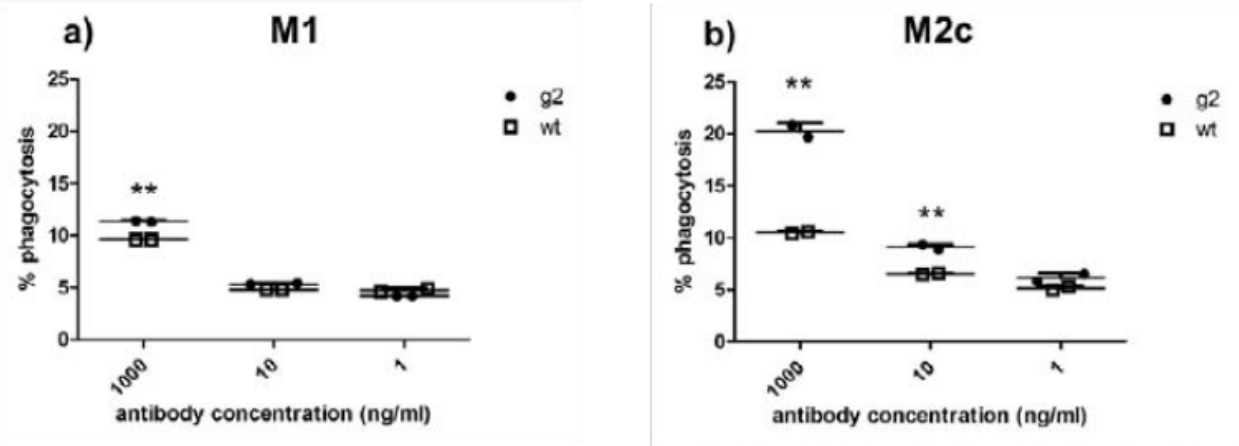


Binding to human macrophages: Binding of glycoengineered GA101 (RO5072759), (circle) and of GA101 WT, rituximab and ofatumumab (squares) labeled with AlexaFluor647 to polarized human monocyte-derived macrophages of M1 (a, c) and M2c (b, d) in the absence (a, b) or presence (c, d) of 10 mg/ml Redimune. Representative data from one binding experiment are shown. Statistical significance, Student t test, ***p<0.001.

The ability of GA101 g2 and GA101 wt mAbs to activate ADCP using the different macrophage preparations was evaluated. In this experiment, a dose-dependent increase in Raji cell phagocytosis was observed using GA101 g2, with both the M1 and M2c macrophage cell types (Figure 4).

Figure 4 GA101-mediated ADCP

(Figure excerpted from Study Report 1053079)



Antibody-dependent phagocytosis (ADCP) of tumor target cells. Raji cells were incubated for 4 h in the presence of GA101 GE (RO5072759) or GA101 WT and 10 mg/ml Redimune with M1 or M2c macrophages (E:T 3:1). The percentage of phagocytosed target cells was determined by flow cytometry. Duplicates of a representative experiment are shown. Statistical significance, Student t test, **p<0.005.

The following two graphs (Figure 5) were provided in the Herter et al. paper (refer to Figure 4 of the paper).[16] The ADCP results provided in the paper support the finding presented in Report 1053079 that GA101 exerts a dose-dependent increase in ADCP activity in Raji cells. The results shown also indicate that GA101 has similar effect on ADCP induction as rituximab and ofatumumab. Results using M2c cells are the upper set of graphed lines in panel F below:

[16] Herter S. et al. (2013) *Ibid.*

Figure 5 GA101 does not display superior ADCP than rituximab

(Figure excerpted from Herter et al. 2013)

Direct cell death induction

Obinutuzumab can induce cell death directly, which appears to be at least partially independent of caspase activation. The exact molecular mechanism of direct cell death induction is not completely understood presently, and is under investigation.

The Herter et al. paper reported results from experiments that measured Annexin V and propidium iodide (PI) staining of human lymphoma cell lines that were treated with GA101 (reduced fucosylation), rituximab or ofatumumab. The results shown in Figure 6 are following a 24-h treatment of the different anti-CD20 antibodies shown. GA101 treated increased cell in all four cells and was more potent than either rituximab or ofatumumab in 3/4 lines tested.

Figure 6 Cell death following 24h mAb treatment

(Figure excerpted from Herter et al. 2013)

- Evidence to support that cell death induced by GA101 is independent of caspase activation is provided in Report 1025240 (reviewed below). Results from an experiment using z-VAD a pan-Caspase inhibitor demonstrated that GA101's ability to induce Annexin V binding was not affected by z-VAD.
- A paper by Alduaij et al. provides evidence to suggest that GA101 autonomous cell death induction is dependent on actin reorganization and lysosome

disruption, can be abrogated by inhibitors of actin polymerization, and is independent of BCL-2 overexpression and caspase activation.[17]

D. Report 1025131: Phosphatidylserine Exposure and Cell Death Induction of RO5072759 (GA101) in Comparison to Rituximab

The ability of RO5072759 and rituximab to activate cell death in eleven different human lymphoma cell lines was compared.

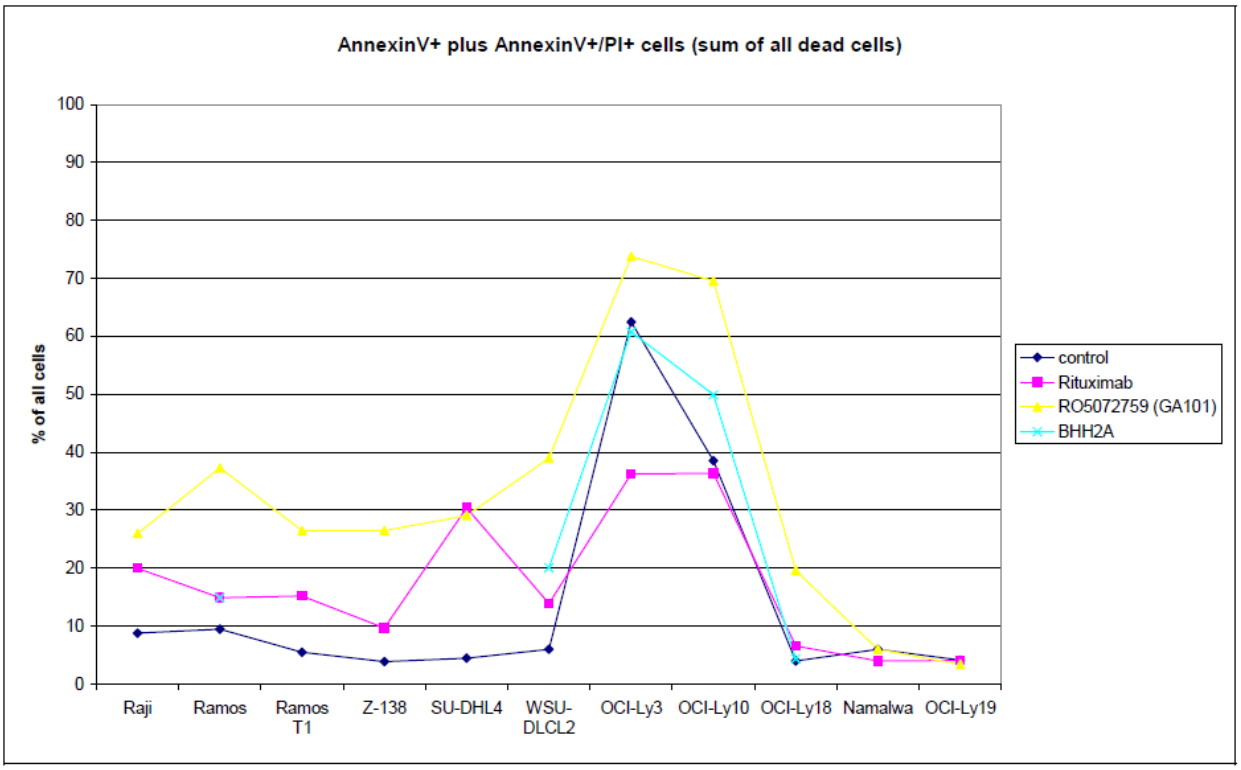
Methods:
Flow cytometry of propidium iodide staining and Annexin V staining was measured. Results are provided for Annexin V-positive cells and double Annexin-V/PI positive cells. There were little to no PI-only positive cells.

Results:
The same concentration of antibody was added to cultures. Antibodies used were: RO5072759, Rituximab or BHH2A (a mutant version of RO5072759). For the majority of cell types, there were fewer than 20% Annexin V-positive cells or Annexin V/PI positive cells for each of the cell types. The figure below summarizes results from the 11 cell lines for Annexin V+ and Annexin V+/PI+ following antibody treatment. Results demonstrate that the majority of cell lines tested (8/11) were more sensitive to RO5072759-induced cell death when compared to rituximab.

[17] Alduaij W et al. Novel type II anti-CD20 monoclonal antibody (GA101) evokes homotypic adhesion and actin-dependent, lysosome-mediated cell death in B-cell malignancies. *Blood* (2011) 117:4519-4529.

Figure 7 Cell death comparing GA101 and rituximab in a panel of human B-cell cancer lines

(figure excerpted from Study Report 1025131)



RO5072759 (GA101) induces cell death (early-stage apoptosis) 3*105 cells were seeded in 24-well plates and either left untreated (control) or treated with 10µg/ml RO5072759 (GA101), Rituximab and BHH2A, respectively, for 72h. The graph shows the percentage of dead cells (Annexin V+ plus Annexin V+/PI+ cells) for 4 Burkitt Lymphoma cell lines, 6 Diffuse Large Cell Lymphoma lines and 1 Mantle Cell Lymphoma line.

E. Report 1025240: Induction of phosphatidylserine exposure and homotypic aggregation of RO5072759 (GA101) in comparison to Rituximab

This study evaluated the autonomous cell death and homotypic aggregation (i.e., clustering of the antibody-bound cells into aggregates) of GA101 in comparison to rituximab. The review of data of this report is limited to the investigation of the contribution of caspases to autonomous cell death induction by GA101.

Methods

The human NHL cell line SU-DHL-4 was incubated with mAbs for 20-24h, stained with Annexin V and PI and analyzed by flow cytometry.

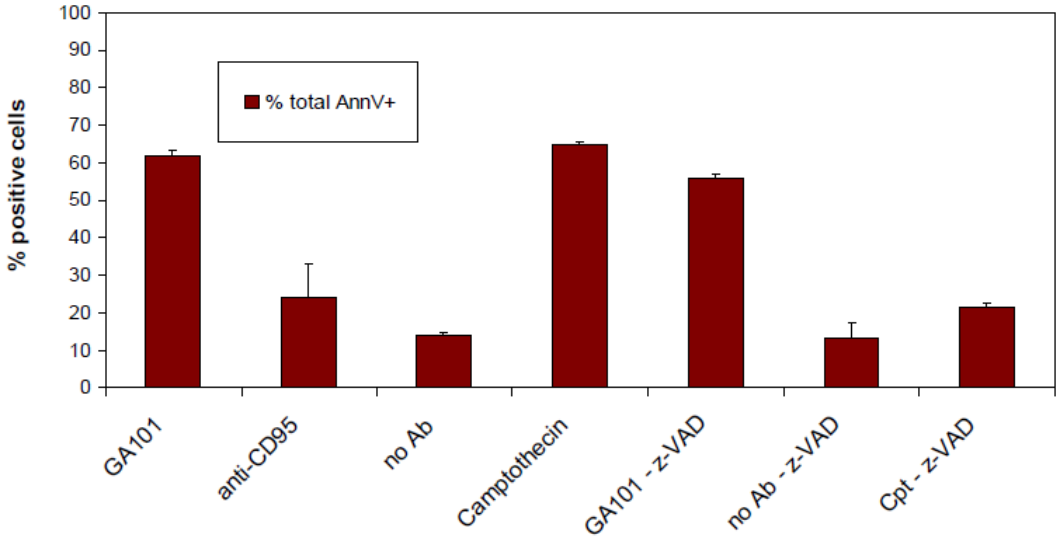
Results

In the figure below, SU-DHL-4 cells were treated with the mAbs shown or camptothecin and both PI+/- Annexin V+ cells were graphed. The addition of the pan-caspase inhibitor z-VAD did not alter the amount of GA101 induced cell death but did so for

camptothecin, suggesting that caspases may not be required for GA101 induced apoptosis.

Figure 8 GA101 induced cell death, caspase independency SU-DHL-4 cells

(figure excerpted from Study Report 1025240)



Complement-Dependent Cytotoxicity

Genentech states that obinutuzumab has a significantly reduced CDC activity relative to rituximab and ofatumumab. However, at mAb concentrations >100 mcg/ml (levels that are achieved clinically), CDC activation by obinutuzumab may play a significant role (see Figure 9 and Figure 10).

F. Report 1025235: Evaluation of the CDC (Complement Dependent Cytotoxicity) Properties of RO5072759 (GA101) in Comparison to Rituximab

This study compared the in vitro CDC activity of GA101 and Rituximab using 5 different human lymphoma lines.

Methods

Raji, Z-138, RAMOS, NAMALWA and SU-DHL-4 human cancer cell lines were used. CD20 levels and complement resistance factor expression were measured for each cell line used. Cells were incubated with various concentrations of the two antibodies 10 minutes prior to addition of complement in order for CD20 binding to equilibrate. Rabbit serum was used as the source of complement to circumvent possible human complement resistance factors present. Cells were then incubated for 2 hours. Cells were prepared in duplicate with and without human unspecified IgG preparations added at physiological concentrations of 4 mg/ml and 10 mg/ml.

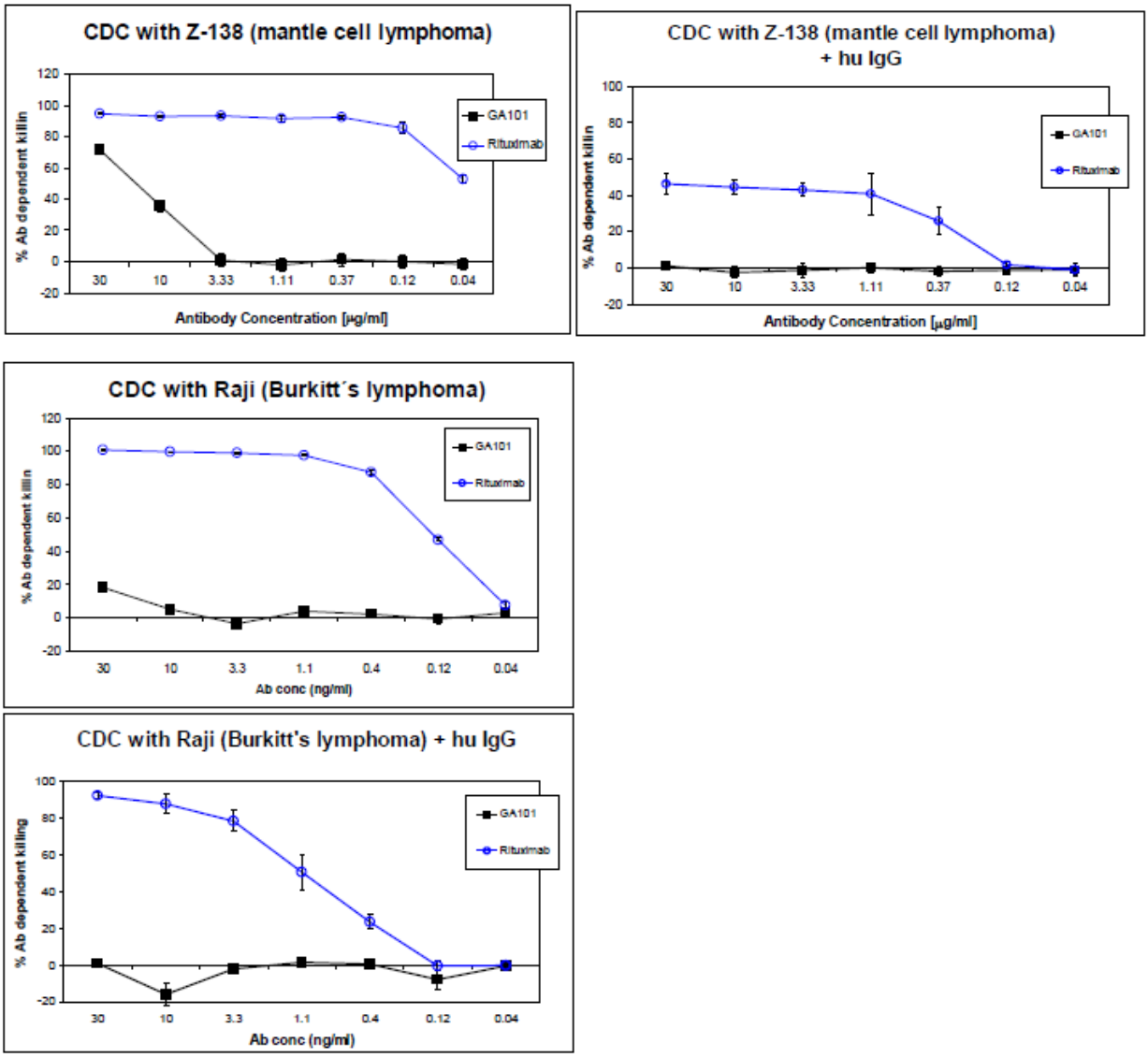
Two different readouts were used: measurement of released LDH into the supernatant and cell viability measured by Alamar Blue.

Results

Dose-response curves displaying CDC activity were provided, but EC_{50} values for each cell line and antibody conditions used were not. Graphs for Z-138 and Raji show that GA101 has less CDC-inducing ability than rituximab in side-by-side comparisons at concentrations of mAb ≤ 30 mcg/ml. The addition of human IgG reduces the amount of CDC activity for either mAb.

Figure 9 In vitro CDC activity for GA101 and Rituximab

(figure excerpted from Study Report 1025235)

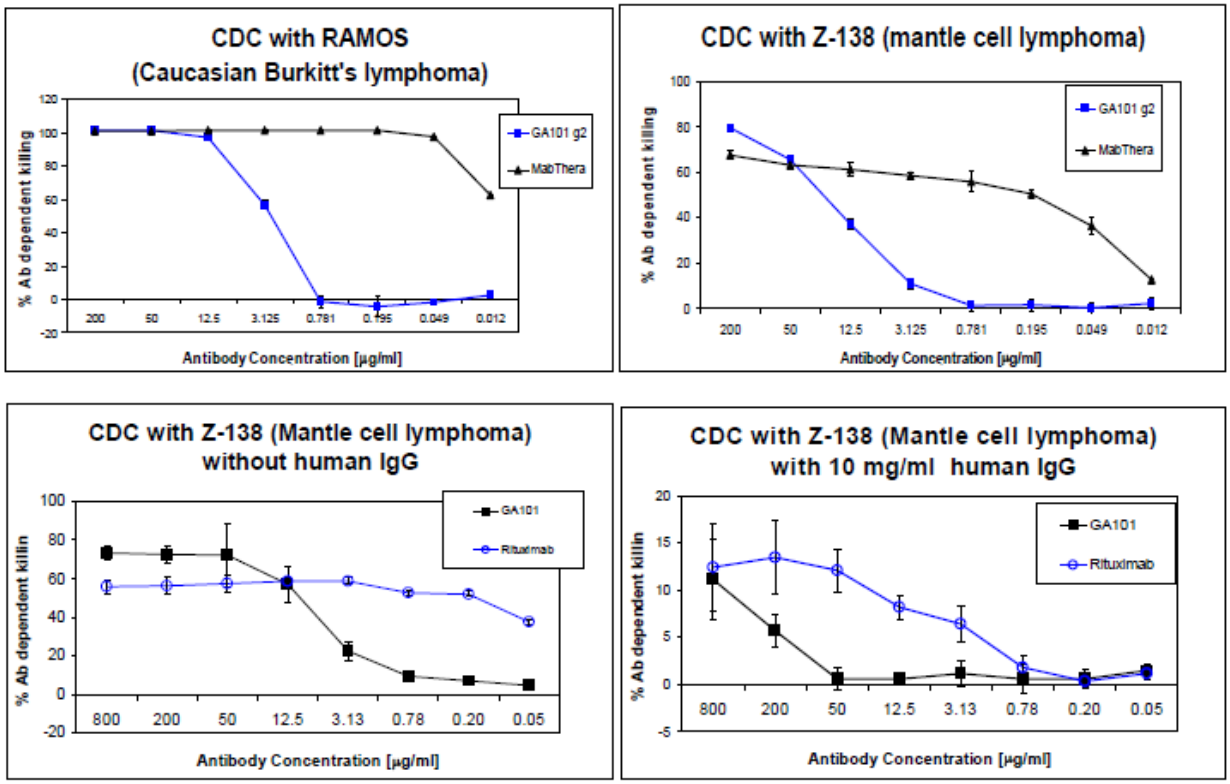


CDC with B-cell lines Raji, RAMOS, Z-138 and SU-DHL-4 Addition of 4 mg/ml of RedImune® human IgG preparation to the experiment led to a loss of potency of the Abs in the CDC assay and abolished CDC activity of RO5072759 (GA101) almost completely.

At higher antibody concentrations (>50 mcg/ml), GA101 exerts similar CDC activity as rituximab.

Figure 10 In vitro CDC activity for GA101 and Rituximab

(figure excerpted from Study Report 1025235)



The figure shows equivalent levels of effector function for RO5072759 (GA101) compared to Rituximab at concentrations higher than 50 mcg/ml in the absence of unspecific IgG. RAMOS cell line showed generally higher susceptibility to CDC compared to Mantle cell lymphoma cell line Z-138. Addition of a human IgG preparation to the experiment reduced potency of RO5072759 (GA101) in CDC and comparable efficacy was reached at concentrations of about 1 mg/ml or higher. EC50 value in the Z-138 experiment for Rituximab shifted from 1.4 ng/ml to 5.1 µg/ml whereas for RO5072759 (GA101) the EC50 value changed from 6.2 mcg/ml to 200 mcg/ml.

Cynomolgus Monkey as a Pharmacologically Responsive Species

Two Reports were reviewed to confirm whether the cynomolgus monkey was an appropriate test species for obinutuzumab safety assessment:

G. Report 1025126: Cynomolgus monkey (*M. fascicularis*) as suitable species for toxicological assessment of the CD20 antibody RO5072759 (GA101)

In this report, the protein sequence of CD20 from human, monkey, rat, dog and other species were aligned. The remainder of this report contains summary information collected from published literature; it does not contain results from original experimentation.

- An analysis of the alignment of the human and cynomolgus monkey amino acid sequences of the extracellular loop between humans and cynomolgus monkeys (residues 60-297) found one amino acid differences between human (Ala-157) and monkey (Val-157) sequences. The sequence of the cynomolgus monkey CD20 used was derived from patent WO2004056312, US20060034835 (Genentech, Inc.). The protein sequence from rat and mouse CD20 shares 63% identity in the large extracellular loop (residues 140-186).
- The core epitope to which GA101 binds (residues 172-176, PSEKN) was determined by Pescan technology and confirmed by X-ray crystallography and site directed mutagenesis.[18] This sequence is conserved between humans and cynomolgus monkeys.
- The binding affinities of GA101 for human and monkey CD20 are comparable based on flow cytometry results using cultured Non Hodgkin Lymphoma cell lines.
- Scatchard plot analysis demonstrated a KD of 4.0nM for GA101 and 4.5 nM for rituximab (Study Report 102538, reviewed below).
- Affinity values of GA101 towards human and cynomolgus FcRn were similar as measured by surface plasmon resonance (Biacore) analysis.

Table 6 Binding affinities of obinutuzumab and rituximab to human and monkey FcγRIIIa

(Table excerpted from Report 1025126)

Compound	*Cynomolgus FcgRIIIa*	*human FcγRIIIa Phe-158*	*human FcgRIIIa Val-158*
RO5072759	104 ± 1 nM	270 ± 3 nM	55 ± 1 nM
Rituximab	710 ± 9 µM	2000 ± 360 nM	660 ±60 nM
Ratio	6.8	7.4	12

The binding affinities (K_D) shown in the table above were determined by Biacore analysis.

- Different binding patterns were described for GA101 and Rituximab.
  - Two fold lower number of binding sites for GA101 in comparison to Rituximab by flow cytometry using NHL cell lines.[19]
- Cross-competition experiments using GA101 and Rituximab determined that both antibodies can interfere with binding of each other to CD20.[20]

H. Report 1025348: Evaluation of the affinity of RO5072759 (GA101) in comparison to Rituximab

[18] Niederfellner, et al. Epitope characterization and crystal structure of GA101 provide insights into the molecular basis for type I/II distinction of CD20 antibodies. *Blood* (2011), 118: 358-67
[19] Roche Report No 1025238, Roche Report No 1025343.
[20] Roche Report No 1025238, Roche Report No 1025130.

This study evaluated the binding characteristics of RO5072759 and rituximab in human lymphoma cell lines and calculated a K_D value for both mAbs using Scatchard analysis.

Methods:

- Flow cytometry analysis cells incubated with fluorescently labeled RO5072759 or rituximab was performed to measure EC50 values.

- Scatchard analysis was conducted using SU-DHL-4 cells that were incubated with Europium-labeled antibodies. The Europium-labeled antibodies (RO5072759 or rituximab) were added at different concentrations for 1 hour at room temperature. Europium has phosphorescence properties that were used to measure bound mAbs with a commercially available fluorescent reagent (Perkin Elmer).

Results:

The binding curves for the two antibodies exhibited similar EC50 values for the cell lines used (see Table 7).

Table 7 EC50 for GA101 or rituximab binding to a panel of NHL cell lines

(Excerpted from Study Report 1025348)

	Rituximab-CY5 EC50 [nM]	GA101-Cy5 EC50 [nM]
SU-DHL-4	9	7
SU-DHL-4 m706	15	9
Z-138	30	22
OCI-LY18	35	43
OCI-LY10	57	74
OCI-LY3	69	78
Raji	79	77
Ramos	36	33
Ramos T1	44	44
Mean	**42**	**43**

Scatchard analysis revealed similar binding affinities for both RO5072759 and rituximab using SU-DHL4 cells. Figure 11 shows one of 3 independent experiments conducted. The K_D values calculated from each of the 3 experiments are shown in

Table 8, and the averaged K_D indicates that binding of both mAbs to SU-DHL4 cells are equivalent.

