

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125326

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Arzerra (ofatumumab)

Date: September 2, 2009

To: File for BLA 125326

From: John K. Leighton, PhD, DABT

Associate Director for Pharmacology

Office of Oncology Drug Products



I have examined pharmacology/toxicology supporting review and labeling provided by Dr. McDougal and supervisory concurrence provided by Dr. Pilaro. I concur with their conclusions that Arzerra may be approved. No additional pharmacology or toxicology studies are necessary for the proposed indication.



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	STN 125326/00
SERIAL NUMBER:	eCTD Sequence # 0000
DATE RECEIVED BY CENTER:	30-JAN-2009
PRODUCT:	Ofatumumab (HuMax-CD20)
INTENDED CLINICAL POPULATION:	patients with chronic lymphocytic leukemia
SPONSOR:	Glaxo Group Limited d/b/a GlaxoSmithKline
DOCUMENTS REVIEWED:	electronic submission (eCTD) 125326/0.0 submitted 1/30/2009 125326/0.9 submitted 3/17/2009
REVIEW DIVISION:	Division of Biologic Oncology Products (HFD-107)
PHARM/TOX REVIEWER:	Andrew J. McDougal, Ph.D., D.A.B.T.
PHARM/TOX SUPERVISOR:	Anne M. Pilaro, Ph.D.
DIVISION DIRECTOR:	Patricia Keegan, M.D.
PROJECT MANAGER:	Raymond Chiang
Date of review submission to RMS-BLA:	7/29/2009

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Recommend approval of Biologics Licensing Application (BLA) STN 125326. No basis for objection was identified in the review of the nonclinical pharmacology and toxicology sections of the original BLA submission and amendments. Nonclinical toxicities of ofatumumab are extensions of the pharmacology of the product, were reflected in the clinical studies, and are monitorable in the clinical setting.

B. Recommendation for nonclinical studies

No additional nonclinical studies are recommended to support the treatment of patients with chronic lymphocytic leukemia (CLL) who have disease refractory to alemtuzumab and fludarabine.

C. Recommendations on labeling

Nonclinical recommendations have been incorporated into the draft label for section 8 (8.1 and 8.3), section 12, and section 13 (13.1 and 13.3). No additional changes to labeling are recommended in this review.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Ofatumumab is an immunoglobulin class G1 kappa (IgG1 κ) human monoclonal antibody specific for an epitope on the cell surface protein cluster of differentiation 20 (CD20). The product is intended for treatment, by intravenous infusion, of patients with B-cell chronic lymphocytic leukemia (CLL).

CD20 is expressed on normal B-cells during their differentiation and maturation, from the pre-B cell stage to the memory B-cell stage. Reportedly, it is also expressed on some CD3⁺ T cells. CD20 is expressed by several hematological tumors that originate from hematopoietic stem cells.

Ofatumumab binds human CD20 with high affinity and specificity; *in vitro* and presumably *in vivo*, binding of ofatumumab causes death of CD20⁺ cells by inducing antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). As a proof-of-concept, ofatumumab was shown to exhibit anti-tumor activity in SCID mice bearing CD20⁺ human tumors.

Ofatumumab bound rhesus monkey and cynomolgus monkey CD20 with the same or slightly higher (2-fold higher) affinity compared to human CD20. Ofatumumab did not bind to CD20 from the other species tested. The pivotal nonclinical *in vivo* testing was limited to one species, the cynomolgus monkey.

In monkeys, the primary effect of ofatumumab treatment was depletion of B-cells; other effects were secondary