Figure 11 Saturation curves and Scatchard Analysis

(Excerpted from Study Report 1025348)

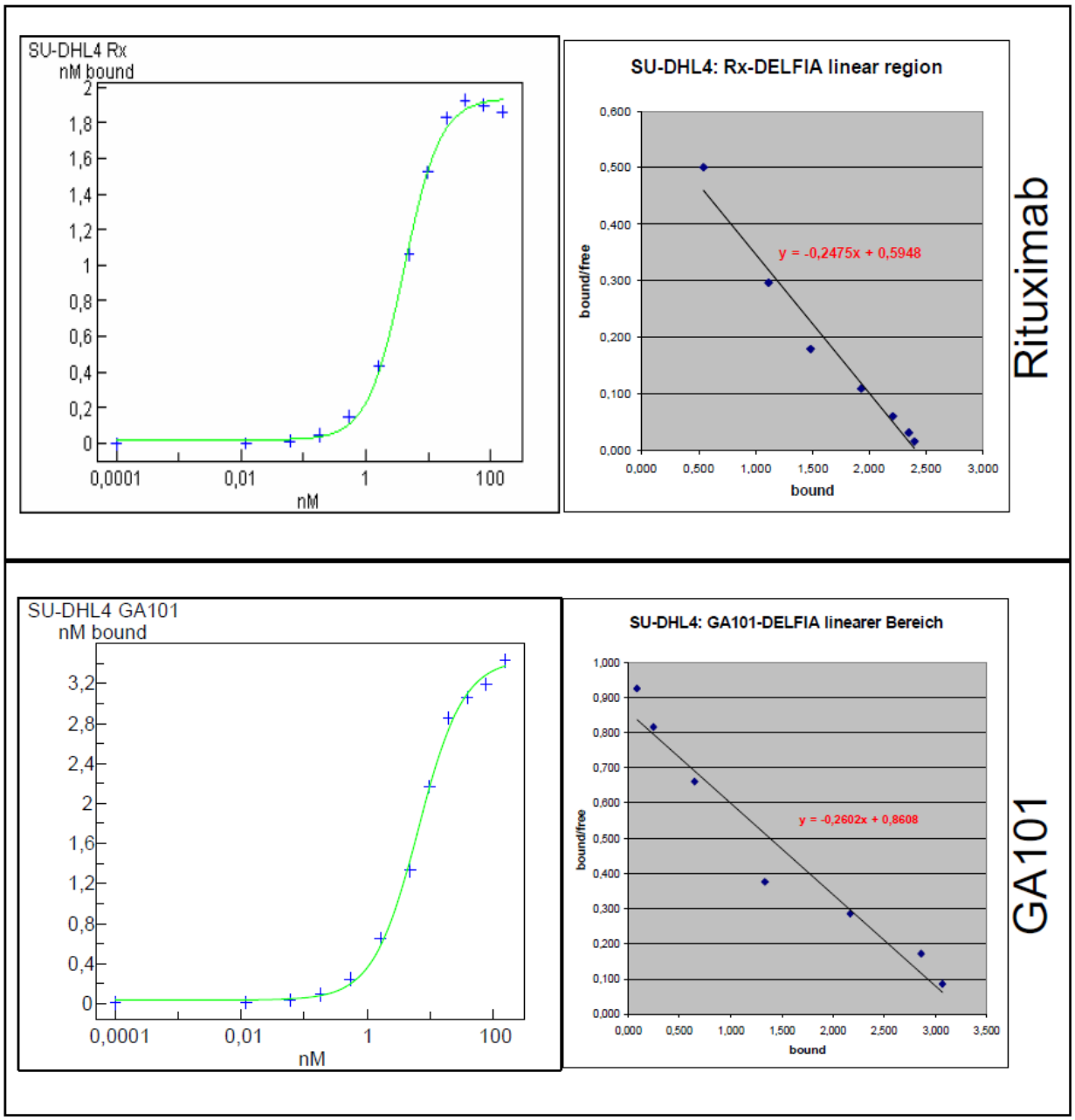


Table 8 K_D values for GA101 and rituximab using SU-DHL4 cells

(Excerpted from Study Report 1025348)

[nM]	Exp.1	Exp.2	Exp.3	Mean
Rx	2,3	4,0	7,1	**4,5**
GA101	7,9	0,4	3,8	**4,0**

I. Report 1025341: Cynomolgus FcγRIIIa receptor binding affinity of RO5072759 (GA101) in comparison to Rituximab

This study evaluated the binding affinity of cynomolgus FcγRIIIa to GA101 and rituximab.

Methods:

Roche cloned the cynomolgus FcγRIIIa gene and purified the recombinant protein. Surface plasmon resonance (Biacore) experiments were performed with either GA101 (Lot. P254034ARS) or rituximab.

Results:

The binding of GA101to cynomolgus FcγRIIIa (K_D = 104±1.07 nM) was up to 7-fold higher than to rituximab (K_D = 706±85 nM).

4.2 Secondary Pharmacology

No secondary pharmacology studies were included in the BLA. Refer to Section 10 (Special Toxicology Studies) for results from tissue cross-reactivity studies using human and monkey tissues.

4.3 Safety Pharmacology

Separate safety pharmacology studies were not conducted in agreement with guidance provided in ICH S6(R1), ICH S9 and ICH S7A. Monitoring for general pharmacological activity on cardiovascular, respiratory and central nervous systems were incorporated in the 13-week and 26-week repeat-dose toxicology studies conducted in monkeys.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Report 1020938: Single IV Dose PK Study in Monkeys
Pharmacokinetics were evaluated in cynomolgus monkeys following a single IV dose of obinutuzumab (2 animals per dose level) at 1 or 10 mg/kg. Accelerated clearance occurred after 216 hours post-dosing in 3/4 animals, from an apparent anti-drug antibody (ADA) response. Noncompartmental analysis demonstrated slow clearance rates and apparent $t_{1/2}$ of 172 and 194 hours, for 1 and 10 mg/kg doses, respectively (Table 9).

Table 9 Estimated PK parameters following a single IV dose in monkeys

(excerpted from Report 1020938)

Animal ID	Dose (mg/kg)	$V_{central}$ (mL/kg)	CL_0 (mL/hr/kg)	V_{periph} (mL/kg)	CL_d (mL/hr/kg)
Hubert	10	33.2	0.173	23.9	0.537
Hasso	10	32.4	0.179	28.1	0.753
Herkules	1	37.8	0.235	27.9	0.775
Heinz	1	38.6	0.222	25.0	0.947

CL_d = the distribution clearance between central and peripheral compartments; CL_0 = the baseline clearance (non-time dependent); $V_{central}$ = volume of central compartment; V_{periph} = volume of peripheral compartment.

No specific distribution, metabolism or excretion studies were conducted.

Note that obinutuzumab can cross the blood-placental barrier in monkeys as shown in the ePPND study.

5.2 Toxicokinetics

Refer to individual toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Not conducted

6.2 Repeat-Dose Toxicity

The following repeat dose toxicology studies were conducted:

1024830	RO5072759 13-Week Intravenous Administration Toxicity Study in the Cynomolgus Monkey with a 37-Week Recovery Phase

1036190	6-Month Infusion Toxicity and Toxicokinetic Study with RO5072759 in Cynomolgus Monkeys with a 37-Week Recovery Period
1024838	RO5072759 4-Week Subcutaneous Administration Toxicity Study in the Cynomolgus Monkey with a Recovery Phase

In addition, a non-GLP, 2-week repeat dose study in cynomolgus monkeys comparing obinutuzumab and rituximab was conducted (Report 1024829). This study was not reviewed, but summary information from the study report is provided as this is the only information regarding direct comparison of the toxicity of rituximab and obinutuzumab in monkeys:

Report 1024829: Preliminary toxicity study by intravenous bolus administration to cynomolgus monkeys

Methods

The preliminary study design was divided into two phases. In Phase I of the study, three cynomolgus monkeys, one male and two females (Group 1) received 1 mg/kg of the test substance, RO5072759, on two occasions (Days 1 and 8). A separate group of one male and one female (Group 3) received 1 mg/kg of rituximab on Days 1 and 8. In Phase II, 10 mg/kg RO5072759 was administered to a further two males and one female (Group 2) and an additional group of one male and one female received 10 mg/kg rituximab (Group 4) on Days 1 and 8.

Group	Treatment	Dosage (mg/kg/occasion)	No. of animals	
			Males	Females
1	RO5072759	1	1	2
2	RO5072759	10	2	1
3	Rituxan	1	1	1
4	Rituxan	10	1	1

Clinical condition, bodyweight, hematology, blood chemistry, B cell counts, cytokine assay, organ weight analysis, macroscopic and microscopic pathology investigations were evaluated.

Results

B-cell depletion of the peripheral blood, spleen and lymph node was observed with RO5072759 and rituximab but there were no differences between monkeys given RO5072759 or rituximab in the rate or extent of depletion of B cells or in rate or extent of recovery. There was evidence of T lymphocyte expansion in the spleen of animals receiving RO5072759, resulting in a greater T:B lymphocyte ratio when compared to rituximab (Rituxan).

Immunological investigations revealed that RO5072759 and rituximab both resulted in the depletion of peripheral blood, spleen and lymph node B lymphocytes after 2 weekly

doses at 1 or 10mg/kg. Peripheral blood recovery was 20-90% complete within 3 weeks of the second dose at 1mg/kg or <10% complete at 10mg/kg respectively. At 1 mg/kg B lymphocyte numbers had recovered in the spleen by 8 weeks after the last dose with both test substances and this was supported by no pathological abnormalities. At 10mg/kg splenic B lymphocytes had not recovered 4 weeks after the second dose of both test substances, confirmed by reduced cellularity of the lymphoid follicles. In the lymph nodes, incomplete recovery of B cell numbers, supported by reduced cellularity of the lymphoid follicles was apparent for both doses and both test substances. Apoptosis of the germinal centers in the mandibular lymph node was apparent for one monkey given 10 mg/kg RO5072759 and all animals given either dose level of rituximab. Depletion of B cells in the mesenteric lymph nodes was confirmed by CD 79a immunostaining.

Study title: 13-week intravenous administration toxicity study in the cynomolgus monkey with a 37-week recovery phase

Study no.:	1024830 ([redacted] (b) (4) # 2136-020)
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	June 6,2006
GLP compliance:	Yes; OECD
QA statement:	Yes, signed
Drug, lot #, and % purity:	RO5072759, Batch #'s GWT0021, GWT0022, GWT0024, GWT0025; Purity: ≥ 98.9% (SDS-PAGE and SE-HPLC)

Key Study Findings

- Two animals (1 male, 1 female) in the 100 mg/kg/week group were euthanized during the recovery phase. The male experienced a severe infection of the gingiva on day 97 of the recovery period and had other signs of poor condition. The female showed unusually strong menstrual bleedings, which resulted in severe anemia (noted at necropsy) and was terminated on recovery day 7 in poor general condition.
- B-cell ablation was evident at all doses. RO5072759 induced transient reductions in NK cells (presumably because of ADCC) in all dose levels following the first dose. Peripheral B-cells began to recover 17 and 23 weeks after the last treatment in the male and female high dose monkey, respectively. At the end of recovery, B-cell counts recovered to a level ~50% of predose values.
- Systemic inflammation was observed that correlated with toxicities in the brain, heart, lung, liver, spleen, stomach and kidney that were partially reversible.

- There was approximately linear increase in plasma concentrations of RO5072759 for both the C_{max} and $AUC_{(0-168h)}$ at all doses tested. There was evidence of immunogenicity, but exposure levels were maintained through Day 78 of the study.

Methods

Doses:	0, 10, 30, 100 mg/kg
Frequency of dosing:	Weekly for 13 weeks (13 total doses)
Route of administration:	Intravenous administration over 30 minutes
Dose volume:	10 mL/kg
Formulation/Vehicle:	20 mM Histidine, 240 mM Trehalose and (b) (4), pH 6.0, Batch #'s GWT0023, GWT0026
Species/Strain:	Cynomolgus monkey (Macaca fascicularis)
Number/Sex/Group:	3/sex/group main-study ; 2/sex/group recovery, control and 100 mg/kg groups only
Age:	3 to 6 years old
Weight:	3.2 to 7.2 kg
Deviation from study protocol:	None affected study validity or interpretation of results
Study design:	See below

Group number	Treatment code *	Group description	Dose level (mg/kg/week)	Animals/group male	Animals/group female	Necropsy after ... 13 weeks	Necropsy after ... 50 weeks
1	G1	control	0	5	5	3 M / 3F	2 M / 2 F
2	G2	low	10	3	3	3 M / 3F	
3	G3	intermediate	30	3	3	3 M / 3F	
4	G4	high	100	5	5	3 M / 3F	2 M / 2 F

* used in the Lims database to present the results

Parameters	Measurement Time
Necropsy:	Day 92 for main study animals and Day 260 for recovery males and 264 for recovery females
Clinical signs:	Two observations daily (one of which is cageside)
Body weights:	Once predose, weekly before dosing, weekly during the recovery phase, on the day before necropsy
Food consumption:	Not conducted
ECG:	Twice predose, before and within 1 hour after dosing on days 1 and 2, in weeks 4 and 13, and at the end of the recovery phase
Ophthalmoscopy:	Once predose and at necropsy
Hematology:	Predose, on day 2, in weeks 4 and 8, every 4 weeks during the recovery phase, at necropsy
Clinical chemistry:	Same as hematology

Coagulation:	Same as hematology
Urinalysis:	Same as hematology
Gross pathology:	Once predose and at necropsy
Organ weights:	At necropsy
Histopathology:	At necropsy
Toxicokinetics:	Main study animals: • Predose on Days 15, 22, 43, and 57 • On Days 1, 29, and 78 predose and at 0.083, 7, 24, 48, 96, and 168 hours after dosing Recovery animals: • Every two weeks after the last dosing until necropsy
Cytokine analysis	Same as hematology; INFγ, INFα, IL-2, IL-4, IL-6, IL-8, IL-10 were measured.
Immuno-phenotyping	Blood samples (approximately 2 mL) were withdrawn from all animals twice during the predose phase, on days 1 (before dosing), 3, and 8 (before dosing), in weeks 3, 5, 7, 9, 11, and 13, and from all surviving animals every second week during the recovery phase; measurements of T-cells (T-helper cells and cytotoxic T-cells), B-cells, and natural killer (NK) cells

Observations and Results

Mortality

There were two unscheduled deaths in the high-dose 100 mg/kg group (HD).

- A male animal was sacrificed moribund after showing a severe infection of the gingiva with multiple signs of a generally bad condition. A mouth swab investigation did not show the presence of known pathogens, but a high load of physiological microbes were detected. Test item induced immunosuppression, and therefore reduced resistance to normally well controlled bacteria, was considered a likely cause of the moribund condition. The severity of the infection was reflected by elevated levels of c-Reactive Protein (CRP), the general condition and by reduced glucose and albumin, as well as elevated bilirubin, creatinine, blood urea, GLDH, AST, ALT, and phosphate levels. Hematological parameters could not be determined.
- A female was terminated on Day 7 of the recovery phase in generally poor condition. She showed unusually strong menstrual bleedings, and severe anemia was noted at necropsy), and displayed dehydration and hypothermia. Clinical chemistry was consistent with dehydration. The strong, long-lasting menstrual bleedings were noted during the pre-dosing phase and were considered an individual variation. A combination of low body weight, severe anemia and possible infection contributed to the moribund state.

Clinical Signs

No test article-related clinical signs in animals with scheduled necropsy. One mid dose

male experienced purulent inflammation that was located on the hand (phlegmon).

Body Weights

Unremarkable

Food Consumption

Not conducted

Ophthalmoscopy

Unremarkable

ECG

Unremarkable

Hematology

Immunophenotyping results demonstrated a rapid loss of CD40+ B cells that persisted throughout the dosing period and during recovery (Table 10). At the end of recovery, B cells partially recovered in both males and females (N=1 for each). Natural killer cells (NK) were also decreased in all groups on Day 3, but the level was greater in GA101 treated animals. Decreases in NK cells persisted in males at the end of dosing.

Table 10 Changes in CD40+ B-cell numbers

Group	Pre-dose	Males					
		Day 3		Day 87/89		Day 232 (recovery)	
	10E9/L	10E9/L	%Δ	10E9/L	%Δ	10E9/L	%Δ
1	0.08	0.78	-3	0.85	6	0.48	-40
2	1.07	0.02*	-98	0.00*	-100		
3	0.09	0.01**	-99	0.00*	-100		
4	0.66	0.01***	-98	0.00**	-100	0.26	-61

Group	Pre-dose	Females					
		Day 3		Day 87/89		Day 232 (recovery)	
	E9/L	10E9/L	%Δ	10E9/L	%Δ	10E9/L	%Δ
1	0.69	0.84	22	0.54	-21	0.83	20
2	0.85	0.03*	-96	0.12*	-85		
3	0.43	0.01**	-98	0.00*	-100		
4	0.66	0.01***	-98	0.00**	-100	0.17	-74

*p≤0.05; **p≤0.01; ***p≤0.001
%Δ = percent change from predose value within the same group
Group 1 – control; Group 2 – 1 mg/kg; Group 3 – 3 mg/kg; Group 4 – 10 mg/kg

Table 11 Changes in CD16+ NK cell numbers

Group	Pre-dose	Males					
		Day 3		Day 87/89		Day 232 (recovery)	
	10E9/L	10E9/L	10E9/L	10E9/L	%Δ	10E9/L	%Δ
1	1.24	1.06	-15	1.35	9	1.23	-1
2	1.44	0.27	-81	0.8	-44		
3	1.38	0.47	-66	0.96	-30		
4	1.11	0.49	-56	0.77	-31	0.25	-77

Group	Pre-dose	Females					
		Day 3		Day 87/89		Day 232 (recovery)	
	10E9/L	10E9/L	%Δ	10E9/L	%Δ	10E9/L	%Δ
1	1.55	0.97	-37	1.16	-25	0.92	-41
2	1.84	0.44	-76	1.54	-16		
3	0.85	0.24*	-72	1.03	21		
4	1.72	0.55	-68	1.48	-14	0.96	-44

*p≤0.05
%Δ = percent change from predose value within the same group
Group 1 – control; Group 2 – 1 mg/kg; Group 3 – 3 mg/kg; Group 4 – 10 mg/kg

Clinical Chemistry

Elevations in creatinine, AST and ALT were observed during dosing Days 28-92 in the male mid-dose animal with the purulent hand. There were no values that demonstrated a dose-dependent or consistent difference when compared with controls.

Cytokine analysis showed great variation among control and treated animals, and no dose-related correlations were apparent.

Urinalysis

Unremarkable

Gross Pathology

The following findings occurred at a higher incidence in treated than control animals.

Table 12 Treatment-related macroscopic findings

No. of Animals Affected		Dosing								Recovery			
Organ	Finding	M (N=3)				F (N=3)				M (N=2)		F (N=2)	
	Group	1	2	3	4	1	2	3	4	1	4	1	4
Spleen	Large			1							1		
Adrenals	Discolored			1							1		
	Enlarged										1		
Liver	Discolored Focus				1								
	Mottled										1		
Testes	Discolored			1	1								
	Soft			1	2								
Ovaries	Cyst								1				1

Group 1 = control; Group 2 = 10 mg/kg; Group 3 = 30 mg/kg; Group 4 = 100 mg/kg

- At necropsy the moribund high dose female was thin, with watery blood and pale inner organs and mucous membranes. She had a moderate large uterus and moderate mucous discharge from the nose.

- The early decedent high dose male showed marked purulent lesion at the gingiva, generalized anemia with watery blood, large spleen, adrenals, and lumbar lymph nodes, mottled liver, pale skeletal muscle, hydropericardium and few red foci of the lung.

Organ Weights

- Elevated liver/brain weight ratios in the high dose (100 mg/kg/week) animals that were not seen by liver/body weight ratios.
- The mid-dose male with the purulent hand had markedly elevated liver and spleen weights.

Histopathology

Adequate Battery: Yes

Peer Review: Yes, signature provided.

Histological Findings:

Table 13 Select Microscopic Findings that Occurred at Higher Incidence in Treated than Control Animals (Dosing Phase)

Treatment-Related Microscopic Findings - Dosing Phase			No. of Animals Affected							
Organ	Finding	Severity	Males (N=3)				Females (N=3)			
Group			1	2	3	4	1	2	3	4
Brain	Inflammatory Cell Foci	minimal			2	2	2			2
		slight				1				
	Neuroaxonal Dystrophy	moderate			1					
	Pigment	slight			1					
	Neuronal Degeneration	moderate			1					
Spinal Cord Cervical	Inflammatory Cell Foci	minimal				1				1
Epididymides	Inflammation, Chronic	slight				1				
Eyes	Degeneration, Retina	minimal								1
	Keratitis	minimal								1
Gingiva		present			3					
Heart	Congestion	minimal								1
	Vacuolation	minimal						1		
		slight			1					
Kidneys	Inflammation, Subacute	slight			1					
	Hematopoiesis, Extramedullary	minimal			1					
	Mineralization/Cortex	minimal							1	
	Dilatation, Tubule (s)	slight						1		

Treatment-Related Microscopic Findings - Dosing Phase			No. of Animals Affected							
Organ	Finding	Severity	Males (N=3)				Females (N=3)			
Group			1	2	3	4	1	2	3	4
Intravenous Site	Inflammatory Cell Foci	minimal								2
LN, Mandibular	No germinal center	present		1	3	3		2	2	1
	Depletion B cells	minimal								3
		slight			2			2	1	
		moderate		1						
	Hematopoiesis, Extramedullary	minimal		1	1		1		1	
		slight						1		2
	CD20 positive	minimal							1	3
		slight	1				1	1		
		moderate	2				2	1		
	CD20 negative	present		3	3	3		1	2	
LN, Mesenteric	No germinal center	present		3	3	3		1	2	3
	Depletion B cells	slight			1			1		
		moderate			1					
	CD20 positive	minimal							1	3
		slight					1	1		
		moderate	2				2	1		
		marked	1							
	CD20 negative	present		3	3	3		1	2	
Liver	Pigment, Macrophages	minimal				1				
	Inflammation, Acute	moderate				1				
	Sinus Leucocytosis	minimal				1				
		moderate			1					
	Vacuolation, Hepatocyte	minimal	1							
		moderate								1
	Hematopoiesis, Extramedullary	minimal			1					1
	Fibrosis	minimal		1			1		1	
	Hyperplasia, Kupffer Cell	minimal			1					
	Vacuolation/Necrosis	minimal						1		
Lung	Fibrosis	minimal				2				
	Inflammation, Acute	minimal								1
		slight						1		
Parathyroids	Cyst	minimal								1
Prostate	Inflammation, Subacute	minimal				1				
		slight				1				
	Inflammatory Cell Foci	minimal	1	2	2	2				
Skin	Scab	present							1	1
	Inflammation, Acute	minimal								1
		slight							1	
	Inflammation, Subacute	minimal				1				
		slight			1					
Sciatic nerve	Inflammatory Cell Foci	minimal			2	2	1		1	2
		slight				1				
Spleen	No germinal center	present		2	3	3		2	3	3

Treatment-Related Microscopic Findings - Dosing Phase			No. of Animals Affected							
Organ	Finding	Severity	Males (N=3)				Females (N=3)			
Group			1	2	3	4	1	2	3	4
	Depletion B cells	minimal				1				
		slight		2	1	1		1	1	1
		moderate			2				2	1
		marked						1		
	CD20 positive	minimal								1
		slight						1		
		moderate	2				3	1		
		marked	1							
	CD20 negative	present		3	3	3		1	3	2
Spinal Cord Thoracic	Inflammatory Cell Foci	minimal								2
Stomach	Erosion	minimal						1		1
	Inflammation, Acute	moderate								1
	Dilated Gland	minimal				1				
Testes	Adolescent/Immature	present			1	1				
Thyroid	Infiltrate, lymphocytes	minimal	1			3	1			
	Foamy Histiocytes	minimal				1				
	Hyperplasia, Follicular Cell	minimal				2	1			
	Cyst, Ultimobranchial	minimal								1
Thymus	Involution	minimal	1				1			
		slight			1		2		1	
		moderate			1					
		marked			1					
		severe						1		
	CD20 positive	minimal	2				3	2	1	
		slight	1							
	CD20 negative	present		3	3	3			2	3

Group 1 = control; Group 2 = 10 mg/kg; Group 3 = 30 mg/kg; Group 4 = 100 mg/kg

Table 14 Select microscopic findings that occurred at higher incidence in treated than control animals (Recovery Phase)

Treatment-Related Microscopic Findings – Recovery Phase			No. of Animals Affected			
Organ	Finding	Severity	Males (N=2)		Females (N=2)	
Group			1	4	1	4
Adrenals	Hyperplasia	slight				1
		moderate		1		
Brain	Inflammatory cell foci	Minimal	1	1		1
Gingiva	Inflammation, Subacute	moderate		1		
	Ulcer	moderate		1		
Intravenous Site	Inflammation, Subacute	minimal				1
		moderate		1		
Heart	Inflammation, Subacute	minimal				1
		slight		1		
	Degeneration	minimal				1

Treatment-Related Microscopic Findings – Recovery Phase			No. of Animals Affected			
Organ	**Finding**	**Severity**	**Males (N=2)**		**Females (N=2)**	
Group			**1**	**4**	**1**	**4**
Kidneys	Inflammation, Subacute	moderate		1		
	Dilatation, Tubule(s)	slight		1		
	Fibrosis	moderate		1		
LN, Mandibular	No germinal center	present		1		1
	Depletion B cells	marked		1		
	CD20 positive	minimal		1		
		moderate	2	1	2	1
	CD20 negative	present				1
	Inflammation, Subacute	moderate		1		
	Inflammation, Chronic	minimal		1		
	Fibrosis, Capsule	moderate		1		
	Vacuolation	minimal				1
LN, Mesenteric	No germinal center	present		1		1
	Depletion B cells	marked		1		
	CD20 positive	minimal		1		
		slight		1		
		moderate	2		2	1
	CD20 negative	present				1
Liver	Inflammation, Acute	moderate		1		
	Sinus Leucocytosis	minimal				1
		slight		1		
	Vacuolation, Hepatocyte	minimal				1
		moderate		1		
	Hematopoiesis, Extramedullary	slight		1		
	Hyperplasia, Kupffer Cell	moderate		1		
	Necrosis	marked		1		
Lung	Hemorrhage	minimal		1		
Ovary	Cyst, Follicle	marked				1
Prostate	Inflammatory Cell Foci	minimal		2		
SG, Mandibular	Degranulation	slight		1		
		moderate				1
Sciatic nerve	Inflammatory Cell Foci	minimal		1		1
Spleen	No germinal center	present		1		1
	Depletion B cells	marked		1		1
	Congestion	moderate		1		
	CD20 positive	minimal		1		
		moderate	2	1	2	1
	CD20 negative	present				1
	Lymphoid Atrophy	severe				1
Stomach	Dilated Gland					1
Thyroid	Infiltrate, lymphocytes	minimal		1		
	Fibrosis	minimal		1		

Treatment-Related Microscopic Findings – Recovery Phase			No. of Animals Affected			
Organ	**Finding**	**Severity**	**Males (N=2)**		**Females (N=2)**	
Group			**1**	**4**	**1**	**4**
	Hyperplasia, C-cell	minimal		1		
Thymus	Involution	minimal	1			
		moderate				1
		marked		1		
	CD20 positive	minimal			1	1
		slight	2	1	1	
	CD20 negative	present		1		1

Group 1 = control; Group 4 = 100 mg/kg

- In the moribund recovery male, subacute inflammation was found in all organs of the digestive system and parasite granuloma in the stomach.

- Inflammatory cell foci were observed in the brain, spinal cord, and sciatic nerve in intermediate and high dose groups. The toxicological significance of this finding is unclear.

- No observable effects on bone marrow.

- The expected pharmacological effect of lack of germinal centers, B cell depletion, and depletion of CD20-positive cells was observed microscopically during the dosing and recovery phases.

- Microscopic findings in the liver, including inflammation, sinus leukocytosis, and hepatocyte vacuolation were observed in the dosing and recovery phases at doses ≥ 30 mg/kg and moderate Kupffer cell hyperplasia and marked necrosis were observed in the moribund high-dose male.

Immunogenicity

Anti-RO5072759 antibodies were determined using an ELISA. In group two (10 mg/kg), one male (23539M) and two females (23690F and 23716F) had detectable anti-RO5072759 antibodies on days 22-57, and Day 78, respectively. The higher dose groups (30 and 100 mg/kg) were not tested or produced negative results due to the high concentrations of RO5072759 that would interfere with the ELISA assay.

- It is likely that one male from the 30 mg/kg group developed anti-RO5072759 antibodies, since from day 15 onwards the measured RO5072759 concentration levels were significantly lower compared to those of the other study animals of this group. The same is true for one male in the 100 mg/kg group in which RO5072759 concentrations were significantly lower and/or decreased more quickly from day 29 onwards compared to the other subjects of the same group.

Toxicokinetics

Overall, exposure levels were maintained throughout the duration of the study based on the C_{max} and AUC_{0-168h} levels measured on day 78 of the study. There was an approximately linear increase in plasma concentrations of RO5072759 for both the C_{max} and $AUC_{(0-168h)}$ at all doses tested during the first dosing interval. After weekly administration, anti-drug antibody formation appeared to impact RO5072759 serum concentrations in low dose group, particularly for females.

Table 15 Mean TK parameters (mean) for RO5072759 (13-week study)

Dose	Males			Females		
	Day 1	Day 29	Day 78	Day 1	Day 29	Day 78
10 mg/kg						
AUC0-168h (hr*µg/mL)	22000	37400	59900	20100	7410	10900
Cmax (µg/mL)	268	588	606	273	299	332
30 mg/kg						
AUC0-168h (hr*µg/mL)	69100	134000	154000	72700	118000	139000
Cmax (µg/mL)	907	1320	1610	966	1470	1470
100 mg/kg						
AUC0-168h (hr*µg/mL)	219000	456000	470000	226000	368000	462000
Cmax (µg/mL)	3080	4970	5110	3350	4460	5210

AUC and Cmax means were rounded by the reviewer.

Dosing Solution Analysis

Dosing solutions were confirmed to be of the expected concentration and purity and remained stable.

Study title: RO5072759 4-week subcutaneous administration toxicity study in the cynomolgus monkey with a recovery phase

Study no.:	1024838
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 19, 20006
GLP compliance:	Yes; OECD
QA statement:	Yes
Drug, lot #, and % purity:	RO507-2759/F01-01, Lot # GWT0024 and GWT0025, Purity 98.9%

Key Study Findings

- One male (120 mg/week dose group) diagnosed with low grade peritonitis/pleuritis and diarrhea was sacrificed on Day 89 of the recovery phase. B-cell depletion and immunosuppression may have reduced the ability to recover from infections.
- Increased/decreased levels of white blood cells, reticulocyte counts, and coagulation parameters occurred during the dosing and recovery phases at 30 or 120 mg/animal/week.
- High creatinine in males and lower BUN in females observed during the dosing phase in some 120 mg/animal/week males and females with no histopathology correlates in the kidney.
- Extensive depletion of circulating blood B cells occurred by Day 3 after the first dose at 30 or 120 mg/animal/week accompanied with reduced number of CD16+ NK cells and increased T-cell populations (CD3+, CD4+, and CD8+). Recovery of B-cell populations was slow and took as long as 28 weeks in the 120 mg/animal/week group.
- Target organs based on the mode of action of RO5072759 included the axillary, mandibular and mesenteric lymph nodes, spleen, and thymus. Additional RO5072759-related microscopic findings included fibrosis in the heart and findings in the liver of hyperplasia of Kupffer cells, sinus leucocytosis and histiocytosis and/or necrosis of individual hepatocytes mostly present in 30 or 120 mg/animal/week monkeys. Microscopic findings in target organs were still present at the end of the recovery. Microscopic findings in the heart, kidney, liver, and testes were present in the single male surviving at the end of recovery

Methods

Doses:	0, 30 or 120 mg/animal/week
Frequency of dosing:	Once weekly (Days 1, 8, 15, 22 and 29)
Route of administration:	Subcutaneous; animals were given up to 4 separate injections on each dosing day with 3 ml/site each.
Dose volume:	Groups 1 and 3 received 12 ml/animal; Group 2 received 3 ml/animal
Formulation/Vehicle:	Sterile aqueous solution containing 20 mM Histidine, 240 mM Trehalose and (b) (4) , pH 6.0
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*) of Mauritian origin
Number/Sex/Group:	5/sex/group (3/main and 2/recovery)
Age:	Male and Female: 4.5 to 6 years
Weight:	Male: 4.4 to 7.2 kg; Female: 2.9 to 4.4 kg
Satellite groups:	None
Unique study design:	3/sex/group necropsied at end of dosing period (4 weeks) and 2/sex/group assigned for 28-week recovery
Deviation from study protocol:	No impact on study conduct or data interpretation

Parameter	Timepoint
Necropsy:	In life phase terminated 3 days after the last dose for main study animals and after the 28 week recovery period.
Clinical signs:	Twice daily.
Body weights:	Twice weekly
Clinical pathology:	Predose, during weeks 2 and 4 during dosing and during week 24 and at the end of the recovery period.
Urinalysis:	Same as hematology.
Toxicokinetics:	Samples were collected pre-dose, 6, 24, 72 and 168 hours after dosing. Samples were collected every two weeks during recovery.
Immuno-phenotyping	Blood samples were taken predose, Days 1, 3, 8, 15, 17, 22 and 29 (before dosing) and every two weeks during recovery. Small pieces of spleen and lymph nodes were also collected at necropsy. Cell types measured are T-, B-, and NK-cells.

Observations and Results

Mortality

One male dosed at 120 mg/week was euthanized on day 89 of the recovery phase in moribund condition. The animal showed severe diarrhea, hypoactivity and severe body

weight loss. The animal was treated with antibiotics on day 77 of recovery to treat a high fever, but continued to deteriorate. Macro observations included distended large intestine with pulpy contents, enlarged liver and semi fluid contents of the gall bladder. Hematology and clinical chemistry showed abnormal values consistent with infection and bleeding (increased WBC, reticulocytes, platelets and decreased Hb and hematocrit). B-cell depletion and consequent impairment of the immune system may have caused an increased susceptibility and inability to recover from infections.

Clinical Signs

No RO5072759-related clinical signs occurred except for clinical signs in the male sacrificed in moribund condition.

Body Weights

Male mean body weight was similar to the control group during the dosing and recovery phase. One 120 mg/animal/week male lost approximately 9% of body weight during the last week of dosing without incidence of clinical signs.

Female mean body weight was similar to the control group during the dosing and recovery phase. Mean body weight for the 120 mg/animal/week female group was statistically significant lower compared to control at the end of the dosing phase because:

- Female monkeys assigned for terminal sacrifice had 0.1 kg less compared to their own body weight at the start of the dosing; and,
- Females assigned for the recovery phase were the heaviest monkeys in the group.

The statically significance is of no biological relevance.

Food consumption was not measured.

Hematology

During the dosing phase, male and female monkeys dosed at 30 or 120 mg/animal/week RO5072759 had reduced mean WBC with statistically significant lower values in males (-1.3 fold) on Dosing Day 13 compared to control. Mean WBC values were comparable to control values or higher by the end of the recovery on Day 200. Mean values for reticulocyte counts, total reticulocyte counts and band neutrophils were increased during the dosing phase with some significantly increased values in 30 mg/animal/week female (Table 16). Mean values for reticulocyte and total reticulocyte counts were reduced (-1.4 fold in males and -1.7 fold in females) in 120 mg/animal/week by the end of the recovery phase. Taken together, these results may reflect inflammation or infection present during the dosing phase.

Mean APTT values were significantly lower in female monkeys (-1.3 to -1.4 fold) at both doses on Dosing Day 13 compared with control, Table 16, with decreased values by the end of recovery. Mean APTT values in males were higher (+1.2 to +1.3 fold) at both

doses by the end of the recovery phase. Mean fibrinogen values were significantly increased in males and increased in 120 mg/animal/week females compared with control on Dosing Day 26. Mean values for fibrinogen in male and female at both doses were decreased (-1.2 to -1.6 fold) compared with control by the end of the recovery phase.

Table 16 Fold-Change in Hematological Parameters in 4-Week Subcutaneous Dosing Monkey Study

Parameter	RO5072759 mg/animal/week			
	30		120	
	M	F	M	F
White Blood Cells – WBC				
DSGN 13	**-1.3**	-1.2	**-1.3**	1
DSGN 26	-1.2	+1.3	-1.2	1
REC 200	+1.4	-1.3	+1.2	+1.1
Reticulocyte counts – RETI				
DSGN 13	+1.8	-1.2	+1.4	-1.1
DSGN 26	+1.5	**+1.9**	+1.7	+1.1
REC 200	+1.3	-1.2	-1.4	-1.7
Absolute Reticulocyte counts –TRET				
DSGN 13	+1.7	-1.2	+1.4	-1.1
DSGN 26	+1.4	**+1.8**	+1.8	+1.1
REC 200	+1.3	-1.2	-1.4	-1.7
Band Neutrophils – BNEU				
DSGN 13	1	+2	+2	+2
DSGN 26	1	+3	+2	**+4**
REC 200	+5	0	0	+2
APTT				
DSGN 13	-1.1	**-1.4**	1	**-1.3**
DSGN 26	-1.1	-1.1	-1.1	-1.3
REC 200	+1.3	-1.3	+1.2	-1.2
Fibrinogen – FIB				
DSGN 13	-1.3	-1.1	-1.1	-1.2
DSGN 26	-1.1	-1.1	**+1.3**	+1.1
REC 200	-1.6	-1.2	-1.2	-1.6

Values in **bold** were significantly different from control
DSGN: dosing; REC: recovery

Clinical Chemistry

Blood samples were taken before dosing, in weeks 2 and 4, Dosing Days 13 and 26, respectively, and on Recovery Days 83, 89, 158, 168 and 200. Individual 120

mg/animal/week male and female monkeys presented high creatinine values on Dosing Day 26 ranging from +1.5 to +3.2 fold increases compared to predose values. Some 120 mg/animal/week females presented significantly lower blood urea nitrogen on Dosing Day 13. High creatinine or lower BUN values had no histopathology correlates in the kidney and values were similar to control animals by the end of recovery.

Male and female monkeys assigned to the 30 and 120 mg/animal/week groups presented high individual bilirubin values at predose and this trend continued during the dosing phase; therefore, the relationship to administration of RO5072759 is unclear. Higher inorganic phosphorous, potassium and chloride values compared to predose values were observed sporadically during the dosing phase.

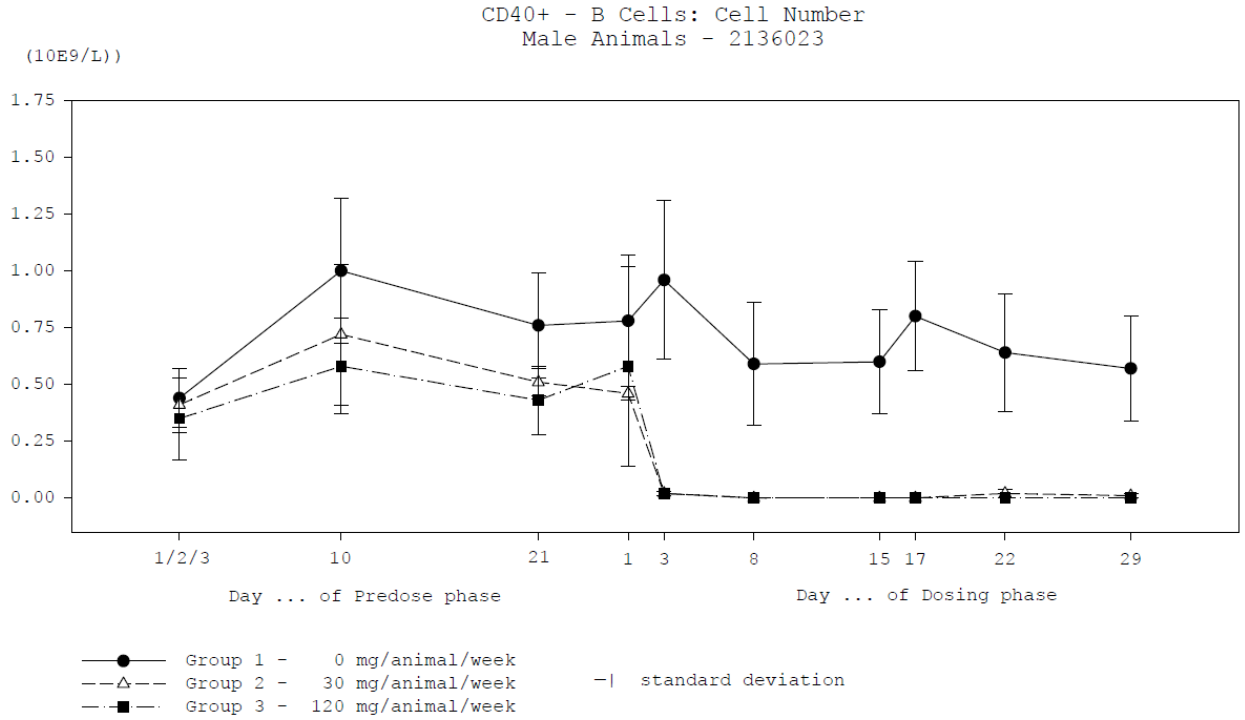
No RO5072759-related significant changes occurred in IgG and IgM measurements.

Immunophenotyping by Flow Cytometry

The intended pharmacology of RO5072759 is depletion/reduction of CD40+ B cells. Subcutaneous administration of RO5072759 produced an almost complete depletion of CD40+ B cells in both male and female monkeys by Day 3 after the first dose at 30 or 120 mg/animal and cells remained depleted during the entire dosing phase (Figure 12). At the 30 mg/animal/week dose, cell numbers in males started recovering by Day 28 and were at similar levels as the control males by Day 98 of the recovery phase (Figure 13). Cell numbers in females at the same dose showed only partial recovery during the recovery phase.

Figure 12 Mean CD40+ B Cells in Monkeys Dosed Subcutaneously with RO5072759

(Excerpted from Applicant's Submission)



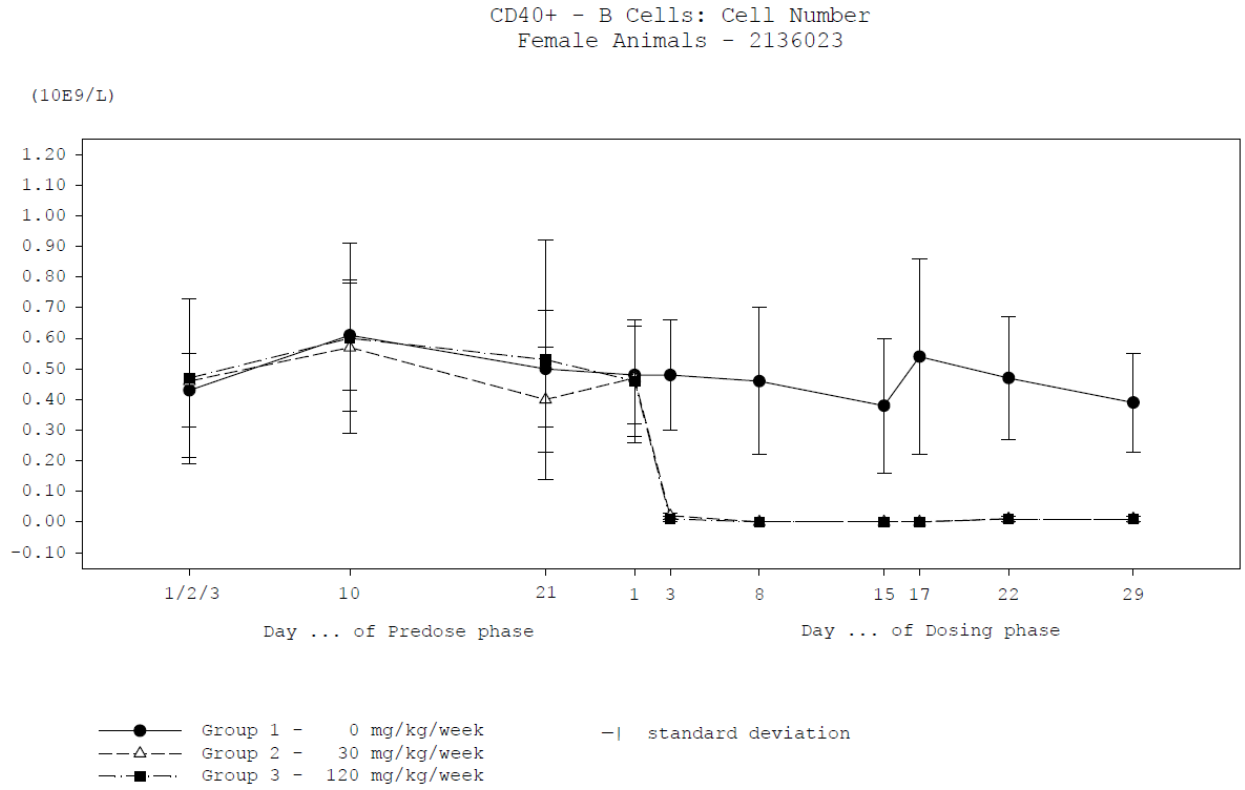
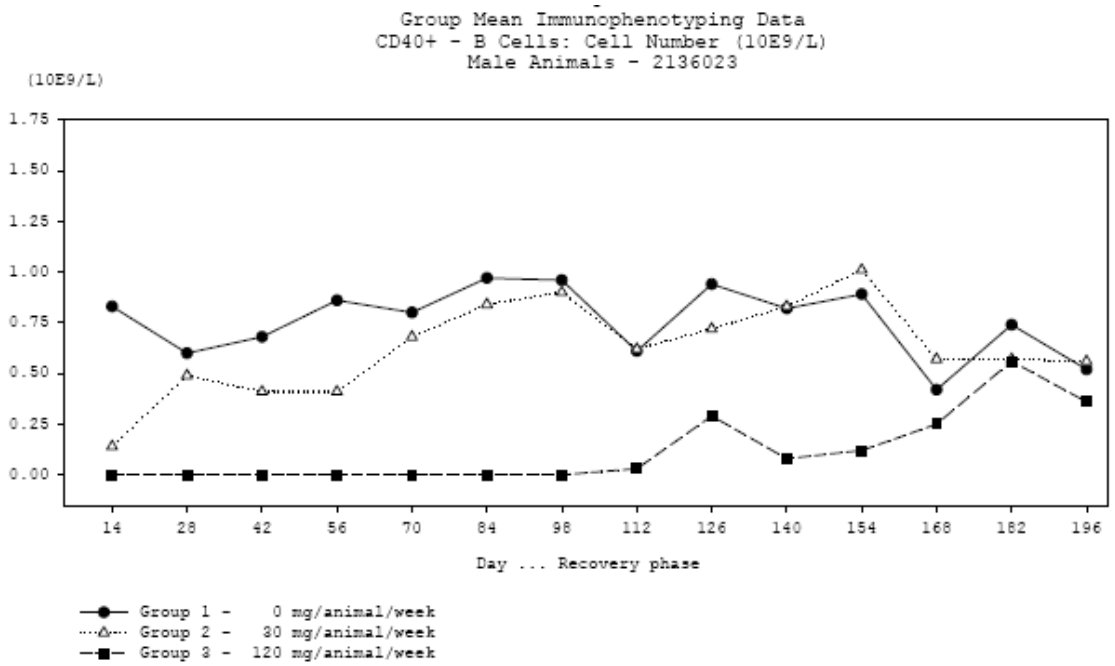
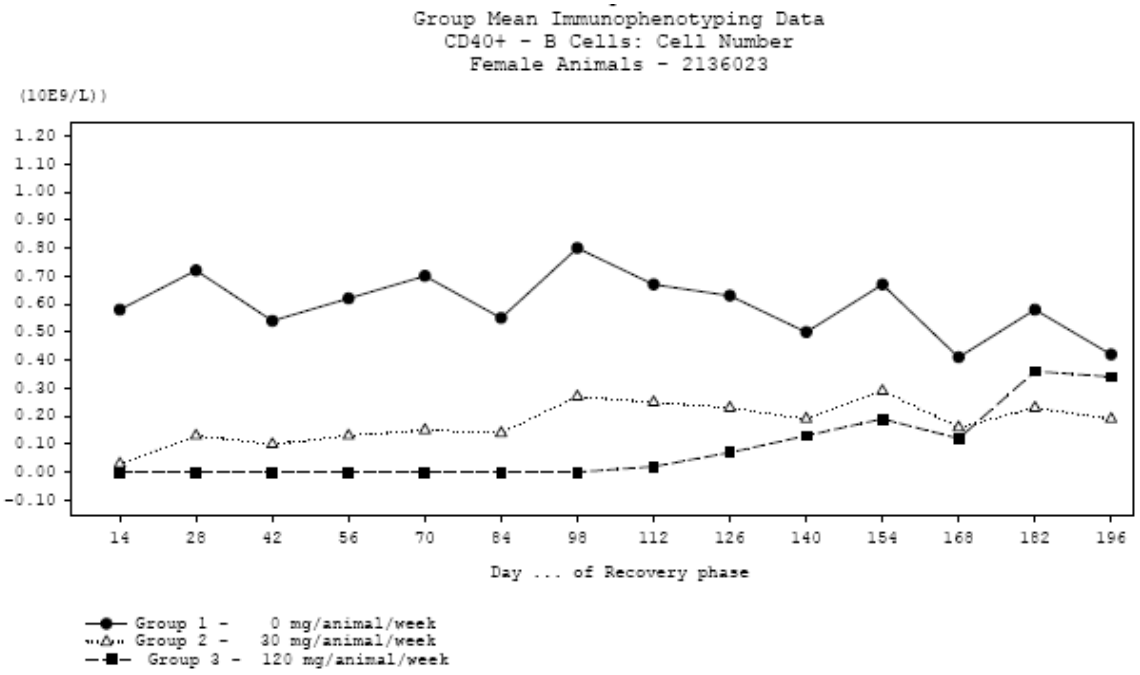


Figure 13 Mean CD40+ B Cells during recovery after subcutaneous administration of RO5072759

(Excerpted from Applicant's Submission)





Depletion of CD40+ B cells was also observed in lymph nodes and spleen tissues from monkeys at 30 or 120 mg/animal/week RO5072759 collected at terminal sacrifice. Examined tissues from recovery animal showed a trend for recovery of CD40+ B cell population. A reduced number of CD16+ NK cells and an increase in other lymphocyte populations were observed, see Table 17.

Table 17 Lymphocytes in Lymphoid Tissues from Monkeys Dosed Subcutaneously with RO5072759

Parameter	RO5072759 mg/animal/week					
	0		30		120	
	M	F	M	F	M	F
	Percent of Lymphocytes (%)					
CD40+ B Cells						
DSGN 1	14.7	11.1	10.7	13.5	13.2	14.8
DSGN 3	16.7	9.7	**0.7**	**0.7**	**0.5**	**0.5**
DSGN 29	15.3	9.6	**0.1**	**0.3**	**0.1**	**0.1**
REC 196	10.4	7.3	10.0	4.6	11.1	7.2
CD16+ NK Cells						
DSGN 1	20.0	25.6	20.6	14.6	17.7	**13.0**
DSGN 3	18.6	26.0	14.6	**11.4**	10.9	**15.4**
DSGN 29	23.0	26.6	23.6	**12.9**	14.0	**12.0**
REC 196	24.8	18.4	21.2	15.6	9.5	12.0
CD3+ T Cells						
DSGN 1	62.8	57.3	66.6	68.6	66.1	66.4
DSGN 3	60.8	59.3	**82.5**	**85.0**	**87.0**	**81.1**
DSGN 29	55.7	59.3	**76.0**	**85.0**	**84.7**	**84.9**
REC 196	58.8	69.4	62.6	75.3	76.7	76.5
CD4+ T Cells						
DSGN 1	35.7	29.7	41.7	**40.0**	32.0	36.7
DSGN 3	33.4	30.4	**52.3**	**49.2**	**46.4**	**45.6**
DSGN 29	33.0	31.5	**46.1**	**50.0**	43.0	**45.9**
REC 196	29.2	35.4	36.4	36.2	36.6	37.4
CD8+ T Cells						
DSGN 1	21.8	21.9	18.1	21.8	27.5	22.0
DSGN 3	22.4	23.8	21.3	27.8	**32.8**	26.3
DSGN 29	20.1	22.9	23.2	28.4	**33.9**	29.3
REC 196	27.2	29.6	20.6	32.8	33.7	32.4

Values in **bold** were significantly different from control
DSGN: dosing; REC: recovery

Urinalysis

Unremarkable

Gross Pathology

Macroscopic findings observed at the end of the dosing and recovery phases are shown in Table 18.

Table 18 Macroscopic findings in Subcutaneous Dosing Study

Tissue / Lesion	Number of Monkeys Affected					
	Males			Females		
Dose (mg/animal/week)	0	30	120	0	30	120
Number of monkeys examined	3	3	2	3	3	3
End of Dosing Phase						
SPLEEN						
Large	0	0	0	0	0	1
CECUM						
Discolored	0	0	0	0	0	1
KIDNEYS						
Discolored focus	0	0	1	0	0	0
Congested vessel	0	0	1	1	1	0
LIVER						
Discolored focus	0	1	0	0	0	0
HEART						
Nodule	0	0	1	0	0	0
Recovery Phase						
Number of monkeys examined	2	2	1	2	2	2
CECUM						
Distended	0	0	0	0	0	1
COLON						
Distended	0	0	1	0	0	0
Abnormal contents	0	0	1	0	0	0
RECTUM						
Distended	0	0	1	0	0	0
Abnormal contents	0	0	1	0	0	0
LIVER						
Large	0	0	1	0	0	0
GALL BLADDER						
Abnormal contents	0	0	1	0	0	0

Organ Weight

Spleen mean organ to body weight ratio was significantly higher (+1.8 fold) in 120 mg/animal/week males and significantly lower (-1.4 fold) in 30 mg/animal/week females compared to control. Spleen ratio values remained high for recovery males but values were similar in females compared to control. Thymus ratio was lower in males at 30 and 120 mg/animal/week and in females at 30 mg/kg/week showing the same trend in recovery animals.

Testes weight was significantly lower in 120 mg/animal/week males with a reduced ratio as well. This male group also presented lower ratios for epididymis and seminal vesicles at the end of the dosing phase. Testes and seminal vesicle ratio values were similar to control by the end of recovery phase and epididymis ratio remained lower compared to control. Organ to brain ratio values followed a similar trend as the organ to body weight ratio values.

Table 19 Fold-Change in Organ Weight Ratios in Monkeys Dosed Subcutaneously with RO5072759

Organ	Ratio Organ/Body Weight			
	Males		Females	
Dose (mg/animal/week)	30	120	30	120
End of Dosing Phase – N=	3	3	3	3
Spleen	+1.1	**+1.8**	**-1.4**	+1.8
Thymus	-1.6	-1.3	-1.3	1.0
Epididymis	1.0	-0.8	--	--
Testis	1.0	-0.8	--	--
Seminal vesicles	+1.7	-1.3		
Recovery Phase – N=	2	1	2	2
Spleen	+1.5	+1.7	1.0	1.0
Thymus	+1.9	+2.6	-1.8	-2.6
Epididymis	+1.1	-0.8	--	--
Testis	+1.2	+1.1	--	--
Seminal vesicles	+2.1	1.0	--	--
	Ratio Organ/Brain Weight			
End of Dosing Phase – N=	3	3	3	3
Spleen	+1.2	+1.6	**-1.4**	+1.5
Thymus	-1.5	-1.4	-1.4	-1.1
Epididymis	+1.2	-1.4	--	--
Testis	+1.1	**-1.5**	--	--
Seminal vesicles	+1.9	-1.4	--	--
Recovery Phase – N=	2	1	2	2
Spleen	+1.2	+1.4	-1.2	1.0
Thymus	+1.5	+2.2	-2.2	-2.9
Epididymis	-1.3	-1.4	--	--
Testis	-1.1	-1.1	--	--
Seminal vesicles	+1.8	-1.2	--	--

-- Tissue no present
Values in **bold** were significantly different from control

Histopathology

Microscopic findings were graded by the pathologist as present and on a scale 1 to 5, minimal<slight<moderate<marked<severe, according to the intensity and extent of change.

Adequate Battery: **YES**

Peer Review: **YES**

Target organs based on the mode of action of RO5072759 included the axillary, mandibular and mesenteric lymph nodes, spleen, and thymus. Additional microscopic findings RO5072759-related included fibrosis in the heart and findings in the liver because they were mostly present in 30 or 120 mg/animal/week monkeys.

Table 20 Microscopic findings in Monkeys after Subcutaneous Dosing

Microscopic Finding			Number of Monkeys Affected					
			Males			Females		
Dose (mg/animal/week)			0	30	120	0	30	120
Number of monkeys examined			3	3	2	3	3	3
End of Dosing Phase								
Aorta	Hyperplasia intima,	minimal	1	0	2	0	1	1
Axillary lymph node	No germinal center B-cell depletion	present	0	3	3	0	3	3
		moderate	0	1	1	0	2	1
		slight	0	1	0	0	1	1
Heart	Fibrosis	minimal	0	0	1	0	0	1
Kidney	Cyst	moderate	0	0	1	0	0	1
Liver	Hyperplasia Kupffer cell	slight	0	0	2	0	0	0
	Sinus leucocytosis	moderate	0	0	0	0	0	1
	Sinus Histiocytosis	minimal	0	0	1	0	0	0
	Necrosis individual	minimal	0	0	1	0	0	1
	hepatocytes	minimal	0	1	0	0	0	0
Mandibular lymph node	No germinal center B-cell depletion	present	0	3	3	0	2	3
		slight	0	0	2	0	1	0
		moderate	0	1	0	0	1	1
Mesenteric lymph node	No germinal center B-cell depletion	present	0	3	3	0	1	3
		slight	0	1	1	0	0	0
		moderate	0	0	0	0	1	2
	Sinusoids dilation	moderate	0	0	0	0	0	1
Pituitary	Cyst	minimal	0	2	0	0	0	2
Spleen	No germinal center B-cell depletion	present	0	3	3	0	3	3
		slight	0	1	1	0	3	1
		moderate	0	2	2	0	0	2
Thymus	Involution	minimal	0	0	0	0	0	1
		slight	1	0	0	0	0	0
		moderate	0	1	0	0	2	1
		marked	0	0	1	0	0	0
	Recovery Phase							
Number of monkeys examined			2	2	1	2	2	2
Heart	Inflammatory cell foci	minimal	0	0	0	0	1	0
		slight	0	0	0	0	1	0
	Subacute inflammation	minimal	0	0	0	0	0	1
	Hemorrhage	slight	0	1	0	0	0	0
	Hypertrophy/ degeneration	slight	0	0	0	0	0	1

Microscopic findings in recovery animals included isolated minimal findings in the target organs lymph nodes, spleen and thymus. Additional findings in the heart were exclusively present in RO5072759-treated monkeys. The single male surviving at the end of the recovery presented subacute inflammation of the epididymides and testes. The same male also presented decreased organ to brain weight ratio for the epididymides and testes.

Immunostaining of Immune Organs (Spleen, Thymus, Lymph Nodes)

CD20:
Staining of B-cells was observed in controls, to a lesser degree in Group 2 animals and minimally in Group 3 animals. Diminished immunoreactivity corresponded to B-cell depletion and the lack of germinal centers in the lymphoid organs seen in H-E sections. Recovery animals did not show differences in immunostaining between controls and treatment groups.

CD3/CD4/CD8:
There were no differences in immunostaining of T-cell populations between the control and treatment groups.

CD16
A clear differentiation between macrophages and NK cells was not possible with CD16 staining alone and sections from control and treatment groups stained similarly.

Immunogenicity

An ELISA was used to determine qualitatively whether ADA formation occurred. Antibodies against RO5072759 were determined in 37/491 serum samples. Five Group 2 animals tested positive (23566M, 23573M, 23603F, 23707F, 23954F) and one Group 3 animal on Day 225 tested positive for ADA formation (23962F). All the pre-dose samples on Day 1 were negative.

Toxicokinetics

After subcutaneous injection administration on Day 1, systemic exposure to RO5072759, as measured by C_{max} and AUC ($_{0\text{-}168h}$) were dose proportional and generally similar in both male and female monkeys.

After repeated dosing (Day 22), high inter-individual variability in serum concentrations and TK parameters (Table 21) was observed caused by the different degrees of immunogenicity. The high variability of data on Day 22 precludes interpretation on accumulation and gender differences after repeated dosing.

Exposure in recovery animals was detected up to week 6 weeks in the 30 mg/animal/week and up to 14 or 16 weeks in males and females at 120 mg/animal/week, respectively.

Table 21 Individual Dose-Normalized TK Parameters After Subcutaneous Administration

(Excerpted from Applicant's Submission)

group	dose [mg/animal]	day	gender	subject	Cmax/dose [ng/mL]/[mg/animal]	AUC(0-168h)/dose [h·ng/mL]/[mg/animal]
G2	30	1	m	23325M	1440	206000
G2	30	1	m	23327M	1880	255000
G2	30	1	m	23330M	1490	209000
G2	30	1	m	23566M	1360	186000
G2	30	1	m	23573M	1910	255000
G2	30	1	f	23603F	2660	376000
G2	30	1	f	23707F	2040	270000
G2	30	1	f	23954F	2010	279000
G2	30	1	f	23959F	1870	258000
G2	30	1	f	23960F	2150	298000
G2	30	22	m	23325M	1520	226000
G2	30	22	m	23327M	923	119000
G2	30	22	m	23330M	2820	360000
G2	30	22	m	23566M	181	22400
G2	30	22	m	23573M	1380	169000
G2	30	22	f	23603F	423	19800
G2	30	22	f	23707F	423	25700
G2	30	22	f	23954F	363	14500
G2	30	22	f	23959F	3600	527000
G2	30	22	f	23960F	2510	363000
G3	120	1	m	23559M	1600	240000
G3	120	1	m	23563M	2130	295000
G3	120	1	m	23571M	1520	230000
G3	120	1	m	23581M	1870	266000
G3	120	1	m	23649M	1450	210000
G3	120	1	f	23593F	2530	312000
G3	120	1	f	23597F	2540	350000
G3	120	1	f	23618F	2720	359000
G3	120	1	f	23958F	1660	230000
G3	120	1	f	23962F	1680	209000
G3	120	22	m	23559M	2730	303000
G3	120	22	m	23563M	1930	234000
G3	120	22	m	23571M	1830	268000
G3	120	22	m	23581M	4280	654000
G3	120	22	m	23649M	1330	193000
G3	120	22	f	23593F	2300	235000
G3	120	22	f	23597F	3870	520000
G3	120	22	f	23618F	4030	478000
G3	120	22	f	23958F	3420	466000
G3	120	22	f	23962F	1860	238000

Dosing Solution Analysis

RO5072759/F01-01 was the formulation code given to the lots of RO5072759 used in the study and prepared in the formulation vehicle as described in Methods. Two parameters were measured for two sample vials from each lot:

1. Protein Content. Measured values of 10.5 and 10.4 mg/mL for lots GWT0024 and GWT0025 showed no significant difference from the respective values given in the certificate of analysis of 10.4 and 10.3 mg/mL, respectively.
2. Purity. Size exclusion chromatography (SEC) measured value of 98.9% monomeric antibody for all samples tested showed no significant difference from the respective value given in the certificate of analysis of 98.9%.

Stability: RO5072759/F01-01 was stable over the entire course of the study based on the results from the concentration measurements and SEC analysis at the end of dosing.

Study title: 6-Month Infusion Toxicity and Toxicokinetic Study with RO5072759 in Cynomolgus Monkeys with a 37-Week Recovery Period

Study report no.:	1036190
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 29, 2009
GLP compliance:	Yes, except for immunohistochemical (IHC) staining, immunogenicity analysis and serum cytokine analysis.
QA statement:	Yes
Drug, lot #, and % purity:	RO5072759, Lot # PDH0000001, Purity: 99.5%

Key Study Findings

- There were 7 unscheduled deaths, 6 were considered test-article related by the Applicant. Three animals died or were euthanized during the dosing phase and 4 during the recovery period following signs of chronic hypersensitivity reactions.
- Test article-related microscopic findings at unscheduled and scheduled necropsies included increased incidence and severity of mononuclear infiltrates, arteritis/periarteritis, and inflammation in several tissues and organs, including immune-complex glomerulonephritis, morphologically consistent with hypersensitivity reactions. At the end of the recovery phase, these changes were less prominent as compared to those in the terminally sacrificed animals, indicating partial recovery.
- B-cell specific lymphoid depletion occurred at all dose level starting on Day 3. The depletion of B cells (blood and lymphoid tissues) was reversed at the end of the recovery phase, except for 1 low dose animal that exhibited only a partial recovery.

Methods

Doses:	0, 5, 25, 50 mg/kg
Frequency of dosing:	Once weekly for 26 weeks
Route of administration:	Intravenous (low and mid dose groups: bolus injection) (control and high dose groups: 30 min. infusion)
Dose volume:	2.0 (control), 0.2, 1.0, 2.0 ml/kg
Formulation:	Formulated RO5072759, huMAb anti CD20 ready-to-use solutions.
Vehicle:	20 mM Histidine, 240 mM Trehalose, 0.02% (w/v) poloxamer 188, pH 6.0
Species/Strain:	Naïve cynomolgus monkeys (*Macaca fascicularis*) from Mauritius
Number/Sex/Group:	6/sex/group; 2/sex/group were kept for a 37 week recovery period
Age:	3 – 8 years
Weight:	6.3 to 10.2 kg for males and 2.9 to 7.6 kg for females
Deviation from study protocol:	None of the deviations affected the integrity or interpretability of the results of the study

Observations and Results

Mortality

Three animals were sacrificed prior to the dosing phase necropsy, and an additional 4 animals were sacrificed during the recovery phase, see table below.

Table 22 Cause of Death Summary (6-Month Repeat Dose Study)

Animal No. (sex)	Dose (mg/kg)	Death	Cause of morbidity (Major Finding)*
I01632 (M)	5	Dosing Day 63	Physical signs consistent with treatment-related anaphylactoid reaction.
I01665 (F)	25	Dosing Day 149	Marked hepatocellular vacuolation. – Clinical signs associated with weight loss and low/no food consumption resulting in marked hepatocellular vacuolation, renal tubular vacuolation, pancreatic acinar cell atrophy, and fat necrosis.
I01642 (M)	25	Recovery Day 85	Glomerulonephritis, inflammation of serosa/adventitia of urinary bladder
I01643 (M)	25	Recovery Day 230	Glomerulonephritis, inflammation of intestine (mucosa)
I01666 (F)	25	Recovery Day 141	Glomerulonephritis, inflammation of serosa/adventitia, thyroid gland, gall bladder, and kidney interstitium
I01670 (F)	50	Dosing Day 181	Inflammation of serosa/adventitia
I01673 (F)	50	Recovery Day 92	Glomerulonephritis, inflammation of the serosa/adventitia, pituitary gland, thyroid gland, intestine (mucosal) and kidney interstitium

*As reported in the Pathology Report.

1. Animal No. I01632 (male 5 mg/kg): On Day 36 of the dosing phase following the sixth dose, the animal had excessive salivation and facial erythema that progressed to the arms, with evident pruritis. Diphenhydramine treatment was administered and animal responded to treatment. Prophylactic treatment prior to seventh and eighth test article doses occurred without incident, but following the ninth dose (on Day 57 of the dosing phase), another reaction occurred despite the diphenhydramine. The animal was necropsied on Day 63. Anti-RO5072759 antibodies were detected and RO5072759/ADA complexes as well as B lymphocyte numbers comparable to controls indicating loss of pharmacological activity. No specific clinical pathology or microscopic findings were observed.
2. Animal No. I01665 (female 25 mg/kg): This animal lost 15% of its body weight between Days 112 and 147 of the dosing phase; this correlated with low/no food consumption during this time, persistent menstruation (almost every day between Days 121 and 147 of the dosing phase), and observations of few feces. Necropsy was performed on Day 149 following observations of hunched posture and ataxic/hypoactivity. Grossly, the liver was diffusely discolored yellow and microscopically, there was marked hepatocellular vacuolation, renal tubular vacuolation, pancreatic acinar cell atrophy, and fat necrosis. The Applicant stated that these findings were consistent with the well-recognized fatal fasting syndrome of

macaques and this death was considered incidental and unrelated to the test article.[21]

Reviewer's comment: It is plausible that fatal fasting syndrome and weight loss following of low/no food consumption is drug treatment-related since similar observations were made in other early decedents.

3. Animal No. I01642 (male 25 mg/kg): Based on weight loss over the previous week, pale gums and marked anemia the animal was sacrificed. Microscopically, major findings were glomerulonephritis, perivascular serosal/adventitial infiltrates or inflammation, and interstitial inflammation of the epididymides. Hematological and clinical chemistry findings included decreases in RBC counts, hemoglobin, glucose, total protein and AG ratio, and an increase in cholesterol. Marginal (2+) protein secretion in urine was also reported in this animal.
4. Animal No. I01643 (male 25 mg/kg): On Day 230, the animal had notable edema of the scrotum and hind limbs (not previously noted for the animal) and was cold-to-touch in the hind limbs. These findings were consistent with severe renal damage; therefore, the animal was sacrificed. Microscopically, the major relevant finding was marked glomerulonephritis, inflammation of intestine and Hematological and clinical chemistry findings included decreases in RBC counts, hemoglobin, increases in BUN, creatinine, and cholesterol, decreases in total protein, albumin, globulin, AG ratio, and phosphorus.
5. Animal No. I01666 (female 25 mg/kg): Based on chronic weight loss during the recovery phase (17% loss from start of recovery), lower food consumption, being hunched and thin, and decreased total protein, albumin, and hematocrit, the animal was sacrificed for humane concern over chronic distress. Microscopically, major findings were glomerulonephritis and inflammation in the thyroid gland, gall bladder, kidney interstitium, and serosa/adventitia. Hematological and clinical chemistry findings included decreases in RBC, hemoglobin, total protein, albumin, and globulin.
6. Animal No. I01670 (female 50 mg/kg): The animal lost 16% of its body weight between Days 147 and 175 of the dosing phase; this correlated with low/no food consumption and observations of liquid or no feces. On Day 181 of the dosing phase, the animal was also noted as cold-to-touch and with pale skin of the gums, and the animal was sacrificed in moribund condition. Microscopically, the major finding was serosal/adventitial inflammation. Clinical chemistry findings included an increase in BUN, and a decrease in total protein, serum albumin, globulin, calcium, and phosphorus.
7. Animal No. I01673 (female 50 mg/kg): Based on weight loss over the previous 2 weeks, lower food consumption and marked anemia the animal was sacrificed. Microscopically, major findings were glomerulonephritis and mononuclear inflammation in the thyroid gland, pituitary gland, kidney interstitium, intestine

[21] Bronson, R. T., et al. Ibid.

(mucosal), and serosa/adventitia. Hematological and clinical chemistry findings included decreases in RBC counts, hemoglobin, total protein, albumin and globulin.

Clinical Signs, Physical Exams and Neurobehavioral Observations

Clinical Signs: Twice daily cage side observations/detailed observations predose and weekly thereafter through the dosing and recovery phases.

- In addition to the findings discussed above for early decedents, the following individual clinical signs were noted:

Animal No. (sex)	Dose (mg/kg)	Clinical Signs	Correlating Findings
I01644(M)	50	Thin on or after Day 175	Body weight loss after Day 147; serosal inflammation
I01662(F)	25	Low food consumption and clinical decline beginning on Day 133; recumbent position on Day 176	Body weight loss; glomerulonephritis
I01664(F)	25	Low food consumption and clinical decline beginning on Day 133; broad spectrum antibiotics administered and condition improved.	Body weight loss from Days 133-161; no microscopic correlates
I01663(F)	25	Cold to touch on Day 78 requiring reversal of ketamine sedation; irregular respiration and ataxia on Day 92 and Day 99; diphenhydramine administered thereafter prior to dosing.	ADA formation and B cell levels comparable to controls indicating loss of pharmacological activity

Physical examinations were conducted by a veterinarian or trained technical staff once predose, during Week 26 and during Week 37.

- Gingivitis was noted at the dosing phase (Day 178) physical examination (2/6 each for males given 5, 25, or 50 mg/kg/dose).

Dose (mg/kg)		0		5		25		50	
Sex		M	F	M	F	M	F	M	F
Gingivitis	predose	0	0	1	0	0	0	0	0
	Dosing phase	0	0	2	0	2	0	2	0
	Recovery phase	0	0	0	0	0	0	0	0

Neurobehavioral observations were performed once during the predose phase, during Week 26 of the dosing phase (within 1 hour postdose) and during Week 37 of the recovery phase by trained technical staff.

- No test article-related findings were noted at dosing or recovery phase neurobehavioral examinations.

Respiration rates were taken predose, during Week 39 (within 1 hour postdose) and during Week 13 of the recovery phase. Blood pressure measurements were recorded

for all animals once during the predose phase, during Week 25 of dosing (within 1 hour of dosing) and during the final week of recovery.

- No RO5072759-related effects noted on blood pressure or respiration rate.

Body Weights

Recorded weekly during the predose phase, on Day -1 (day prior to initiation of dosing), weekly thereafter, and on the days of scheduled sacrifices.

- There were few significant differences in body weight change during the dosing phase, except towards the end of dosing, a few animals besides those sacrificed early (see above), showed notable losses in body weight. Body weight losses correlated with low food consumption in 3 mid dose group animals and 2 control group animals. The treatment group animals had greater losses in total weight and also exhibited microscopic findings shown below:

Table 23 Body Weight Loss (6-Month Toxicology Study)

Animal No. (sex)	Dose (mg/kg)	Body weight loss during specified interval	Microscopic findings
I01628 (M)	0 (control)	↓11% from Days 147 to 168 of the dosing phase	
I01653 (F)	0 (control)	↓15% from Days 147 to 182 of the dosing phase	
I01662 (F)	25	↓25% from Day 147-182 dosing phase	Glomerulonephritis
I01664 (F)	25	↓24% from Days 133 to 161 of the dosing phase; with antibiotic treatment, weight recovered over the remainder of the dosing phase	Serosal inflammation
I01644 (M)	50	↓17% from Day 147-182 dosing phase	Arteritits/periarteritis

Body weight changes during the recovery phase were consistent with controls.

Feed Consumption

Assessed once daily during all phases.

- Multiple animals, particularly females, were noted with multiple instances of low or no food consumption throughout the dosing phase, see above.

Ophthalmoscopy

Eye exams were performed once during the predose phase and during Weeks 13 and 26 using an indirect ophthalmoscope and a slit lamp microscope.

- No ophthalmic lesions were noted during the dosing or recovery phase.

ECG

Electrocardiography results were recorded 3 times during the predose phase, during Weeks 12 and 25 and during Week 37.

- No test-article related changes were observed during the dosing or recovery phase.

Hematology

Blood samples were collected 3 times predose, during Week 13 of the dosing phase and prior to scheduled sacrifice.

- There were no treatment effects on hematology parameters measured in the routine clinical pathology sampling during the dosing or recovery phases. At the end of dosing necropsy, one high dose male displayed changes that were consistent with treatment-related serosal/adventitial inflammation (increased WBC and neutrophil counts, and decreased RBC counts and Hb).
- As part of the Immunophenotyping analysis, total lymphocytes were measured with greater frequency than the clinical pathology sampling. The Immunophenotyping results demonstrated a transient decrease in mean absolute lymphocyte counts on day 3 that recovered to predose values after Day 8.

Immunophenotyping for peripheral blood lymphocyte subsets was conducted on samples collected 3 times predose, on Days 1, 3 and 8 of the dosing phase and every 3 weeks thereafter and prior to scheduled sacrifice. Lymphocyte subsets were quantitated using flow cytometry (B cells, CD3-CD20+; NK cells CD3-CD16+; T cells, CD3+; Helper T cells, CD3+CD4+; Cytotoxic T cells, CD3+CD8+).

- **B Cells**: Marked decreases in absolute and relative CD20+ B cell values were observed starting on Day 3 of the dosing phase for all treated animals. The decrease in B cells correlated with a transient decrease in mean absolute lymphocyte counts on Day 3 with total lymphocyte counts being similar to predose values and controls by Day 8. B cell ablation continued throughout the dosing phase, with the exception of animals that developed detectable anti-RO5072759 antibodies (Animal Nos. I01632, I01633, I01634, I01657, and I01660 at 5 mg/kg/dose and Animal No. I01663 at 25 mg/kg/dose). By the end of recovery, B-cell levels reversed to peak levels of 64%−140% of baseline for the remaining individual animals, with the exception of 1 animal No. I01637 given 5 mg/kg (peaked at 7% of baseline).

- **NK Cells**: A transient marginal reduction of CD 16+ NK cells in all the treated groups observed on Day 3. The NK cell values were similar to predose values by Day 8 of the dosing phase.

Immunophenotyping of lymphoid tissues: At necropsy, the spleen and mandibular lymph node were collected for immunophenotyping analysis from all animals at scheduled sacrifices. Single-cell suspensions of each spleen and mandibular lymph node were prepared. Total cellularity (spleen only) and viability were determined for each spleen and lymph node sample.

- Moderately to markedly decreased CD20+ B cell values occurred in all main study treatment groups, when compared with controls. Animals with detectable anti-RO5072759 antibodies had B cell values comparable to controls in the spleen and lymph nodes. Overall, the B cell values were similar to control values at the end of the recovery phase, with the exception of the one animal at 5 mg/kg/dose (Animal No I01637), with depressed peripheral B-cell counts, mentioned above.

No effects were seen on absolute T cell populations in peripheral blood or tissue samples.

Clinical Chemistry

Sampling times same as for hematology.

- Similar to unscheduled sacrifice animals and presented in the Mortality section (see above), treatment-related individual serum chemistry changes were observed in individual animals at scheduled necropsies, during the dosing or recovery phases, and correlated with RO5072759-related glomerulonephritis or inflammation in other tissues observed microscopically in these animals and/or their poor physical condition.

Animal No. (sex)	Dose (mg/kg)	Serum chemistry finding	Reason
I01662 (F) Main study	25	↑ BUN, ↑creatinine, ↑cholesterol, ↑triglycerides, ↓glucose, ↓total protein, ↓albumin and ↓AG ratio.	Glomerulonephritis
I01644 (M) Main study	50	↓ total protein, ↓serum albumin, ↓AG ratio, ↑cholesterol and ↑triglycerides.	Serosal/adventitial inflammation
I01649 (M) Recovery	50	↓ total serum protein and albumin	Glomerulonephritis

- Glomerulonephritis occurrence (in both early decedents and scheduled necropsies) correlated with decreased total serum protein in 6 of 7 affected animals and increased blood urea nitrogen and serum creatinine in 2 of 7 affected animals. These animals were: I01662, I01642, I01673, I01666, I01643, I01649 and I01672.
- Serum cytokines were evaluated from blood samples collected 3 times during the predose phase and approximately 1 and 4 hours postdose on Day 1 and during Weeks 4, 13 and 26. Also, predose and postdose samples were collected from Group 3 female No. I01663. Cytokines measured were INF-g, TNF-a, IL-2, IL-4, IL-6, IL-8 and IL-10.
- Histamine levels were measured in samples collected from Group 3 female No. I01663 collected for cytokine analysis.

There were no remarkable findings

Urinalysis

Urine samples were collected once during predose phase, during Week 13 of the dosing phase, and prior to scheduled sacrifices.

- Increased urinary excretion was observed in two males with glomerulonephritis (I01642, early decedent, 25 mg/kg/dose; and I101649, recovery group, 50 mg/kg/dose).

Organ Weights

Weights of the following organs (when present) were recorded at each scheduled sacrifice from all animals. Paired organs were weighed together.

adrenal (2)
brain
epididymis (2)
heart
kidney (2)
liver with gallbladder (drained)
lung
ovary (2)
pituitary gland
prostate
spleen
testis (2)
thymus
thyroid (2 lobes) with parathyroid
uterus

Table 24 Gross Findings in Scheduled Necropsies from 6-Month Study

Animal No. (sex)	Dose (mg/kg)	Finding	Correlation
I01662 (F)	25	Increased kidney weight.	These changes were considered secondary to glomerulonephritis
I01649 (M)	50		
I01644 (M)	50	Decreased epididymides, testes and thyroid/parathyroid gland weights.	Correlated with intratubular cellular debris in these tissues. This animal had serosal/adventitial and interstitial inflammation affecting several tissues.

Histopathology

Adequate Battery **YES**

- Hematoxylin and eosin staining was performed for all tissues, and special stains were used for select specimens at the request of the study pathologist.

Peer Review **YES** (by pathologists employed by Hoffman La Roche)

Histological Findings

- **Lymphoid depletion** with the absence of germinal centers was observed in the spleen and mesenteric and mandibular lymph nodes in all treatment groups.

- **Glomerulonephritis (minimal-marked)** was observed in 1 animal (female given 25 mg/kg/dose) at the end of the dosing phase and in 6 animals sacrificed at an

unscheduled interval during the recovery phase or at the scheduled recovery phase necropsy. Severity ranged from minimal to marked with no apparent dose-response relationship between the 25 and 50 mg/kg dose groups. The glomerulonephritis was primarily characterized by global, diffuse increase in mesangial hypercellularity, thickened glomerular basement membranes (membrano-proliferative) and reactive parietal epithelial cells. Other changes that were secondary to glomerulonephritis were hyaline (protein) and cellular casts in the cortical and medullary regions along with tubular epithelial degeneration/regeneration. In Animal No. I01643 (male, 25 mg/kg), the glomerulonephritis was marked with glomerular crescent formation and glomerulosclerosis; glomerulonephritis and the consequent renal failure was considered the primary cause of the observed physical signs. Glomerulonephritis was present at the end of the recovery phase indicating the change did not reverse.

- **Arteritis/periarteritis** in small blood vessels was observed at the end of the dosing phase in two animals (male 5 mg/kg) and (female 25 mg/kg). This finding was present in two recovery group animals, one male unscheduled sacrifice (25 mg/kg) and one 50 mg/kg male at the end of recovery, indicating the finding did not reverse.

- **Fibrin thrombi** were observed in the lungs and/or kidneys of 3 unscheduled sacrifice recovery animals: 1 male and 1 female at 25 mg/kg, and 1 female at 50 mg/kg. These changes may be secondary to test article-related systemic inflammation observed in these animals.

- **Mononuclear infiltration and inflammation** were observed in all treatment groups. Increased incidence and/or severity of these changes were observed in many tissues and organs, including: sciatic nerve, heart, lung, kidney, liver, pancreas, brain (choroid plexus), small and large intestines, pituitary, urinary bladder, epididymides, prostate, and serosal/advential surface of tissues in the peritoneal, pelvic and pleural cavities. These inflammatory changes appeared systemic in nature, were not target organ-specific. Some were associated with immune complex deposition. These changes were still present in animals at the recovery phase sacrifice.

Table 25 Selected microscopic findings 6 month study–Dosing phase (4/sex/group including early decedents)

Target tissue	Dose (mg/kg)							
	0		5		25		50	
	M	F	M	F	M	F	M	F
Kidney								

Target tissue	Dose (mg/kg)							
	0		5		25		50	
	M	F	M	F	M	F	M	F
Glomerulonephritis	0	0	0	0	0	1(+)	0	0
Mononuclear infiltrate	2 (+)	2(+) 1(++)	2(+) 1(++)	1(+) 1(++)	1(+) 2(++)	2(+) 2(++)	1(+) 2(++)	2(+)
Inflammation, interstitial, mononuclear	0	0	0	0	0	0	1(++)	0
Sciatic nerve								
Mononuclear infiltrate perivascular	0	1(+)	1(+)	0	1(+)	3(+)	2(+)	1(+)
Pituitary								
Mononuclear infiltrate perivascular	0	0	0	0	0	0	1(++)	1(+)
Inflammation	0	0	0	0	0	1(+++)	0	1(++)
Epididymides								
Mononuclear infiltrate	3(+)	--	2(+)	--	2(++) 1(+)	--	2(++)	--
Inflammation, interstitial	0	--	1(+)	--	0	--	1(+++) 2(++)	--
Brain (choroid plexus)								
Mononuclear infiltrate	0	0	0	0	0	0	1(+)	0
Heart								
Myocardial degeneration/fibrosis	0	0	0	0	0	0	1(+)	0
Lung								
Fibrosis/thrombosis	0	0	0	0	1(++)	1(+) 1(++)	1(++)	1(++)
Pancreas								
Acinar cell atrophy	0	0	0	0	0	1(+++)	0	0
Bone Marrow								
Myeloid hyperplasia (sternum and rib)	0	0	0	0	0	0	1(++)	0
Urinary bladder								
Mononuclear infiltrate	2(+)	0	2(+)	0	1(+)	1(++)	2(++)	0

Target tissue	Dose (mg/kg)							
	0		5		25		50	
	M	F	M	F	M	F	M	F
Prostate								
Inflammation	0	--	0	--	1(++)	--	1(+++)	--
Serosa/adventitial surface (Includes tissues in peritoneal, pelvic, and pleural cavities)								
Mononuclear infiltrate/inflammation	0	0	0	0	0	0	3	1
Small blood vessels								
Arteritis/periarteritis	0	1	1	0	0	2	0	0

Code: 0: organ examined, no pathology finding noted.
-- : Tissue not present; +: minimal, ++ slight, +++ moderate, ++++: marked

Table 26 Selected microscopic findings 6 month study– Recovery phase (2/sex/group)

Target tissue	Dose (mg/kg)							
	0		5		25		50	
	M	F	M	F	M	F	M	F
Kidney								
Glomerulonephritis	0	0	0	0	1 (+++) 1(++++)	1(+)	1(++)	2(+)
Mononuclear infiltrate	1(+)	1(+)	2(+)	2 (+)	1(++)	1(+)	1(+) 1(++)	1(++)
Inflammation, interstitial, mononuclear	0	0	0	0	1(+++)	1(++++)	0	1(++++)
Thrombosis/fibrosis	0	0	0	0	1 (+++)	1 (+++)		1 (+++)
Pituitary								
Mononuclear infiltrate perivascular	0	0	0	0	1(+)	0	1(+)	0
Inflammation	0	0	0	0	0	1(++)	1(+)	1(++)
Epididymides								
Mononuclear infiltrate	2(+)	--	2(++)	--	0	--	2(++)	--

Target tissue	Dose (mg/kg)							
	0		5		25		50	
	M	F	M	F	M	F	M	F
Inflammation, interstitial	0	--	0	--	1(++)	--	0	--
Brain								
Mononuclear infiltrate	0	0	0	0	0	0	1(+)	0
Bone Marrow								
Myeloid hyperplasia (sternum)	0	0	1(+)	0	0	0	0	0
Pancreas								
Acinar cell atrophy	0	0	0	0	0	1(+++)	0	1(+++)
Urinary bladder								
Mononuclear infiltrate	0	2(+)	1(+)	1(++)	0	1(++)	0	1(+++)
Prostate								
Inflammation	0	--	0	--	0	--	0	--
Serosa/adventitial surface (Includes tissues in peritoneal, pelvic, and pleural cavities)								
Mononuclear infiltrate/inflammation	0	0	1	0	2	1	0	1
Thyroid gland								
Inflammation	0	0	0	0	0	1(++)	0	1(+++)
Small blood vessels								
Arteritis/periarteritis	0	0	0	0	1	0	1	0
Gall bladder								
Inflammation	0	0	0	0	0	1(+++)	0	0
Lung								
Thrombosis/fibrosis	0	0	1(++)	0	0	0	0	0

Code: 0: organ examined, no pathology finding noted.
-- : Tissue not present; +: minimal, ++ slight, +++ moderate, ++++: marked

Additional Pathology Evaluations:

1. Immunohistochemical staining was conducted by (b) (4) Study No. 8210512/11067) on the kidneys from all animals at the recovery phase necropsy (sections from the right kidney that contained the cortex and medulla). Antibodies again against monkey IgG, IgM or complement protein C3 were used on sections from all of the 7 animals with glomerulonephritis. Controls used were donor monkeys and not from the study control group 1.
    - IHC staining displayed increased IgG, IgM and/or C2 in glomerular, tubular epithelial cell, interstitial and/or peritubular capillary granular deposits from RO5072759-treated animals but not in untreated donor monkey comparators. The granular deposits can be interpreted as the histological correlate of immune complexes. The presence of glomerular granular deposits correlated with the presence of glomerular histopathology.
    - The presence of tubular epithelial cell and/or intersitital/peritubular capillary monkey IgG and IgM granular deposits had partial overlap with tubulointerstitial histopatholgic changes. The interstitial infiltrate/inflammation and fibrosis could be the result of different causes, but based on the pattern of granular deposition and evidence of concurrent glomerular deposits, the Pathologist concluded these were secondary to immune complex deposition.
    - The presence of RO5072759 in the immune complexes was not demonstrated by IHC. The lack of staining could be the result of lack of sensitivity of the assay or loss of the target epitope.

2. Transmission electron microscopy (TEM) on formalin-fixed kidney tissue from two control males and three males with glomerulonephritis (I01642, I01643 and I01649). Glomerular ultrastructural changes were evaluated. Direct immunofluorescence (IF) analysis for monkey IgG was conducted on fresh frozen tissue from 1 control male (I01631) and 1 high dose male with glomerulonephritis (I01649). These analyses appear to have been conducted by (b) (4) but the primary from the TEM and IF results are not provided.
    - TEM: Animal Nos. I01642 and I01649 had prominent subepithelial and intramembranous electron dense deposits within the glomerular capillary basement membranes consistent with immune-complex deposition. Animal No. I01643 had glomerular involution with thickened basement membranes consistent with chronic inflammation, but no obvious immune deposits. The control animals (Animals Nos. I01629 and I01631) did not have any evidence of immune deposits in the glomeruli.
    - IF: The treated male had intense granular capillary wall monkey IgG deposits, but the control did not display any glomerular deposition of monkey IgG.

Additional Special Evaluations for Reproductive Assessment

Individual daily vaginal swab (for the presence of blood) and weekly hormone data (prolactin, luteinizing hormone, follicle-stimulating hormone, estradiol and progesterone

levels), and mean and individual semen analysis and testicular volume data were collected.

Female reproductive effects:

- No differences were noted in average menstrual cycle length. One low dose female (I01657) was observed with no ovaries at the time of necropsy, and did not display menstrual cycles.
- No treatment-related differences were observed in female hormone levels during the dosing or recovery phase.

Male reproductive effects: Testicular size was recorded for all males once during the predose phase, during Week 26 and during Week 37. Semen was collected from all males 3 times during the predose phase, during Week 26 and during Week 37.

- No RO5072759-related effects were noted at the dosing or recovery phase necropsy for mean sperm motility, density (count), morphology, or testicular volume.

Toxicokinetic and Immunogenicity Analyses

Serum samples for RO5072759 were collected were collected predose and approximately 1, 7, 24, 48, 96 and 168 hours postdose on Day 1 and during weeks 13, 26. Single samples were collected once during Week 1 of the recovery phase and every 2 weeks thereafter and at recovery necropsy. Analysis was conducted under GLP.

Anti-RO5072759 antibodies (ADAs) were measured using a validated ELISA method under non-GLP conditions. During the process of confirmative assay, extended characterization of the nature of ADAs (anti-Fc or anti-CDR) was performed for select samples. An exploratory immune complex assay was performed on select samples to investigate whether circulating complexes correlated with significant findings. Additionally, residual serum from each sample was tested for anti-RO5072759 antibodies (ADAs).

- RO5072759 exposure was maintained until the end of the study in all animals given 50 mg/kg/dose, but 5/12 group 2 animals and 1/12 animals Group 3 animals showed accelerated elimination of RO5072759 and repletion of B cells, that correlated with detectable ADA formation (see Table 28).
- C_{max} and AUC_{0-168h} values increased in an approximately dose-proportional manner on Days 1, 85 and 176.
- Plasma drug concentration accumulated approximately 2- to 3-fold by the end of the dosing phase (Day 176).
- No sex-related differences were apparent.

Table 27 Mean Toxicokinetic Parameters (Day 176, final dosing)

Day	Dose	C_{max} (mcg/ml)		$AUC_{0\text{-}168h}$ (mcg·hr/ml)	
		M (n)	F (n)	M (n)	F (n)
1	5	178 (6)	174 (6)	12700 (6)	13300 (6)
	25	902 (6)	907 (6)	70000 (6)	68200 (6)
	50	1800 (6)	1800 (6)	137000 (6)	128000 (6)
85	5	327 (3)	429 (4)	32100 (3)	41600 (4)
	25	1620 (6)	1700 (5)	171000 (6)	172000 (5)
	50	3230 (6)	2900 (6)	335000 (6)	260000 (6)
176	5	309 (3)	427 (4)	33400 (3)	44500 (4)
	25	1770 (6)	1170 (4)	220000 (6)	128000 (4)
	50	3170 (6)	2670 (6)	379000 (6)	303000 (6)

Figure 14 Individual animal serum RO5072759 concentrations vs. time (5 mg/kg Group)

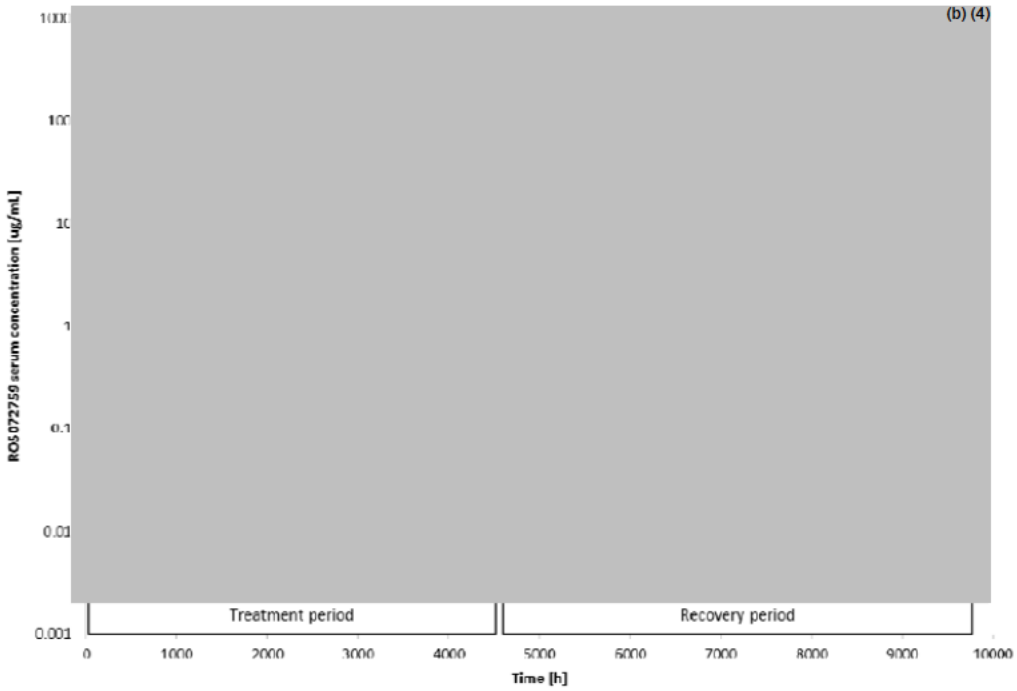


Figure 15 Individual animal serum RO5072759 concentrations vs. time (25 mg/kg Group)

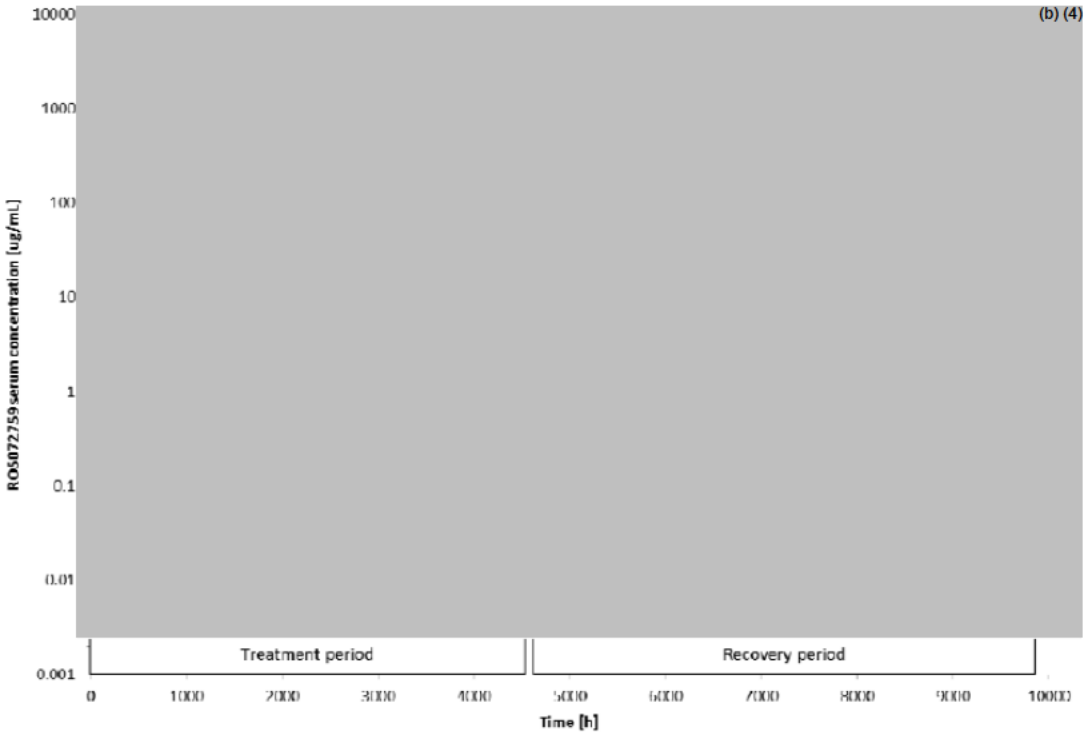
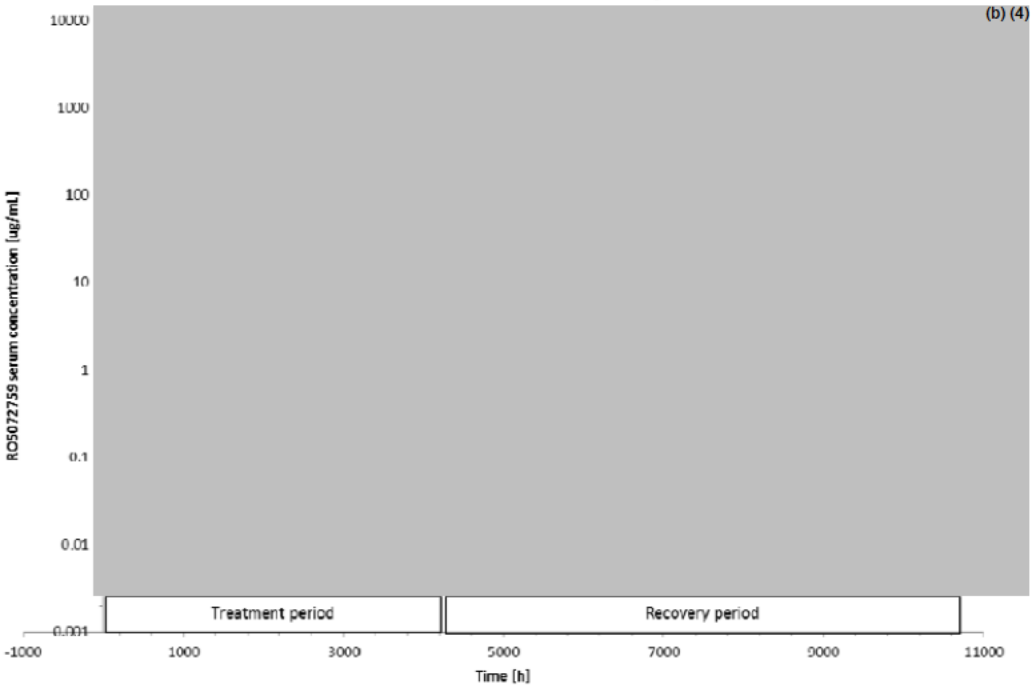


Figure 16 Individual animal serum RO5072759 concentrations vs. time (50 mg/kg Group)



- ADAs were detected in 7 animals (see Table 28). Of the 6 Group 2 animals that developed ADA, 3 were ADA+ on Days 86 and 176, 1 on the day of early sacrifice and one during recovery week 26. One Group 3 animal developed ADA beginning on Day 85. (6 in Group 2 and 1 in Group 3).
- The ADA epitope specificity testing revealed the generated ADAs were directed against the variable Fab region of RO5072759 in all animals and not against the constant Fc portion.
- Circulating immune complexes were identified samples from 7 of the 16 animals analyzed (Table 28). Two of these animals developed anaphylactic or anaphylactoid reactions, 2 had glomerulonephritis, and 1 had inflammatory changes in tissues. Two animals with circulating immune complexes showed no reaction. The Applicant stated that the weak correlation of the presence of immune complexes in serum and tissue deposition and damage may be related to the size of the complexes, intermittent presence of immune complexes, or "in situ" formation[22].

[22] Solling, J. and S. Olsen, Circulating immune complexes in glomerulonephritis. *Clin. Nephrology,* (1981), 16: 63-74.

Table 28 Positive immunogenicity results for 6 month study

Animal No. (Sex)	Dose mg/kg (Phase)	ADA-positive	Circulating Immuno-Complexes (+ or -)	Accelerated clearance after 85 and 176 days of dosing (+ or -)	Related Severe Findings
I01632(M)	5 Dosing	Day 63	+	-	Early decedent (Day 63); anaphylactoid reaction; inadequate response to diphenhydramine pretreatment
I01633(M)	5 Recovery	Days 85 onward	+	+	-
I01634(M)	5 Dosing	Days 85 and 176	NA	+	-
I01657(F)	5 Dosing	Days 85 and 176	NA	+	-
I01659(F)	5 Recovery	Day 357	-	-	-
I01660(F)	5 Dosing	Days 85 and 176	NA	+	-
I01643(M)	25 Recovery	No	+	-	Early decedent (Day 406); marked glomerulonephritis
I01663(F)	25 Dosing	Day 85 onwards	+	+	Anaphylactoid reaction; responded well to diphenhydramine pretreatment
I01666(F)	25 Recovery	No	+	-	Early decedent (Day 141); glomerulonephritis; inflammation of serosa/adventitia, kidney interstitium, gall bladder, thyroid
I01644(M)	50 Dosing	No	+	-	Serosal/advential inflammation of the kidneys, liver, GI tract and others
I01648(M)	50 Recovery	No	+	-	-

NA: not analyzed

Dosing Solution Analysis

Protein content:
The protein content of RO5072759 was determined in 2 sample vials (batch/lot PDH0000001) of test article (RO5072759). The measured values of 25.7 mg/ml for the two samples are similar to the value of 26.3 mg/ml given in the certificate analysis.

Purity:
The purity of RO507275 was determined in 2 sample vials (see above) by size exclusion chromatography. The value of 99.2% monomeric antibody obtained from both vials is slightly lower than the value of 99.5% given in the certificate of analysis. The decrease of 0.3% of monomer is mainly related to an increase of the low molecular weight species.

Stability:

The result from concentration measurements and analysis of end-of-dosing samples demonstrate that RO5072759 has been stable over the whole course of the study.

Summary

- There were 7 unscheduled deaths, 6 were considered test-article related by the Applicant. Three animals died during the dosing phase and 4 during the recovery period.
    - One low dose group animal experienced an anaphylactoid response following the 6th dose and responded to diphenhydramine treatment. After the 9th treatment, the animal had another reaction despite prophylaxis. ADA and immune complex formation was confirmed.
    - Five mid- or high-dose group animals displayed worsening clinical conditions requiring early termination. They were found to have immune-complex mediated glomerulonephritis, arteritis/periarteritis and/or inflammation of other tissues, including serosa/adventitia. Three of these animals had fibrin thrombi of the lungs and/or kidneys.
    - One mid-dose animal was described as having fatal fasting syndrome that was considered unrelated to the test article.[23] However, a treatment related cause cannot be ruled out.
- Renal changes were observed in this study at the end of the dosing period (1 monkey dosed at 25 mg/kg/dose), and during and at the end of the recovery period (6 monkeys, 25 and 50 mg/kg/dose). Microscopically, bilateral glomerulonephritis and interstitial inflammation were reported in kidneys of 7 animals. No renal changes were noted at 5 mg/kg and in the control group. Changes in clinical pathology parameters relevant to kidney findings included decreased total serum protein in 6 of 7 affected animals and increased blood urea nitrogen and serum creatinine in 2 of 7 affected animals.
- Severity of glomerulonephritis was ranged from minimal to marked with no clear dose-response relationship between the 25 and 50 mg/kg dose groups. Detection of immunocomplexes as electron-dense deposits by electron microscopy and by positive immunohistochemical or immunofluorescent staining for monkey IgG, IgM, and/or complement protein C3 in the glomeruli of some animals with glomerulonephritis confirm the immune-mediated nature of these changes.
- Other treatment-related microscopic findings in unscheduled and scheduled necropsies included arteritis/periarteritis and increased incidence and severity of mononuclear infiltrates/ inflammation in multiple tissues and organs. The infiltration and inflammation was primarily mononuclear composed of lymphocytes, macrophages, plasma cells with variable numbers of neutrophils and eosinophils. The perivascular orientation of the infiltrates and the tissue inflammation were

[23] Bronson, R. T., et al., Fatal Fasting Syndrome of Obese Macaques, *Laboratory Animal Science*, (1982) 32:187-192.

prominent in the serosa/adventitia, epididymis (interstitium), pituitary, kidney and thyroid gland. In more severe cases, the inflammation was diffuse.

- At the end of the recovery period, mononuclear infiltration and inflammation and the arteritis/periarteritis affecting small blood vessels was observed in some treated animals at the end of the recovery period indicating these change did not completely reverse but did trend toward a decreased incidence and severity.
- Administration of RO5072759 resulted in treatment-related pharmacologically-mediated B-cell specific lymphoid depletion at all dose level. The depletion of B cells (blood and lymphoid tissues) was reversed at the end of the recovery phase, except for 1 low dose animal that exhibited only a partial recovery.
- Serum concentrations were dose proportional, but 5 animals appeared to have anti-RO507279 antibody (ADA) mediated clearance on Days 85 and 176. ADAs were not detected in serum of animals with tissue inflammation, neither in the dosing nor in the recovery period. Administration of RO5072759 at 5 mg/kg/dose via IV injection was associated with an AUC (0-168h) of 33,400 (µg·h)/mL for males and 44,500 (µg·h)/mL for females on Day 176 of the dosing phase.

Dose (mg/kg)	Phase	ADA-positive	accelerated clearance after 85 and 176 days of dosing
5	Dosing	+	+
5	Dosing	+	+
5	Dosing	+	+
5	Dosing	+	+
5	Dosing	+	-
5	Recovery	+	-
25	Dosing	+	+

9 Reproductive and Developmental Toxicology

9.1 Enhanced Pre and Postnatal Development

Study title: RO5072759 (huMAb anti-CD20) An intravenous administration study for effects on embryo-fetal and pre- and postnatal development in cynomolgus monkeys (enhanced design)

Study no.:	1045612
Study report location:	eCTD 4.2.3.5
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 10, 2010
GLP compliance:	Yes; OECD
QA statement:	Yes
Drug, lot #, and % purity:	RO507-2759/F06-01, Lot # H0002, Purity 96%; Vehicle supplied as a ready-to-use aqueous formulation, Lot PDH0000058 composition not provided)

Key Study Findings

Maternal

- Maternal mortality was 3/19 (16%) in the 25 mg/kg group and 3/18 (17%) in the 50 mg/kg group during gestation and lactation.
- RO5072759 concentration in milk was low compared to the corresponding maternal or infant serum concentration.
- RO5072759-related histopathological findings occurred at low incidence and included lymphoid and bone marrow depletion and lack of germinal centers in lymphoid tissues accompanied with inflammation of several organs such as kidney and liver as well as minimal to slight intestinal crypt micro abscesses and/or esophageal hyperkeratosis as immune-mediated lesions.

Neonatal

- Neonate mortality was 1/9 and 2/12 at 25 and 50 mg/kg/dose, respectively.
- Significantly lower mean body weight and body weight gain occurred in the 50 mg/kg/dose infants.
- RO5072759-related complete depletion of CD20+ B-cells by PND 28 and decreased percent lymphocytes and basophils accompanied with increased percent monocytes and neutrophils.
- RO5072759 serum concentration in infant monkeys was variable on Day 28 p.p. and was BLQ on Day 168 p.p.

Methods

Doses:	0, 25, 50 mg/kg/dose
Frequency of dosing:	Once weekly from Day 20 post-coitum (p.c.) until delivery
Dose volume:	1.0 or 2.0 mL/kg/dose
Route of administration:	Intravenous infusion
Formulation/Vehicle:	Supplied as a ready-to-use aqueous formulation; Composition not provided
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*) of Asian origin
Number/Sex/Group:	Control: 18; 25 mg/kg/dose: 19; 50 mg/kg/dose: 18 pregnant females
Satellite groups:	None
Study design:	Enhanced EFD study to include pre- and postnatal development to day 240.

Observations

Standard in-life examinations (clinical signs, morbidity, mortality, pregnancy monitoring, body weight, and food consumption) were conducted in maternal animals with periodically ultrasonograms to examine the progression of fetuses; neonatal infant's examinations included general, morphological, neurobehavioral, grip strength, and X-ray examinations for skeletal development. Blood samples were taken for hematology, immunophenotyping, antigenic challenge, maternal and infant toxicokinetics, antidrug antibodies, and milk sampling. Further details of these observations are included in Attachment B of this review.

Results

F_0 Dams - Gestation

Survival:	Control: 18/18; 25 mg/kg/dose: 18/19; 50 mg/kg/dose: 17/18
Clinical signs:	Salivation during and after dosing and nasal bleeding and/or bloody discharge
Body weight:	Unremarkable
Food consumption:	Social housing, not measured
Uterine content:	Not evaluated
Necropsy observation:	Dams kept alive through lactation <u>Early decedents</u>: − Lack of germinal centers in lymphoid tissues − Inflammation of several organs such as kidney and liver
Toxicokinetics:	Mean C_{max} and $AUC_{(0\text{-}168h)}$ values were higher (approximately 1.7 to 2.0 fold) on Day 139 p.c. Maximum serum concentrations at ~7 h
Dosing Solution Analysis	Unremarkable
Other:	Higher prenatal loss incidence at 25 mg/kg/day

F_0 Dams - Lactation

Survival:	Control: 18/18; 25 mg/kg/dose: 16/19; 50 mg/kg/dose: 15/18
Clinical signs:	Nasal bleeding and/or bloody discharge
Body weight:	Unremarkable
Food consumption:	Social housing, not measured
Uterine content:	Not evaluated
Necropsy observation:	<u>Early decedents</u>: − lack of germinal centers in lymphoid tissues − Inflammation of several organs such as kidney and liver − intestinal crypt micro abscesses and/or esophageal hyperkeratosis <u>Scheduled Sacrifices</u>: • inflammatory cell foci, acute, sub-acute or chronic inflammation and hemorrhage in different organs, fibrosis, atrophy, basophilic, dilation and/or vacuolation of tubule cells in the kidney, hepatocytes vacuolation and liver necrosis, and lymphocyte depletion in different organs
Toxicokinetics:	Serum concentrations Day 28 p.c. Group 2 (25 mg/kg/dose) 394±333 µg/mL Group 3 (50 mg/kg/dose) 144±90.6 µg/mL
Dosing Solution Analysis	Unremarkable

F_1 Generation

Survival:	Infants born control: 13; 25 mg/kg/dose: 9; and 50 mg/kg/dose: 12 Early decedents: One infant from the control and one infant from the 50 mg/kg/dose group were found dead on Day 0 and 2 p.p. One infant from the 25 mg/kg/dose and two infants from the 50 mg/kg/dose early sacrificed in moribund condition
Clinical signs:	Unremarkable in surviving infants
Body weight:	Significantly lower mean body weight and body weight gain in the 50 mg/kg/dose infants
Food consumption:	Social housing, not measured
Toxicokinetics:	Serum concentrations Day 28 p.c. Group 2 (25 mg/kg/dose) 86.6±51.6 µg/mL Group 3 (50 mg/kg/dose) 241±166 µg/mL
Ratio Mother Milk to Infant Serum:	Group 2 (25 mg/kg/dose) 0.00124±0.00181 Group 3 (50 mg/kg/dose) 0.000823±0.000651
Physical development:	Normal
Neurological assessment:	Normal
Reproduction:	Not evaluated
Other:	RO5072759-related: Decreased percent lymphocytes and basophils accompanied with increased percent monocytes and neutrophils Complete depletion of CD20+ B-cells by PND 28 accompanied with increased percent of different T-cell types

Maternal Examinations

Mortality

Gestation

One female at 25 and 50 mg/kg/dose were euthanized during the gestation phase. Infection/inflammation of different tissues was the cause of morbidity, Table 29. Histopathological findings of lymphoid depletion corresponded with the mode of action of RO5072759.

Lactation

Two females in each dosing group that presented hypersensitivity-like responses and/or secondary opportunistic infections were euthanized during the lactation phase, Table 30. Histopathological findings of lymphoid depletion corresponded with the mode of

action of RO5072759. One additional 50 mg/kg/dose female that presented spontaneous cestode cysts during rearing phase was euthanized on Day 91 p.p.

Table 29 Unscheduled Maternal Deaths during Gestation Dosing Phase

(Excerpted from Applicant's Submission)

Animal No.	RO5072759 mg/kg/wk	Day of Early Sacrifice	Cause of morbidity & major histopathology findings
16332	50	Day 113 *post-coitum*	Necrotizing gingivitis Lack of germinal centers in lymphoid follicles/lymphoid depletion/thymus atrophy
16416	25	Day 176 *post-coitum*	Interstitial inflammation, kidney, glomerulopathy, liver biliary Lack of germinal centers in lymphoid follicles/lymphoid depletion/thymus atrophy

Table 30 Unscheduled Maternal Deaths during the Lactation Dosing Phase

(Excerpted from Applicant's Submission)

Animal No.	RO5072759 mg/kg/wk	Day of Early Sacrifice	Cause of morbidity & major histopathology findings
16001	50	Day 88 *post-partum*	• Interstitial inflammation in the kidney and gallbladder • Upper alimentary tract mycosis, laryngeal inflammation, ulcer and hyperkeratosis. • Lack of germinal centers in lymphoid follicles/lymphoid depletion/thymus atrophy
15092	50	Day 133 *post-partum*	• Interstitial inflammation liver, biliary, kidney, urinary bladder, glomerulopathy • Serosal (& subserosal) inflammation, intestines • Lack of germinal centers in lymphoid follicles/lymphoid depletion/thymus atrophy • Larynx inflammation/ulcer
16363	25	Day 93 *post-partum*	• Serosal (& subserosal) inflammation, heart, GI tract, urinary bladder, intestines • Lack of germinal centers in lymphoid follicles/lymphoid depletion/thymus atrophy
15304	25	Day 96 *post-partum*	• Interstitial inflammation liver, biliary, kidney, • Serosal (& subserosal) inflammation, intestines • Lack of germinal centers in lymphoid follicles/lymphoid depletion/thymus atrophy • Nasal cavity inflammation

Clinical Signs

Gestation
Clinical signs of soft/fluid feces, thin hair and/or crusted lesions were common among groups with similar incidence and frequency. RO5072759-related clinical signs of salivation during and after dosing and nasal bleeding and/or bloody discharge had higher incidence and frequency in the 50 mg/kg/dose monkeys, Table 31.

Table 31 RO5072759-related Maternal Clinical Signs during Gestation

Clinical Sign	RO5072759 Doses (mg/kg/dose)					
	0		25		50	
	N= 18		N= 19		N= 18	
	N*	No. Days	N*	No. Days	N*	No. Days
Breathlessness severe	0	0	1	1	0	0
Salivation during/after dosing	0	0	1	1	1	2
Nasal bleeding/bloody discharge	0	0	1 1	8 9	1 1 1 1	3 6 5 20
Hypoactivity	0	0	0	0	1	2

* Number of monkeys with the corresponding number of days presenting the clinical sign

Lactation
RO5072759-related clinical signs of nasal bleeding and/or bloody discharge had higher incidence and frequency in the 50 mg/kg/dose monkeys, Table 32.

Table 32 RO5072759-related Maternal Clinical Signs during Lactation

Clinical Sign	RO5072759 Doses (mg/kg/dose)					
	0		25		50	
	N= 12		N= 9		N= 12	
	N*	No. Days	N*	No. Days	N*	No. Days
Nasal bleeding/bloody discharge	1 1	1 3	1 1 1	4 4 4	1 1 1 1	29 17 3 19

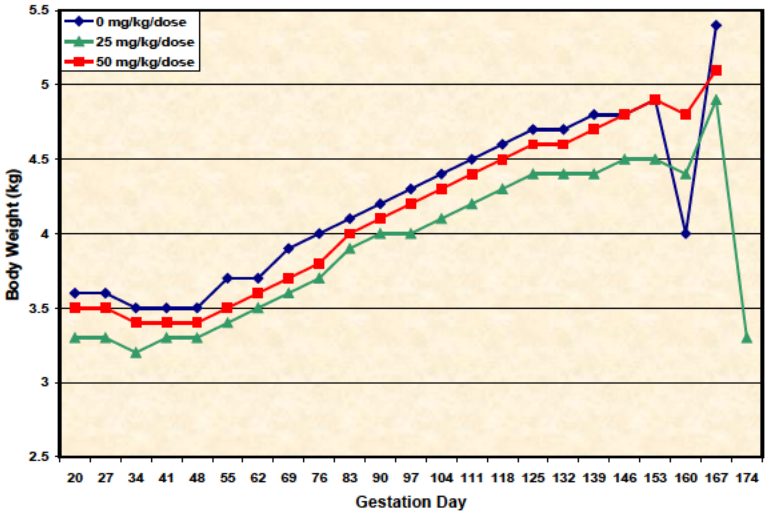
* Number of monkeys with the corresponding number of days presenting the clinical sign

Body Weight

Gestation
Although the mean body weight of 25 mg/kg/dose female monkeys was lower compared to control from the start of the study, the mean body weight changes were similar during gestation (post-coitum Days 20 to 146-174) among groups with a total mean body

weight increase of 1.2, 1.2 and 1.3 kg in the control, 25 and 50 mg/kg/dose groups, respectively.

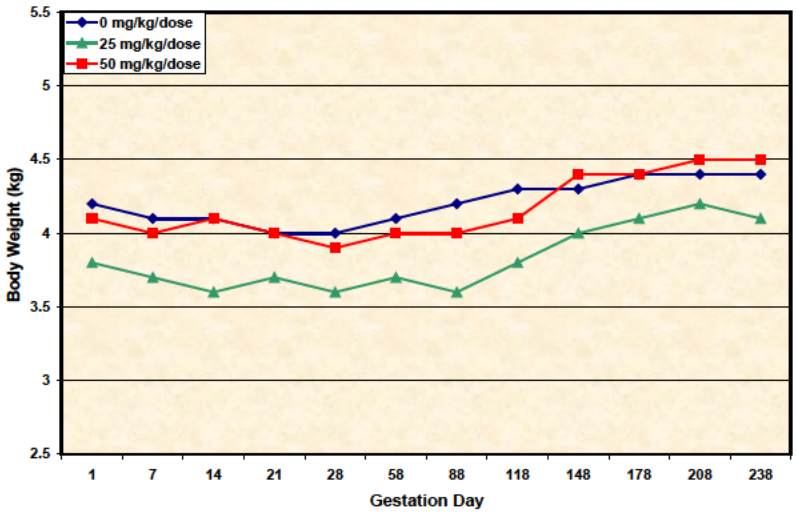
Figure 17 Mean Body Weight of Monkeys dosed with vehicle control or RO5072759 during Gestation



Lactation

Although the mean body weight of 25 mg/kg/dose female monkeys was lower compared to control from the start of the lactation period, the mean body weight changes were similar among groups during lactation (post-partum Days 1 to 238) with a total mean body weight increase of 0.2, 0.3 and 0.2 kg in the control, 25 and 50 mg/kg/dose groups, respectively.

Figure 18 Mean Body Weight of Monkeys dosed with vehicle control or RO5072759 during Lactation



Pregnancy Monitoring and Outcome

Ultrasonographic examinations were used to monitor monkey's pregnancy on Days 30, 44, 58, 72, 86, 100, 114, 128, 142 and 156 p.c. Additional examinations were conducted when signs of abortion were observed. Vaginal smears were taken daily until delivery. Pregnancy outcome is shown in Table 33.

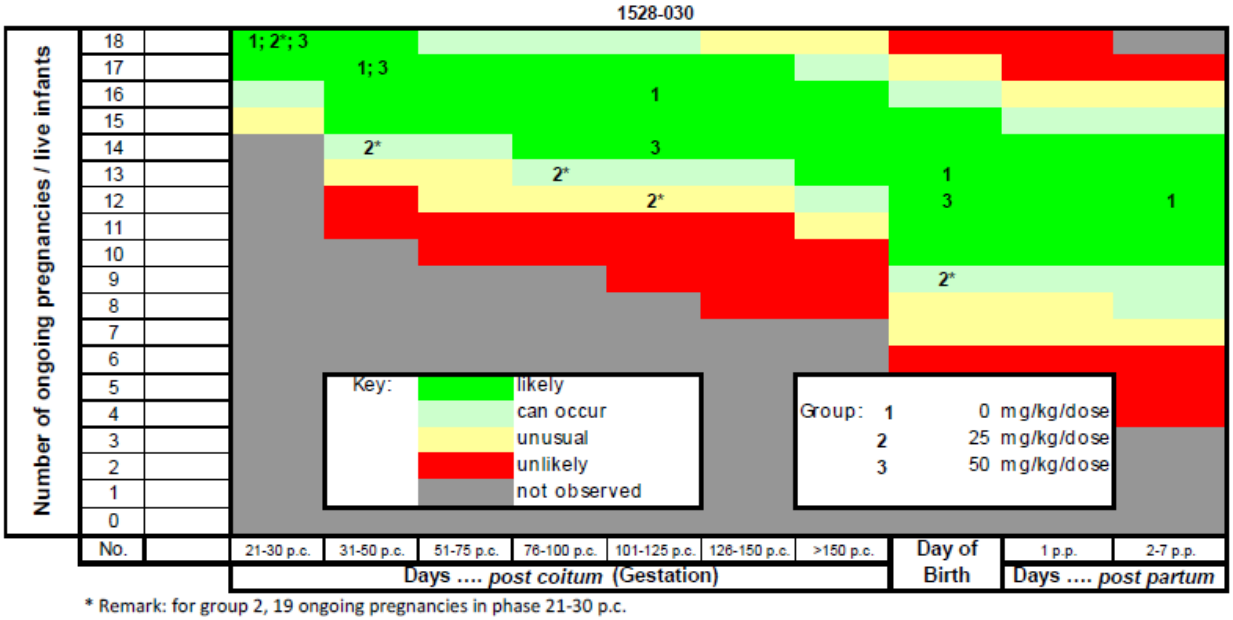
Table 33 Pregnancy Outcomes

Day 20 p.c. to delivery	RO5072759 (mg/kg/dose)		
	0	25	50
Total number of pregnant females	**18**	**19**	**18**
Number of females with abortion	1	6	4
Number of females with stillbirth	3	3	1
Number of females with cesarean section	1	0	0
Number of females with breech delivery	0	1	0
Number of females with early delivery	0	0	1
Number of females with delivery	13	9	12

The incidence of prenatal loss at 25 mg/kg/dose was higher (31.6%) compared to control (5.6%) or 50 mg/kg/dose (22.2%). The Applicant states that these values are within the range of past experience with monkeys at this testing facility (b) (4). The lower rate of successful pregnancies in the 25 mg/kg group was characterized by the CRO as "unusual" and not "likely" according to this analysis provided in the Study Report.

Figure 19 Normogramm of pregnancy outcomes

(Excerpted from Applicant's Submission)



* Remark: for group 2, 19 ongoing pregnancies in phase 21-30 p.c.

Explanation for the normal distribution of outcomes: "likely" contains more than 70%, "can occur" contains approximately 20%, "unusual" contains approximately 8% and "unlikely" contains approximately 2% of outcomes. Jarvis P, et al. (2010), Weinbauer GF, et al. (2011a) and Weinbauer GF, et al. (2011b)

Neonatal/Infant Examinations

Fetal Examinations using Ultrasound

Morphological measurements such as crown-rump length, biparietal and fronto-occipital diameter, femur length were taken and heart rate monitored during gestation from Day 30 through 170, see the appendix for the complete schedule of morphological measurements. All measurements were within or close to the variations noted in the control group and no RO5072759-related differences were observed.

Mortality

A total of 13, 9, and 12 infants were born in the control, 25 and 50 mg/kg/dose groups, respectively. One infant from the control and one infant from the 50 mg/kg/dose group were found dead on Day 0 and 2 p.p., respectively. A total of three infants were sacrificed early, Table 34.

Infant 16330/3 presented abnormal liquid contents of the large intestine; infant 16135/3 presented adhesion of abdominal organs; and infant 16363/2 presented adhesion of pericardium to the heart, liver to diaphragm and lung lobes to each other and pleura. The mother of the 25 mg/kg/dose infant was also sacrificed early on Day 93 post-partum. Microscopic findings of lymphoid tissues atrophy and inflammation of multiple tissues suggest potential immune-depression or hypersensitivity reactions similar to maternal morbidity.

Table 34 Unscheduled Deaths of Infants

(Excerpted from Applicant's Submission)

Animal No.	RO5072759 mg/kg/wk	Day of Early Sacrifice	Cause of morbidity & major histopathology findings
16330/3	50	88 *post-partum*	• Thymus atrophy • Larynx ulcer, pulmonary edema and multiple foci of inflammation centered in airways
16135/3	50	Day 93 *post-partum*	• Serosal inflammation (lung, heart-pericardium/ epicardium, gastro-intestinal tract, liver) • Lymphoid atrophy thymus, spleen • Inflammation/ulcer skin mammary region
16363/2	25	Day 182 *post-partum*	• Interstitial inflammation, kidney • Serosal inflammation (lung, heart-pericardium/ epicardium, gastro-intestinal tract, liver, urinary bladder) • Lymphoid atrophy thymus, spleen

Clinical Signs

Clinical signs of thin hair, reddening of some body parts, crusted lesions, and/or bloody tail followed by amputation and antibiotic treatment were common among groups with similar incidence and frequency.

The 25 mg/kg/dose infant (16363/2) euthanized on Day 182 presented diarrhea, inflated abdomen, dermatitis on several body parts, swelling of mandibular/lymph nodes, severe reddening of face skin, bronchial noises and labored respiration, deformed head by Day 149 p.p., body weight loss starting on Day 153 p.p., hypothermia and hypoactivity by the day of sacrifice.

The 50 mg/kg/dose infant (16330/3) euthanized on Day 88 presented no remarkable clinical signs before Day 77 p.p. However; the infant's condition started deteriorating with diarrhea and body weight loss, presented severe sunken eyes, apathy, weakness and poor physical condition until the day of early sacrifice.

The 50 mg/kg/dose infant (16135/3) presented no remarkable clinical signs and was found dead on Day 93.

Body Weight

Infant mean body weight gain for the 50 mg/kg/dose group was significantly lower compared to control starting on Days 58-88 and corresponded to lower mean body weight, Figure 20 and Table 35. This group gained 15.6% less weight during lactation, Days 1 to 239 after birth, than infants in the control group.

Figure 20 Infant Mean Body Weight during Lactation

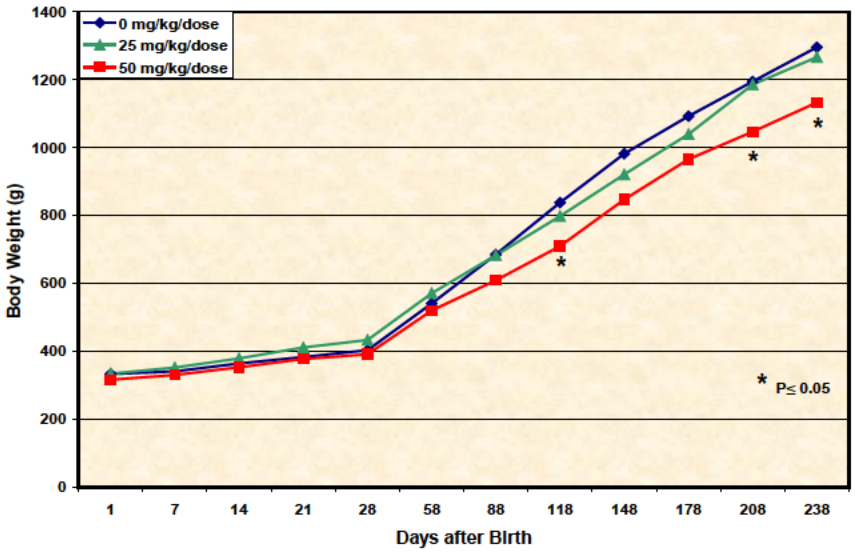


Table 35 Infant Mean Body Weight Gain during Lactation

Day Ranges after Birth	Statistics	RO5072759 (mg/kg/dose)		
		0	25	50
1-7	Mean	9	18	14
	SD	24	10	16
	N	12	9	12
7-14	Mean	23	27	23
	SD	13	12	13
	N	12	9	12
28-58	Mean	138	138	130
	SD	28	40	20
	N	12	9	12
58-88	Mean	144	112	**89****
	SD	28	42	58
	N	12	9	12
88-118	Mean	153	115	**95***
	SD	28	42	52
	N	12	9	10
1-239	Mean	964	933	**814***
	SD	89	144	120
	N	12	8	10
* P≤ 0.05; ** P≤ 0.01; *** P≤ 0.001				

Values in **bold** were significantly different from control

Infant Neurobehavioral Assessments

Parameters evaluated on Days 1 and 7 p.p. for observed and elicited postural tones included respiration rate, head extended, hip and shoulder adduction, elbow, knee, fingers and toes flexed, and wrist and ankle elicited dorsiflexion, see appendix for the complete schedule of neurobehavioral assessments. No RO5072759-related alterations in neurobehavioral assessments were present.

Infant Skeletal Development

X-ray images were taken during week 13 and 16 after birth. No RO5072759-related pathological findings were present. All infants presented a skeletal development according to age.

Infant Hematology

Absolute counts and percent lymphocytes as well as other few parameters were statistically different from control infants, Table 36. The reduced percent lymphocytes corresponded with the significant reduction of CD20+ B cells observed through PND 184.

Table 36 Fold-Change in Hematological Parameters in Infants

Parameter	Maternal Doses RO5072759 mg/kg/dose	
	25	50
Hematocrit (%)		
PND 28	**+1.1**	+1.1
N=	9	12
Mean Corpuscular Hemoglobin concentration (mmol/L)		
PND 28	**-1.1**	**-1.1**
N=	9	12
PND 84	**-1.1**	**-1.1**
N=	9	10
Lymphocytes (%)		
PND 84	**-1.2**	**-1.3**
PND 112	**-1.2**	**-1.1**
PND 140	**-1.1**	**-1.2**
N=	9	10
Monocytes (%)		
PND 84	**+2**	**+2.4**
PND 140	+1.5	**+2**
N=	9	10
Neutrophils (%)		
PND 84	**+1.7**	**+1.9**
PND 112	**+1.8**	**+1.4**
PND140	+1.5	**+1.5**
N=	9	10
Basophils (%)		
PND 84	**-1.7**	**-2.5**
N=	9	10

PND= postnatal day
Values in **bold** were significantly different from control

Infant Immunophenotyping by Flow Cytometry

Administration of RO5072759 to mothers during pregnancy resulted in complete depletion of CD20+ B cells at both doses of 25 or 50 mg/kg/dose. CD20+ population recovered by PND 112 and 140 at 25 or 50 mg/kg/dose, respectively. CD16+ NK cells were not impacted but an increase in other lymphocyte populations were observed, see Table 37.

Table 37 Percent of Lymphocytes cells in Infant monkeys

Parameter	Maternal Doses RO5072759 mg/kg/dose		
	0	25	50
CD20+ B Cells			
PND 28	26.7	**0.0**	**0.0**
PND 84	30.9	**15.0**	**1.8**
PND112	30.9	27.8	**20.1**
PND 238	25.8	35.5	**36.3**
CD3+ T Cells			
PND 28	66.5	**92.2**	**88.6**
PND 84	64.8	**81.0**	**92.6**
PND 238	68.5	58.9	**56.4**
CD4+ T Cells			
PND 28	50.9	**74.3**	**70.9**
PND 84	45.8	**59.6**	**68.3**
PND 238	42.6	36.7	36.6
CD8+ T Cells			
PND 28	12.9	15.7	15.0
PND 84	15.6	18.9	**22.1**
PND 238	18.9	18.1	15.9

Group 2, N= 9 at all intervals
Group 3, N= 12 on PND 28
11 on PND 84
9 on other intervals

Values in **bold** were significantly different from control

Antigenic Challenge in Infants

Infants were challenged with anti-KLH immunoglobin G and M on PND 181 and 212 ± 1. T-cell dependent antibody responses on different days after KLH stimulation showed no RO5072759-related effects. Large titer inter-individual variation was observed with low and high responders in the control and RO5072759-treated groups indicating there were no long-lasting RO5072759 effects on immune function in the offspring. It is important to note that all 3 early sacrificed infants occurred before the first antigenic challenge and no response data was collected for those animals.

Toxicokinetics

Maternal Animals

During the dosing phase, blood samples for TK analysis were collected before dosing on Days 20 p.c. and 139 p.c. at 7, 24, 96 and 168 h. Blood samples were also collected on Day 28 p.p. and during the last week prior to necropsy.

RO5072759 was found in 23/194 samples collected from Group 1 monkeys that received the vehicle control. Exposure in vehicle control monkeys was <0.012% of the RO5072759 concentration measured in 25 mg/kg/dose monkeys at the same sample

time. RO5072759 was also found in 12/38 predose samples with an individual assigned to the 50 mg/kg/dose group presenting an unusual high concentration of 1980 µg/mL. *No explanation was found for these vehicle or predose positive samples.*

Exposure to RO5072759, as measured by C_{max} and $AUC_{(0-168h)}$, increased as the dose increased in a dose-proportional manner on Days 20 and 139 p.c. Table 38. After 119 days of dosing, mean C_{max} and $AUC_{(0-168h)}$ values were higher (approximately 1.7 to 2.0 fold) suggesting some degree of accumulation of RO5072759 after repeated dosing. RO5072759 was detectable at all time points and the maximum serum concentrations were generally observed at the 7 hour time point.

Table 38 TK Parameters of RO5072759 in serum of pregnant Cynomolgus monkeys after intravenous administration

Group	Dose (mg/kg/dose)	Mean C_{max} (µg/mL)	C_{max} Accumulation ratio	Fold increase	Mean AUC (0-168h) (µg*h/mL)	AUC Accumulation ratio	Fold increase
Day 20 post-coitum							
2	25	709	--	1.00	66900	--	1.00
3	50	1460	--	2.06	125000	--	1.83
Day 139 post-coitum							
2	25	1220	1.72	1.00	122000	1.87	1.00
3	50	2470	1.69	2.02	250000	2.05	2.00

Infant Animals

Blood samples were collected on Days 28 and 168 p.p. and during the last week prior to necropsy.

RO5072759 serum concentration in infant monkeys was variable on Day 28 p.p. and was BLQ on Day 168 p.p. and prior to necropsy collection. Mean infant serum concentration increased as the dose increased from 25 to 50 mg/kg/dose in a higher than dose proportional (~2.8 –fold) manner. Mean maternal and infant serum concentration values overlapped on Day 28 p.p. at both dose levels, Table 39.

Table 39 Concentration of RO5072759 in serum of infant monkeys after intravenous administration during pregnancy

Group 2: 25 mg/kg/dose			
Animal No.	**Day 28 post-partum**	**Day 168 post-partum**	**Prior Necropsy**
	Concentration (µg/mL)		
15304 infant	(b) (4)		
16102 infant			
16314 infant			
16339 infant			
16356 infant			

Group 2: 25 mg/kg/dose			
Animal No.	**Day 28 post-partum**	**Day 168 post-partum**	**Prior Necropsy**
	Concentration (µg/mL)		
16363 infant	(b) (4)		
16365 infant			
16868 infant			
16876 infant			
N	9	9	8
Mean	86.6	0.00158	0.00146
SD	51.6	0.00473	0.00414
Maternal Mean	394	-	-
Maternal SD	333	-	-

Group 3: 50 mg/kg/dose			
Animal No.	**Day 28 post-partum**	**Day 168 post-partum**	**Prior Necropsy**
	Concentration (µg/mL)		
14216 infant	(b) (4)		
15091 infant			
15092 infant			
15260 infant			
16001 infant			
16129 infant			
16135 infant			
16193 infant			
16199 infant			
16225 infant			
16330 infant			
16845 infant			
N	11	10	10
Mean	241	0.00179	NC
SD	166	0.00296	NC
Maternal Mean	144	-	-
Maternal SD	90.6	-	-

* Sample taken on Day 30 p.p. was not included in the mean calculation

Milk Samples

RO5072759 concentration in milk was low compared to the corresponding maternal or infant serum concentration. Milk to maternal or infant serum ratios were in general below 0.005 indicating a milk concentration less than 0.5% compared to serum concentration. The proportion of RO5072759 in the serum of infant compared to its mother was variable especially in the 25 mg/kg/dose group, where concentrations were generally higher in mother serum. Serum concentration values in infants were greater than their mothers in the 50 mg/kg/dose group where the ratio of RO5072759 in serum of infant to mother was higher.

The high concentration of RO5072759 in the serum of infants is likely the result from RO5072759 crossing the blood-placenta barrier since the concentration of RO5072759 in the serum of infants was much higher than the concentration of RO5072759 in the milk, Table 39 and Table 40.

Table 40 Ratio of RO5072759 in female Cynomolgus monkey mother serum, mother milk, and infant serum after intravenous administration

Group 2: 25 mg/kg/dose				
Animal No. (Mother)	Animal No. (Infant)	Mother Milk/ Mother Serum	Infant Serum/ Mother Serum	Mother Milk/ Infant Serum
		Concentration (µg/mL)		
15304	15304 infant	(b) (4)		
16102	16102 infant			
16314	16314 infant			
16339	16339 infant			
16356	16356 infant			
16363	16363 infant			
16365	16365 infant			
16868	16868 infant			
16876	16876 infant			
N		9	9	9
Mean		0.000447	0.764	0.00124
SD		0.000708	1.09	0.00181

Group 3: 50 mg/kg/dose				
Animal No. (Mother)	Animal No. (Infant)	Mother Milk/ Mother Serum	Infant Serum/ Mother Serum	Mother Milk/ Infant Serum
		Concentration (µg/mL)		
14216	14216 infant	(b) (4)		
15091	15091 infant			
15092	15092 infant			
15260	15260 infant			
16001	16001 infant			
16129	16129 infant			
16199	16199 infant			
16225	16225 infant			
16330	16330 infant			
16845	16845 infant			
N		10	9	9
Mean		0.00125	2.44	0.000823
SD		0.000676	1.88	0.000651

Antidrug Antibody Analysis

A total of 8/279 tested samples were positive for anti-RO5072759 antibody in the screening/confirmatory assay. Two ADA positive animals belonged to the 25 mg/kg/dose group (maternal animal 16314 and infant animal 16876), and six ADA

positive animals belonged to the 50 mg/kg/dose group (maternal animals 15091, 15260, 16225, 16845 and infant animals 15260, 16339).

Dosing Solution Analysis

End of dosing concentration was conducted using UV-spectrometric analysis and purity determined using SE-HPLC analysis. All protein concentration evaluation were within ±5% of the certificate of analysis value and the percent of monomer (99.4%) was similar to the purity determined at the beginning of the study (99.7%), see tables below. Concentration and purity determinations showed that formulated RO5072759 was stable during the time of study conduct.

Table 41 Protein Concentration End of Dosing Samples

(Transcribed from the Study Report)

	Description	Protein content (mg/mL)	Mean Protein content (mg/mL)	Delta from the CoA value (%)	Within ±5% of CoA value
1	hMA anti CD20 RO507-2759/F06-01 Batch H0002	25.37	25.4	-1	Yes
		25.48			
		25.44			
2	hMA anti CD20 RO507-2759/F06-01 Batch H0002	25.60	25.5	0	Yes
		25.49			
		25.56			

Table 42 Purity of RO5072759 huMAb End of Dosing Samples

(Transcribed from the Study report)

Description	Peaks	Percent Area
hMA anti CD20 RO507-2759/F06-01 Batch H0002	Sum of HMW	(b) (4)
	Monomer	
	Sum of LMW	
hMA anti CD20 RO507-2759/F06-01 Batch H0002	Sum of HMW	
	Monomer	
	Sum of LMW	

Necropsy

Unscheduled Maternal Deaths

One female each at 25 and 50 mg/kg/dose were euthanized during the gestation phase and two females each at 25 and 50 mg/kg/dose were euthanized during the lactation phase, see Table 29 & Table 30 under Maternal Examinations-Mortality. One additional 50 mg/kg/dose female was euthanized on Day 91 p.p. because of spontaneous cestode cysts infection.

Organ Weight
Absolute organ weight and organ weight to body or brain weight ratios were unremarkable in early maternal decedents.

Macroscopic findings
RO5072759-related macroscopic findings included adhesions of liver, spleen, intestine and heart to adjacent organs and pleural or pericardial surfaces (16363), discolored/ mottled kidneys (16001, 15304), round edges of liver (15092, 15304), abnormal contents (semi-liquid/liquid) or discolorations of large and small intestine (16001, 16416, 15304). These changes corresponded with histomorphological changes consistent with evidence of hypersensitivity-like reaction. In addition, one animal (16001) had mycosis in the GI track indicative of an opportunistic infection secondary to their immune-compromised status of the animal.

Microscopic findings
RO5072759-related histopathological and immune-mediated lesions were noted in the gastrointestinal tract, liver and gallbladder, kidney, visceral serosae and pericardium/ epicardium of early decedents. Histopathological findings included lymphoid and bone marrow depletion and lack of germinal centers in lymphoid tissues accompanied with inflammation of several organs such as kidney and liver as well as minimal to slight intestinal crypt micro abscesses and/or esophageal hyperkeratosis as immune-mediated lesions.

Unscheduled Infant Deaths

One infant from the control and one infant from the 50 mg/kg/dose group were found dead on Day 0 and 2 p.p., respectively. A total of three infants were sacrificed early, see Table 34 under Infant Examinations-Mortality.

Macroscopic findings
RO5072759-related macroscopic findings included abnormal liquid contents of the large intestine, adhesion of abdominal organs such as adhesion of pericardium to the heart, liver to diaphragm and lung lobes to each other and pleura.

Microscopic findings
RO5072759-related histopathological findings included similar findings as the unscheduled maternal deaths, see paragraph above.

Scheduled Maternal Necropsies

Macroscopic findings
No unusual macroscopic findings suggestive of target organ toxicity were noted.

Microscopic findings
RO5072759-related histopathological findings included inflammatory cell foci, acute, sub-acute or chronic inflammation and hemorrhage in different organs, GALT hyperplasia and crypt microabscess, fibrosis, atrophy, basophilic, dilation and/or vacuolation of tubule cells in the kidney, hepatocytes vacuolation and liver necrosis, and

lymphocyte depletion in different organs. Few of these microscopic findings were graded moderate to severe and occurred at low incidence, one-to-two animals, in mothers at 25 or 50 mg/kg/dose.

Scheduled Infant Necropsies

Organ Weight
Absolute organ weight and organ weight to body or brain weight ratios were unremarkable in terminal infant sacrifices.

Macroscopic findings
No unusual macroscopic findings suggestive of target organ toxicity were noted.

Microscopic findings
RO5072759-related histopathological findings included inflammatory cell foci, acute, sub-acute or chronic inflammation and hemorrhage in different organs, crypt microabscess, fibrosis in several organs, inflammatory cell foci, atrophy, basophilic, dilation and/or vacuolation of tubule cells in the kidney, hepatocytes vacuolation and individual hepatocytes necrosis, and lymphocyte depletion in different organs. Few of these microscopic findings were graded moderate to severe and occurred at low incidence, one-to-two animals, in infants at 25 or 50 mg/kg/dose.

10 Special Toxicology Studies

1 Study title: RO5072759: Cross-Reactivity Study of RO5072759-000 (huMAb_CD20) with Normal Human Tissues

Study no.:	1024159
Study report location:	BLA Section 4.2.3.7.7
Conducting laboratory and location:	(b) (4) [redacted]
Report Date:	January 2, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RO5072759, GWT0022, 98.9%; negative control used was Human IgG1, kappa (Sigma)

Cryosections of normal human tissues (3 tissues from 3 or more donors) were stained with two antibody concentrations (5 mcg/ml and 30 mcg/ml). Expected specific cross-reactivity was observed localized in the membrane of the following tissues and cell types:

- Lymphocytes in
  - Breast (mammary gland)
  - GALT in the small intestine and stomach
  - Lymph node
  - Spleen
  - Thymus
  - Tonsil
  - Bone marrow
  - Thyroid

Unexpected cross-reactivity was observed localized in the membrane and cytoplasm of the following human tissues:

- Liver epithelium (bile canaliculi/ducts)
- Salivary gland (glands and ductular/duct basal reserve cells)
- Lung endothelium

The following tissues were evaluated:

Adrenal	Lung	Spinal Cord
Blood Cells	Lymph Node	Spleen
Blood vessels (endothelium)	Ovary	Striated muscle (skeletal)
Bone Marrow	Fallopian Tube (oviduct)	Testis
Brain – cerebrum (cortex)	Pancreas	Thymus
Brain – cerebellum	Parathyroid	Thyroid
Breast (mammary gland)	Peripheral Nerve	Tonsil
Eye	Pituitary	Ureter
Gastrointestinal Tract	Placenta	Urinary Bladder
Heart	Prostate	Uterus- (endometrium)
Kidney (glomerulus, tubule)	Salivary Gland	Uterus- cervix
Liver	Skin	

[1] Blood cells include granulocytes, lymphocytes, monocytes and platelets.

[2] Gastrointestinal tract includes the colon (large intestine), esophagus, small intestine, and stomach.

2 Study title: RO5072759: Cross-Reactivity Study of RO5072759-000 (huMAb_CD20) with Normal Cynomolgus Monkey Tissues

Study no.: 1024159
Study report location: BLA Section 4.2.3.7.7
Conducting laboratory and location: (b) (4)
Report Date: January 8, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: RO5072759, GWT0022, 98.9%; negative control used was Human IgG1, kappa (Sigma)

Cyrosections of normal cynomolgus monkey tissues (2 donors per tissue if available) were stained with two antibody concentrations (5 mcg/ml and 30 mcg/ml). Expected cross-reactivity with RO5072759 was observed localized in membranes of the following tissues and cell types:

- Lymphocytes in
  - Lymphoid tissues and thyroid

Unexpected staining was observed localized in the membrane and cytoplasm of:

- Endothelial cells found in the
  - Small intestine
  - Kidney
  - Lung

- Ovary
- Pancreas
- Pituitary
- Prostate
- Salivary gland
- Testis
- Endometrium

The following tissues were evaluated:

Adrenal	Lung	Spinal Cord
Blood vessels (endothelium)	Lymph Node	Spleen
Bone Marrow	Ovary	Striated muscle (skeletal)
Brain – cerebrum (cortex)	Fallopian Tube (oviduct)	Testis
Brain – cerebellum	Pancreas	Thymus
Breast (mammary gland)	Parathyroid	Thyroid
Eye	Peripheral Nerve	Tonsil
Gastrointestinal Tract	Pituitary	Ureter
Heart	Placenta	Urinary Bladder
Kidney (glomerulus, tubule)	Prostate	Uterus- (endometrium)
Liver	Salivary Gland	Uterus- cervix
	Skin	

Reviewer comment: The staining of endothelium was unexpected in both human and monkey tissues. The salivary gland and lung were overlapping between the two species. Staining of the liver was seen only in human tissue.

3 Study title: Effect of RO5072759 on Cytokine Release and Neutrophil Activation in Human Whole Blood (including Amendment 1)

Study no.:	1025124
Study report location:	BLA Section 4.2.3.7.7
Conducting laboratory and location:	Pharma Research Basel PRBN-T, Immunosafety F. Hoffmann-La Roche Ltd. Basel, Switzerland
Report Date:	December 18, 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	RO5072759, GWT0025

This study was conducted to compare the effects of RO5072759 on cytokine release using fresh undiluted heparinized human blood from healthy donors. TNF secretion was used as the key biomarker for identifying risk because TNF, rather than INF-γ or IL-6, because TNF was elevated in patients that experienced cytokine release syndrome

after rituximab infusion.[24] CD11b was described as the second most important marker because TNF triggers neutrophil activation. Upregulation of CD11b by more than 30% is considered a positive signal.[25]

Methods:

- Fresh whole blood from healthy donors was collected with added heparin as an anti-coagulant and kept at room temperature until usage (within 1-4 hours). Blood was added in triplicate to U-bottom wells of a 96-well plate containing the antibody to be tested. Final antibody concentrations ranged from 0.01 to 200 mcg/ml. An endotoxin capture reagent was added to exclude potential interference from endogenous endotoxins.
- TNF, IL-6, INF-ɣ and CD11b were measured following 2 hour incubation with antibody. A commercial kit was used to detect TNF, INF-ɣ and IL-6 (CBA-KIT: BD™ CBA Human Th1/Th2 Kit II (Cat. No. 551809), BD Pharmingen). The lower limit of detection of the CBA assays was TNF-α: 3.6pg/ml, IFN-γ: 17.2 pg/ml, IL-6: 5.0 pg/ml. The lower limit of quantitation (LLOQ) was: TNF-α: 13.2 pg/ml, IFN-γ: 81.1 pg/ml, IL-6: 21.4 pg/ml.
- CD11+ neutrophils were measured using flow cytometry and calculated as a percentage of CD11b positive cells per 1500 CD16+CD45+ neutrophils.
- Alemtuzumab (Campath), muromonab-CD3 (Orthoclone IKTr3) and rituximab were used as positive controls. Palivizumab (Synagis) was used as a negative control.
- Two tests were conducted:
    - Study 1: Samples from 22 donors were tested with RO5072759, alemtuzumab and muromonab-CD3.
    - Study 2: Samples from 19 donors were tested with RO5072759, rituximab or alemtuzumab.

Results:

- Study 1 results showed that 5/22 donors exhibited minor elevation of TNF, and 1/6 exhibited moderate elevation of TNF. CD11b-positive neutrophils were observed in these 6 donor samples. Alemtuzumab was positive in 22/22 samples and TNF levels were 3-30 times higher than those observed with RO5072759.
- Study 2 results showed that 4/19 donors exhibited minor elevations of TNF and CD11b-positive neutrophils following RO5072759. Rituximab did not induce cytokine secretion or neutrophil activation above thresholds in any of the samples tested. Alemtuzumab triggered TNF secretion in 16/19 donor samples at 3-20 fold higher levels than RO5072759.

Conclusion:

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[24] These citations were provided in support of using TNF as the key biomarker: Bienvenu J, et al. *Hematol J*, (2001) 2: 378-84; Tournamille JF, *Bull Cancer*, (2005) 92: 769-71; Winkler U, et al. *Blood*, (1999)94:2217-24.
[25] Report 1025484 was cited in support of the 30% cut-off.

- RO5072759 induced cytokine secretion in approximately 20-25% of the donor samples, at a lower level than alemtuzumab but at a higher level than rituximab.

Reviewer's comment: This assay did not include a set of experiments using immobilized antibodies. By not doing, the assay may not adequately mimic the in vivo conditions that may give rise to cytokine release.

4 Study title: Effect of R05072759 on Cytokine Release in a 24-hour Human Whole Blood Assay

Study no.:	1045703
Study report location:	BLA Section 4.2.3.7.7
Conducting laboratory and location:	Pharma Research Basel PNSIB, Immunosafety F. Hoffmann-La Roche Ltd. Basel, Switzerland
Report Date:	Sept. 7, 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	RO5072759/F01-01, # 78262500

The main difference between this assay from Study 1025124 reviewed above is the incubation time was increased from 2 hours to 24 hours.

Methods:

- Fresh human blood from healthy donors was collected, heparanized and processed within 3 hours. Blood samples were incubated with antibodies for 24 hours at 37 ºC. Antibodies were not immobilized to the U-bottom 96 well plates.
- Plasma samples were analyzed for TNF, IL-6 and IL-8 with a 4-plex commercial kit (MesoScale Discovery Multi-Array™).
- RO5072759 was tested and compared to alemtuzumab, cetuximab (Erbitux®) or a TGN1412-like anti-CD28 recombinant IgG4 antibody. Cetuximab served as a negative control. Four different concentrations of each antibody were used (0.1, 1, 10 and 100 mcg/ml).

Results:

Number of blood samples with elevated cytokines		
Cytokine	RO5072759	Alemtuzumab
IL-6	10/10	10/10
IL-8	9/10	10/10
TNF	10/10	10/10

Reviewer's comment: These results suggest that RO5072759 is likely to elicit cytokine release.

5 Study title: RO5072759-000 : *In-Vitro* hemolysis and plasma

precipitation and turbidity tests with human heparinated blood and plasma

Study no.:	1025140
Study report location:	BLA Section 4.2.3.7.7
Conducting laboratory and location:	Pharma Research F.Hoffmann-LaRoche Ltd. Basel, Switzerland
Report Date:	March 26, 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	RO5072759, GWT0025, 98.9%

An in vitro hemolysis test was conducted using erythrocytes. Measurement of hemoglobin in the test medium indicates whether hemolysis occurred. A range of concentrations were used (0.07-5 mg/ml) and no hemolysis occurred. A positive control was not included.

11 Integrated Summary and Safety Evaluation

The mechanism of action of anti-CD20 monoclonal antibodies including rituximab and obinutuzumab involves a combination of (1) antibody-dependent cell-mediated cytotoxicity and phagocytosis (ADCC and ADCP) (2) caspase-independent apoptosis or direct cell death induction and (3) complement-dependent cytotoxicity (CDC).

The pharmacokinetics of obinutuzumab in monkeys displayed linearity and were characterized by a low clearance and a small volume of distribution with long terminal $t_{1/2}$. Exposure values were similar in pregnant monkeys compared to non-pregnant monkeys indicating pregnancy had no impact on pharmacokinetics. ADA formation was prevalent and was associated with rapid clearance of obinutuzumab. B-cell depletion correlated with circulating obinutuzumab levels.

The estimated exposure margins represented by the doses administered during the 26-week toxicology study exceed the estimated population prediction for a 1000 mg clinical dose every 28 days (Table 43). Significant morbidity and mortality were seen at doses considered ~1.8 fold greater but these findings were determined to be the result of cross-species reactivity to repeated IV administration of a foreign protein.

Table 43 Estimated Safety Margins based on Animal Exposures

(Excerpted from the BLA)

Species	Dose (mg/kg)	$AUC_{(0-168\ hr)}$ (µg • hr/mL)	AUC_{4wk} [a] (µg•day/mL)	Exposure Margin [b]
Cynomolgus monkey	5	39800	6630	0.39
	25	183000	30500	1.8
	50	344000	57300	3.4

[a] Extrapolated AUC4wk by $AUC_{(0-168\ hr)}$ x4 ÷24 hr

[b] Based on population predictions for 1000 mg clinical dose every 28 days (Cycle 6), AUC_{tau} = 17040 µg.day/mL, (see 5.3.3.5 Population PK Study Report)

Species	Dose (mg/kg/dose)	AUC (0-168h) [a] (µg*hr/mL)	AUC [b] (µg*day/mL)	Exposure Margin [c]
Cynomolgus monkey (Pregnant)	25	122000	24605	2.6
	50	250000	50420	5.3

[a] Exposure after 119 days of daily dosing

[b] Exposure calculated AUC(0-168h) x 24 h / 119 days

[c] Based on population predictions in CLL patients receiving 1000 mg clinical dose every 28 days, AUC_{tau} = 9547 µg*day/mL

The primary effects of obinutuzumab include marked decreases in circulating B cells observed after the first dose in all animals. Corresponding lymphoid tissue B-cell depletion occurred in the spleen and lymph nodes. Both circulating and lymphoid tissue B cells remained depleted well into the recovery phase. Exceptions included individual animals that developed anti−obinutuzumab antibodies and, therefore, had considerably reduced exposures.

Table 44 Summary of Major Findings from Repeat-Dose Toxicology Studies

GLP Toxicology Study	Weekly Doses (mg/kg)	Major Findings
13-Week IV (#1024830)	0, 10, 30,100	• 2 HD (1M/1F) unscheduled deaths possibly resulting from infection secondary to immunosuppression (gingivitis in the HD male) or hypersensitivity reactions based on histomorphologic findings; HD female also experience unusually strong menstrual bleeding • B-cell ablation after 1st dose in all groups that continued through recovery, but showing reversal by the end of the recovery period

GLP Toxicology Study	Weekly Doses (mg/kg)	Major Findings
		• Transient reduction in NK cells after first dose. Systemic inflammation was observed that correlated with toxicities to major organs (brain, heart, and lung) that were partially reversible.
26-Week IV (#1036190)	0, 5, 25, 50	• 7 unscheduled deaths: 3 MD/2 HD deaths resulting from chronic hypersensitivity reactions leading to immune-complex mediated glomerulonephritis or inflammation in other tissues, or both; 1 LD death following anaphylactoid reaction following 6th dose; 1 MD F death attributed to fatal fasting syndrome but treatment-related cause cannot be ruled out. • Dose-related incidence of glomerulonephritis, serosal/adventitial inflammation in multiple tissues and/or arteritis/periarteritis. • Gingivitis in all treated males (all doses) • B-cell ablation at all doses that continued into recovery unless neutralizing ADA developed. • Transient reduction in NK cells after 1st dose • Hematological changes included anemia, lower total protein, albumin and hematocrit; increased BUN and creatinine; urinary protein excretion—findings consistent with inflammation associated with hypersensitivity reactions or poor clinical condition or both. • Microscopic changes included immune-complex glomerulonephritis, arteritis/periarteritis, and an increased incidence and/or severity of mononuclear infiltrates/inflammation in multiple tissues. Inflammatory changes were present in multiple tissues and appeared systemic, not target organ specific. • Immune-complex formation confirmed by detection of monkey IgG by immunofluorescence and electron dense deposits by TEM.
4-Week SC (#1024838)	0, 30, 120	• 1 HD unscheduled death resulting from infection secondary to immunosuppression or possible hypersensitivity reactions based on histomorphologic findings • B-cell ablation at both doses used that continued through recovery; some reversal at the end of recovery period. • Transient reduction in NK cells after first dose

GLP Toxicology Study	Weekly Doses (mg/kg)	Major Findings
ePPND (#1036190)	0, 25, 50 (Day 20 p.c. until birth)	• Higher incidence of prenatal loss at LD (31.6%) compared to control (5.6%) or HD (22.2%). • Maternal mortality LD: 3/19 (16%); HD: 3/18 (17%) resulting from infections or immune responses • Glomerulonephritis was less prominent than systemic serosal inflammation (along retroperitoneal and abdominal and, to a lesser extent, thoracic organs), and parenchymal interstitial inflammation (liver/gallbladder and kidney) compared to the 26-week • Significantly lower mean body weight and body weight gain in HD infants but not observed in HD dams • Complete B-cell depletion in neonates that returned to control levels by 112 days (LD) or 168 days (HD) postpartum • Maternal and infant serum concentrations were similar on Day 28 postpartum indicating that RO5072759 crosses the blood-placental barrier.

LD, MD, HD: low, mid and high dose group

Excluding the toxicities associated with immune complex deposition, the toxicity findings observed were, in general, consistent with the intended pharmacology of obinutuzumab: B-cell ablation, transient NK cell reduction, and opportunistic infections secondary to immunosuppression. Immune-complex-mediated glomerulonephritis and hypersensitivity reactions were noted at all doses in the 26-week study. These findings were attributed to cross-species reactivity to obinutuzumab. Results from the in vitro analysis of cytokine release predicted first infusion-related cytokine release which is clinically manageable with premedication, splitting the first dose over two days and modulating the infusion rate.

12 Appendix/Attachments

A. Studies submitted that were not reviewed:

	Study Report	Title
Primary Pharmacology		
1	1024727	Comparison of Antitumor Activity of Anti-CD20 Antibody RO5072759 (GA101) and Rituximabe in SUDHL-4 SCID Beige Mouse Xenograft
2	1025127	Redistribution of CD20 to Lipid Rafts Upon Binding of RO5072759 (GA101) in Comparison to Rituximab
3	1025128	Homotypic Aggregation Mediated by RO5072759 (GA101) in comparison to Rituximab
4	1025129	CD20 Does not Internalize Upon binding of RO5072759 (GA101) and Rituximab
5	1025130	RO5072759 (GA101) and Rituximab Compete for Binding to CD20
6	1025132	Down-Modulation of CD19 Upon Binding of RO5072759 (GA101) in Comparison to Rituximab - A Possible Mechanism of Action
7	1025133	Half Binding Capacity of Non-Hodgkin's Lymphoma Cells for RO5072759 (GA101) Compared to Rituximab\Pre-Clinical Study Report
8	1025134	Properties of Non-Hodgkins Lymphoma Cell Lines: Bead-Based CD20 Receptor Quantification\Pre-Clinical Study Report
9	1025135	Evaluation of the Inhibition of Cell Proliferation by RO5072759 (GA101) in Comparison to Rituximab
10	1025237	Phosphatidylserine Exposure and Cell Death Induction of RO5072759 (GA101) in Comparison to Rituximab in Leukemic Patient Samples
11	1025238	CD20 Binding and Competition for Binding on Human NHL Cell Lines of RO5072759 (GA101) in Comparison to Rituximab
12	1025239	Autologous B-Cell Depletion in Fresh Whole Blood and Fate of B-Cells by RO5072759 (GA101) in Comparison to Rituximab
13	1025242	CD20 Binding on Human vs. Cynomolgus B Cells of RO5072759 (GA101) Compared to Rituximab
14	1025243	Cynomolgus In Vitro ADCC Activity and Ex Vivo Autologous B Cell Depletion of RO5072759 (GA101) in Comparison to Rituximab
15	1025244	B-Cell Depletion in Whole Blood of Leukemic Patients - RO5072759 (GA101) Precursors in Comparison to Rituximab
16	1025304	Evaluation of the Antitumor Activity of RO5072759 (GA101) and Rituximab (Mabthera) in the Human OCI-Ly-18 NHL (DLBCL) Xenograft I.V. Model in SCID Beige Mice
17	1025305	Evaluation of the Antitumor Activity of RO5072759 (GA101) and Rituximab (Mabthera) in the Human OCI-Ly-18 NHL (DLBCL) Xenograft S.C. Model in SCID Beige Mice
18	1025306	Evaluation of the Antitumor Activity of RO5072759 (GA101) and Rituximab (Mabthera) in the Human Z138 NHL (MCL) S.C. Xenograft Model in SCID Beige Mice
19	1025307	Evaluation of the Antitumor Activity of RO5072759 (GA101) and Rituximab (Mabthera) in the Human Z138 NHL (MCL) I.V. Xenograft Model in SCID Beige Mice
20	1025308	Evaluation of Apoptosis Induction of RO5072759 (GA101) and Rituximab (Mabthera) after Single Treatment against Advanced S.C. Human Z138 Mantle Cell Lymphoma Xenografts in SCID Beige Mice
21	1025309	Dose Related Anti-Tumor Activity of RO5072759 (GA101) and Rituximab (Mabthera) in the Human Z138 NHL (MCL) S.C. Xenograft Model in SCID Beige Mice

	Study Report	Title
22	1025310	Anti-Tumor Activity of RO5072759 (GA101), GA101 WT and Variant BHH2A Against the Human Z138 NHL (MCL) S.C. Xenograft Model in SCID Beige Mice
23	1025312	Evaluation of the Antitumor Activity of Rituximab (Mabthera) and RO5072759 (GA101) in the Human Raji NHL Advanced Xenograft S.C. Model in SCID Beige Mice
24	1025313	Evaluation of Apoptosis Induction of Rituximab (Mabthera) and RO5072759 (GA101) After Single Treatment Against Advanced S.C. Human Raji NHL Xenografts in SCID Beige Mice (Study CD20-PZ-RAJI-006)
25	1025314	Evaluation of the Anti-Tumor Activity of RO5072759 (GA101) and Rituximab (MabThera) Against Raji NHL Cells After I.V. Transplantation into Female SCID Beige Mice (Survival Model)
26	1025316	Dose Related Anti-Tumor Activity of RO5072759 (GA101) and Rituximab (MabThera) in the Human OCI-LY18 NHL (DLBCL) S.C. Xenograft Model in SCID Beige Mice
27	1025318	Anti-Tumor Activity of RO5072759 (GA101), Compared to Rituximab Against the Human RL Follicular NHL S.C. Xenograft Model in SCID Mice
28	1025328	Evaluation of the Therapeutic Properties of RO5072759 (GA101) in Comparison to Rituximab
29	1025329	Evaluation of the Therapeutic Properties of RO5072759 (GA101) in Comparison to Rituximab
30	1025330	CD19 Receptor Modulation and B-Cell Depletion on Normal, Human B-Cells by RO5072759 (GA101) in Comparison to Rituximab
31	1025331	Humanization of RO5072759 (GA101) and Elbow-Hinge Mutation
32	1025342	Cloning and Sequence Analysis of the Cynomolgus FcgRIIIa Gene
33	1025343	GA101ge, GA101-WT and Rituxan: Comparison of Receptor Expression and In Vitro B Lymphocyte Depletion
34	1026442	CD20 Receptor Quantification on Human, Non-Malignant B Cells from Volunteers
35	1026567	Anti-CD20 Mediated Killing of CD20 Positive Tumor Cells by Differentiated Peritoneal Monocytes - RO5072759 (GA101) in Comparison to Rituximab
36	1027582	CD19 Modulation and B-Cell Depletion of Primary Human B-Cells Induced by RO5072759 (GA101) in Comparison to Rituximab
37	1028080	Histopathological Parameters and Antitumor Activity of RO5072759 (GA101), GA101 WT and Variant BHH2A After Single Administration Against the Human Z138 NHL (MCL) S.C. Xenograft Model in SCID Beige Mice
38	1028081	Anti-Tumor Activity of RO5072759 (GA101), After Alternative Dose Scheduling Against the Human Z138 NHL (MCL) S.C. Xenograft Model in SCID Beige Mice
39	1028082	Antitumor Activity of RO5072759 (GA101) after Single Administration at Different Doses against Advanced S.C. Z138 MCL Xenografts in SCID Beige Mice (168 hrs)
40	1028083	Evaluation of Apoptosis Induction of RO5072759 (GA101) or Rituximab After Single Treatment Against SU-DHL-4 DLBC Lymphoma Xenografts Transplanted S.C. onto SCID Beige Mice
41	1028084	Antitumor Activity of RO5072759 (GA101) Compared to Rituximab, 2F2 (Humax-CD20) and 2H7 Variant 114 Against SU-DHL-4 DLBC Lymphoma Xenografts Transplanted S.C. onto SCID Beige Mice
42	1029348	Antitumor Activity of Second Line Treatment with RO5072759 (GA101) Following First Line Rituximab Therapy Against SU-DHL-4 DLBC Lymphoma Xenografts in Female SCID Beige Mice

	Study Report	Title
43	1029350	Antitumor Activity of RO5072759 (GA101) in Comparison to Treatment with Cyclophosphamide/Vincristine and/or Doxorubicine Against Subcutaneous WSU-DLCL2 Lymphoma Xenografts in Female SCID Beige Mice
44	1029352	Evaluation of the Therapeutic Properties of RO5072759 (GA101) in the Orthotopic WSU-DLCL2 Model Comparison to Rituximab
45	1029353	Evaluation of the Contribution of Cell Mediated Anti-Tumor Activity to the Therapeutic Properties of R5072759 (GA101), GA101-WT and Rituximab in a S.C. DLCL SUDHL-4 Model
46	1029354	Antitumor Activity Evaluation of Rituximab and GA101 in a SCID Beige Mice - Z138 Xenograft S.C. Model
47	1029363	Evaluation of Cell Death Induction in CD20 Positive Lymphoma Cells After CD20 Crosslinking
48	1029364	Evaluation of Cell Killing Activity of Purified Human NK Cells on Lymphoma Cells in the Presence of CD20 Antibodies (RO5072759; GA101) and Derivatives
49	1029375	Evaluation of Antitumor Activity of: Rituximab, Non-Glycoengineered RO5072759 (GA101), RO5072759 (GA101) and the Glycoengineered Variant BHH2 A in a Z138 Xenograft I.V. Model in SCID bg Mice
50	1029376	RO5072759 (GA101) Therapeutic Dosage Schedule Evaluation in a Z138 Xenograft I.V. Model in SCID Beige Mice
51	1029377	Evaluation of Antitumor Activity of: Mabthera, GA 101-WT and GA101-Glycoengineered in a Z138 Xenograft I.V. Model in SCID bg Mice
52	1030198	Comparison of B Cell Depletion Mediated by RO5072759 (GA101) and Rituximab in Cynomolgus Monkey
53	1030199	Evaluation of Wildtype and Afucosylated-Variant RO5072759 (GA101) Compared to Rituximab Cynomolgus Monkey: B Cell Depletion Efficacy and Durability
54	1030200	B Cell Depletion Mediated by RO5072759 (GA101) in Human CD20 Transgenic Mice
55	1032916	Anti-Tumor Efficacy of RO5072759 (GA101) or Rituximab Against SU-DHL6 Lymphoma Xenografts After S.C. Transplantation in Female SCID Beige Mice
56	1034645	Antitumor Activity of GA101, Rituximab and the F(ab)2 Parts of GA101, Rituximab and BHH2 A WT CD20 Antibody against SUDHL-4 Lymphoma Xenografts in Female SCID Beige Mice
57	1034799	Evaluation of the Binding Properties of RO5072759 (GA101) Towards FcgIIb in Comparison to Rituximab
58	1035139	Evaluation of the Binding Properties of RO5072759 (GA101) Towards Human FcRn and Cynomolgus FcRn
59	1035988	Exposure Assessment in the Study GA101_1 Comparison of B Cell Depletion Mediated by RO5072759 and Rituximab (RO0452294, Mab Thera®) in Cynomolgus Monkeys RDR No. 1030198
60	1035992	Exposure Assessment in the Study GA101_02 Evaluation of Wild Type and Afucosylated-Variant RO5072759 (GA101) Compared to Rituximab (RO0452294, Mab Thera®) in Cynomolgus Monkeys: B Cell Depletion Efficacy and Durability, RDR No. 1030199
61	1036128	Antitumor Activity of RO5072759 (GA101) Applying Different Treatment Schedules against Subcutaneous SUDHL-4 DLBC Lymphoma Xenografts (350 mm^3) in Female SCID Beige Mice
62	1036129	Antitumor Activity of RO5072759 (GA101) applying Different Treatment Schedules against Subcutaneous SUDHL-4 DLBC Lymphoma Xenografts (700 mm^3) in Female SCID Beige Mice

	Study Report	Title
63	1036131	Antitumor Activity of First Line Treatment with Bendamustine Followed by a Second Line Therapy with GA101 Against Subcutaneously Implanted Z138 (MCL) Xenografts in Female SCID Beige Mice (CD20-PZ-Z138-009)
64	1036297	Evaluation of the Binding Properties of RO5072759 (GA101) Towards the Isolated Extracellular Loop of CD20 in Comparison to Rituximab
65	1038384	Antitumor Activity Evaluation of RO5072759 (GA101) in a Model of Z138 Mantel Cell Lymphoma in SCID huCD16 Transgenic Mice
66	1038385	Preclinical Study Report on the Crystal Structure of RO5072759 (GA101)
67	1038386	Preclinical Study Report on Epitope Characterization of RO5072759 (GA101) and Other CD20 Antibodies
68	1038388	Mode-of-Action Studies with GA101 Antibody (RO5072759)
69	1038394	Antitumor Activity of Second Line Treatment with RO5072759 (GA101) following First Line Rituximab Therapy against SUDHL-4 Lymphoma Xenografts in Female SCID Beige Mice
70	1038395	Antitumor Activity of RO5072759 (GA101) Compared to Rituximab, 2F2 (Ofatumumab) and the bcl2-Inhibitor ABT-263 against SUDHL-4 Lymphoma Xenografts in Female SCID Beige Mice
71	1038396	Antitumor Activity of RO5072759 (GA101) or Rituximab (MabThera) Against Subcutaneous OCI-Ly3 Lymphoma Xenografts in Female SCID Beige Mice (Study CD20-PZ-OCI-Ly3-001)
72	1038397	Dose Dependant Antitumor Activity after a Single Administration of RO5072759 (GA101) against Different Advanced Subcutaneous SUDHL-4 Lymphoma Xenografts in Female SCID Beige Mice
73	1038398	In Vivo Imaging of Cy5 Labeled RO5072759 (GA101) in Differentially Advanced Subcutaneous SUDHL4 Xenografts in Female SCID Beige Mice
74	1038406	Differential Induction of HA and Cell Death by Anti-CD20 Antibodies Analyzed by Confocal Laser Scanning Microscopy
75	1043605	Antitumor Activity of RO5072759 (GA101) Compared to Rituximab and Ofatumumab Against SUDHL-4 Lymphoma Xenografts in Female SCID Beige Mice
76	1043639	CD16 Down-Regulation After GA101 (RO5072759)-Mediated ADCC
77	1043640	Effects of Blood Anticoagulants on GA101 (RO5072759)-, Ofatumumab- and Rituximab-Mediated B-Cell Depletion
78	1043641	Assessment of the Avidity of GA101 (RO5072759) Binding as lgG vs F(ab)2 vs Fab
79	1043690	Evaluation of Antitumor Activity of RO5072759 (GA101) Compared to Ofatumumab, Rituximab and GA101 WT in a SUDHL-4 Xenograft Model in SCID CD16tg Mice
80	1043691	GA101 (RO5072759) and Rituximab Induced Cell Death in Lymphoma Cells In Vitro: Influence of FACS Staining Conditions
81	1043692	Superior In Vitro and In Vivo Activity of the Novel Type II, Glycoengineered Anti-CD20 Antibody Obinutuzumab (GA101) Compared With Rituximab and Ofatumumab
82	1049394	The Novel Type II CD20 Antibody RO5072759 (GA101) Mediates Superior B Cell Depletion in Whole Blood from Healthy Volunteers and B-CLL Patients
83	1049395	GA101 Mediates Superior B Cell Depletion in the Whole Blood of B-CLL Patients Compared to Rituximab
84	1049396	Anti-Tumor Activity of RO5072759 (GA101), Compared to Rituximab or Ofatumumab With or Without Normal Human Leukocytes Against the Human RL NHL S.C. Xenograft Model Sensitive or Resistant to Rituximab in SCID Mice
85	1050113	Assessment of neutrophils in Whole Blood of Healthy Volunteers After Incubation with GA101 (RO5072759) and Rituximab

	Study Report	Title
86	1051515	Antitumor Activity of Second Line Treatment with RO5072759 (GA101), Rituximab or Ofatumumab Following First Line Rituximab Therapy Against SUDHL-4 Lymphoma Xenografts in Female SCID Beige Mice
87	1051689	Obinutuzumab (GA101) Efficacy in Chronic Lymphocytic Leukemia In Vitro is not Diminished in High Risk Patients
88	1053047	Evaluation of Antitumor Activity of RO5072759 (GA101) Compared to Ofatumumab and Rituximab in a Z138 Xenograft I.V. Model in SCID CD16tg Mice
89	1053171	GA101 (RO5072759) Induces NK-Cell Activation and Antibody-Dependent Cellular Cytotoxicity More Effectively than Rituximab When Complement is Present
90	1053421	Study in Whole Blood Assays of the Role of PMN in the Therapeutic Activity of Rituximab and GA101 (Obinutuzumab)
91	1053422	Enhanced Binding Affinity of GA101 (Obinutuzumab) for FcgRIIIb
92	1053423	In Vitro Activity of the CD20-Antibody GA101 in High-Risk CLL
93	1053424	Enhanced Binding of GA101 (Obinutuzumab) to Neutrophile Granulocytes (PMNs) Compared to Wildtype Non-Glycoengineered Antibody
91	1053425	Glycoengineering of GA101 (Obinutuzumab) Does Not Affect FcRn Binding
95	1053638	RO5072759 (GA101) Mediates Superior B Cell Depletion Including the Ability to Deplete Marginal Zone B Cell Subsets and Translating into Superior Suppression of De Novo Antibody Responses, but Leaving Protective Humoral Memory Responses Intact
Pharmacodynamic Drug Interactions		
96	1025315	Antitumor Activity of RO5072759 (GA101) in Combination with Bortezomib or RO4968040-000 Against SU-DHL-4 DLBC Lymphoma Xenografts Transplanted S.C. onto SCID Beige Mice
97	1025317	Combined Treatment of RO5072759 (GA101) and Rituximab (Mabthera) Against the Human Ocily18 NHL (DLBCL) S.C. Xenograft Model in SCID Beige Mice
98	1027588	Preclinical Study Report RO5072759 (GA101): NK Cell Activity After Pre-Treatment with Prednisolone
99	1029347	Anti-Tumor Activity of RO5072759 (GA101) or Rituximab Combined to Cyclophoshpamide Against the Human Rl NHL S.C. Xenograft Model in Female SCID Mice
100	1029349	Antitumor Activity of RO5072759 (GA101) or Rituximab in Combination with Two Different Cytotoxic Treatment Schedules Against SU-DHL-4 DLBC Lymphoma Xenografts in Female SCID Beige Mice
101	1029351	Antitumor Activity of RO5072759 (GA101) or Rituximab in Combination with Lyclophosphamide/Vincristine Against WSU-DLCL2 Lymphoma Xenografts in Female SCID Beige Mice
102	1033914	Evaluation of Antitumor Activity of <CD20> Antibody RO5072759 (GA101) and Bendamustine (Ribomustin) and their Combination Against Z138-NHL Xenografts After S.C. Transplantation in Female SCID Beige Mice
103	1034644	Antitumor Activity of RO5072759 (GA101) or Rituximab in Combination with Doxorubicin Against RL-NHL Xenografts in Female SCID Beige Mice
104	1036130	Antitumor Activity of RO5072759 (GA101) or Rituximab (MabThera) in Combination with the bcl-2 Inhibitor RO4968040 Against Subcutaneous SU-DHL4 DLBC Lymphoma Xenografts in Female SCID Beige Mice (Study CD20-PZ-SUDHL4-013)
105	1036132	Antitumor Activity of RO5072759 (GA101) or Rituximab in Combination with Fludarabine (Fludarabinmedac) Against Subcutaneously Implanted Z138 Xenografts in Female SCID Beige Mice

	Study Report	Title
106	1038389	Antitumor Activity Study of RO5072759 (GA101) and the VEGF Antibody B20-4.1 in SCID Beige Mice Bearing Subcutaneous SU-DHL-4 Human Lymphoma
107	1038390	Antitumor Activity Study of RO5072759 (GA101) and Fludara (Fludarabine) in SCID Beige Mice Bearing Subcutaneous SU-DHL-4 Human Lymphoma
108	1038391	Antitumor Activity Study of RO5072759 (GA101) and Leukeran (Chlorambucil) in SCID Beige Mice Bearing Subcutaneous SU-DHL-4 Human Lymphoma
109	1038392	Antitumor Activity Study of RO5072759 (GA101) and Revlimid (Lenalidomide) in SCID Beige Mice Bearing Subcutaneous SU-DHL-4 Human Lymphoma
110	1038393	Antitumor Activity of RO5072759 (GA101) and Rituximab Alone or in Combination with CD40 Dacetuzumab/SGN-2) Against WSU-DLCL2 Lymphoma Xenograft in SCID Beige Mice
111	1038516	Antitumor Activity Study of RO5072759 (GA101) and the mTOR Inhibitor Torisel (Temsirolimus) in SCID Beige Mice Bearing Subcutaneous SU-DHL-4 Human Lymphoma
112	1038517	Antitumor Activity Study of RO5072759 (GA101) and Treanda (Bendamustine HCl) in SCID Beige Mice Bearing Subcutaneous SU-DHL-4 Human Lymphoma
113	1040649	Investigation of Additive-Syngergistic Cell Death Induction of GA101 Antibody (RO5072759) and the bcl-2 Inhibitors ABT-737 and ABT-263
114	1043604	Antitumor Activity of RO5072759 (GA101) or Rituximab (MabThera) in Combination with the bcl-2 Inhibitor RO5428734 (ABT-263) Against SU-DHL4 DLBC Lymphoma Xenografts in Female SCID Beige Mice
115	1043606	Antitumor Activity of RO5072759 (GA101) or Rituximab in Combination with the bcl-2 Inhibitor RO5428734 (ABT-263) Against Subcutaneously Implanted Z138 Xenografts in Female SCID Beige Mice
116	1043607	Antitumor Activity of RO5072759 (GA101) or Rituximab in Combination with Chlorambucil (Leukeran) Against Subcutaneously Implanted Z138 Xenografts in Female SCID Beige Mice
117	1043638	Investigation of the Effects of PI3K Inhibitors on GA101-Mediated B Cell Depletion
118	1043688	Evaluation of Antitumor Activity of RO5072759 (GA101), the Combination of GA101 with Recombinant Human Interferon-Alpha-A/D and Human Interferon-Alpha-A/D Alone in a Z138 Xenograft I.V. Model in SCID Beige Mice
119	1043689	Evaluation of Antitumor Activity of RO5072759 (GA101), the Combination of GA101 with Recombinant Human Interferon-Alpha-A/D and Interferon Alpha Alone in a Z138 Xenograft I.V. Model in SCID huCD16-tg Mice
120	1048504	Evaluation of Antitumor Activity of a Combination of RO5072759 (GA101) with a CD79b Antibody Drug Conjugate Compared to the Combination with Rituximab in a Z138 Xenograft I.V. Model in SCID Beige Mice
121	1048505	Evaluation of Antitumor Activity of a Combination of RO5072759 (GA101) with a CD22 Antibody Drug Conjugate Compared to the Combination with Rituximab in a WSU-DLCL Xenograft I.V. Model in FcgR3 SCID tg Mice
122	1050168	Chlorambucil does not Impact ADCC Mediated by GA101 (RO5072759) and Rituximab
123	1050208	Combination Efficacy Study of GA101 and GDC-0199 in the SU-DHL-4 Non-Hodgkin's Lymphoma Xenograft Model Grown in SCID Beige Mice
124	1051514	Antitumor Activity of RO5072759 (GA101) or Rituximab in Combination with the MDM2 Inhibitor RO5045337 (Nutlin) Against Subcutaneously Implanted Z138 Xenografts in Female SCID Beige Mice

	Study Report	Title
125	1053048	Evaluation of the Therapeutic Properties of RO5072759 (GA101) as Single Agent and in Combination with a Targeted IL2 Immunocytokine (Fab-IL2wt-Fab) in the Human Mantle Cell Lymphoma Model (Z138) in hCD16 Transgenic SCID Mice
Pharmacokinetics		
126	1026210	RO5072759: Determination of RO5072759 in Cynomolgus Monkey Serum by Enzyme-Linked Immunosorbant Assay (ELISA
127	1031899	O5072759: Semi-Quantitative Determination of Antibodies Directed to R05072759 in Cynomolgus Monkey Serum by Enzyme-Linked Immunosorbent Assay (ELISA)
128	1051562	RO5072759: Validation of an Analytical Method for the Quantification of RO5072759 in Cynomolgus Monkey Serum by Enzyme-Linked Immunosorbent Assay (ELISA)
129	1020938	RO5072759 (Anti-CD20 Human Antibody): Pharmacokinetics and Pharmacodynamics Following Intravenous Administration of 1 or 10 mg/kg RO5072759 to Cynomolgus Monkeys (Study No. 06-0630)
130	1024122	RO5072759 (Anti-CD20 Human Antibody): Pharmacokinetics and Pharmacodynamics Following Subcutaneous Administration of 20 mg/animal RO5072759 to Cynomolgus Monkeys (Study No.06-3896)
131	1024804	RO5072759 (Anti-CD20 Human Antibody): Pharmacokinetics Following Single Intravenous Administration of 1 or 10 mg/kg RO5072759 to Mice (Study No. 06-1377)
Toxicology		
132	1024829	RO5072759 Preliminary Toxicity Study by Intravenous (Bolus) Administration to Cynomolgus Monkeys

B. Additional information from the ePPND Study

Observations

Maternal Animals In-life parameters

Parameter	General information	Frequency (from Day 20 p.c.)
Clinical signs	General: Behavior and appearance by cage site observation Detailed: Any changes while animal in hand Feces (normal/soft/liquid/no feces) Fur inspection	Twice daily Once weekly, and on the Day of necropsy Twice daily Once weekly
Morbidity and mortality	Visual inspection	Twice daily
Monitoring of pregnancy	Determined by means of ultrasonographic examination, where appropriate. If signs of abortion were observed, additional ultrasonography was performed. Vaginal smears were examined, where appropriate.	Days 30, 44, 58, 72, 86, 100, 114, 128, 142, and 156 p.c. ± 1 Daily until delivery
Body weights	Recorded individually (during pregnancy) Recorded individually (after delivery)	Days: 20, 27, 34, 41, 48, 55, 62, 69, 76, 83, 90, 97, 104, 111, 118, 125, 132, 139, 146, 153, 160, 167, and 174 p.c. Days 1, 7, 14, 21, and 28 p.p. Monthly intervals for up to 8 months
Food consumption	Not performed due to social housing	

Ultrasonographic Fetal Examinations

Days of Gestation (± 1 Day)	Crown-Rump length (CRL)	Biparietal Diameter (BPD)	Fronto-occipital Diameter (FOD)	Femur Length, one side (FL)	Heart rate (HR)
30	X				
37	X				
44	X				X
58		X	X		X
72		X	X	X	X
86		X	X	X	X
100		X	X	X	X
114		X	X	X	X
128		X	X	X	X
142					X
156					X
170					X

Neonatal Infant Examination

Parameter	General information	Frequency
Clinical signs	Behavior and appearance	Twice daily
General examination	Sex determination Weighed and examined for external abnormalities	Day 1 p.p. Days 1, 7, 14, 21, and 28 p.p. Monthly intervals for up to 8 months
Parameter	**General information**	**Frequency**
Morphological examinations	Measurement of external organs (head circumference, distance between the eyes, crown-rump length, tail length, chest circumference, length of upper and lower extremities, ano-genital distance, crown-heel length)	Days 1, 21, 58, 88, 178, and 238 p.p.
Neuro-behavioral test battery	Infant in cage – color – initial state - quiet sleep, active sleep, quiet wake, active wake Infant outside cage – elicited tonus – wrist or ankle dorsiflexion – grasp support – righting reflex – prone progression – clasp support – respiration rate - without disturbing the infant – observed muscle tonus - without disturbing the infant – postural tonus (observed) – buildup – following of eyes – lipsmack orientation – sucking – rooting – snout reflex – pupil response – glabellar tap – nystagmus – moro reflex – buildup	Days 1 and 7 p.p.
Grip strength		Day 28 p.p.

Skeletal Development X-ray

Schedule	
Time points	Once (week 13 to 16 p.p.)
General procedures	
Animal conditions	Fasted prior anesthesia Measurements made under ketamine/xylazine hydrochloride anesthesia Performed under non-GLP
Evaluation of X-rays	By Dr. [redacted] (b) (4)

Hematology

Standard parameters evaluated at the same time points for immunophenotyping

Immunophenotyping

Schedule		
Time points	On Days 28, 84, 112, 140, 168, 196, and 238 p.p.	
General procedures		
Blood sampling	Approximately 0.2 mL	
Sample site	Cubital, brachial or femoral vein	
Anticoagulant	EDTA	
Sample mixed	Gently mixed by hand and on a mixer in the lab until analysis	
Method	With specific monoclonal antibodies to comprise T-cells (T-helper-cells and cytotoxic T-cells), B-cells, and natural killer (NK) cells. Analysis of relative cell numbers (percentage of lymphocytes) using the FACSCalibur (Becton Dickinson). Total lymphocyte counts determined on the same Day. Absolute numbers of the lymphocyte subpopulations computed from relative and total numbers.	
Combination of antibodies	**Antibody**	**Determination of...**
	Isotype controls	Background
	CD3, CD4, CD8, CD45	Total T-cells, T-helper-cells, cytotoxic cells,
	CD3, CD16, CD20, CD45	B-cells, NK-cells

Antibody parameter	**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/%	CD20+ B-cells
Natural killer cells	10E9/L/%	CD16+ NK cells

Antigenic Challenge (KLH)

Schedule	
Animals	All Infants
Time points for stimulation	Days 181 and 212 p.p. ± 1 Day
Time points for blood collection	Days 181 (prior to first KLH injection), 188, 195, 202 p.p., 212 p.p. (prior to second KLH injection) Days 215, 219, and 226 p.p. ± 1 Day
General procedures	
Stimulation	Intramuscular injection of 1 mg KLH in 0.9% sodium chloride
Sample site	Cubital, brachial or femoral vein
Blood sampling	1 mL blood
Centrifugation	To be allowed to clot for at least 30 minutes 10 min 1500 *g* +5°C ± 3°C
Sample handling	Serum was divided to equally two plastic tubes "Snap frozen" in dry ice Stored at –20°C ± 4°C
Analysis	ELISA analysis

Milk Sampling
Day 28 postpartum

Antidrug Antibodies
Maternal Animals

Time points	**direct after dosing**	**Sample time (hours after dosing)**	
		96	**168**
Day 20 p.c. (post 1st dose)	X	X	X
Day 139 p.c. (post dosing)	X	X	X
Day 28 p.p.	X		
Last week prior necropsy	X		

Neonatal Infants

Time points
Day 168 p.p.
Prior to necropsy

Toxicokinetic Evaluations
Maternal Animals

Time points		**Sample time (hours after dosing)**			
		7	**24**	**96**	**168**
Day 20 p.c. (1st dose)	X (prior-dosing)	X	X	X	X
Day 139 p.c.	X (prior-dosing)	X	X	X	X
Day 28 p.p.	X				
Last week prior necropsy	X				

Neonatal Infants

Time points
Day 28 p.p.
Day 168 p.p.
Prior to necropsy

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/s/

M S RICCI
09/17/2013

PEDRO L DEL VALLE
09/17/2013

HALEH SABER
09/17/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR BLA 125486

BLA Number: 125486 **Applicant: Genentech, Inc.** **Stamp Date: April 25, 2013**

Drug Name: obinutuzumab **BLA Type: Original BLA**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Animal toxicology studies were limited to repeat dose and embryo-fetal development studies in cynomolgus monkeys. Monkeys are the only animal species available for toxicity testing that is pharmacologically responsive to obinutuzumab.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		Product was administered intravenously or subcutaneously for all animal toxicology studies.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR BLA 125486

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

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

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

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

None

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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

/s/

M S RICCI
05/24/2013

HALEH SABER
05/24/2013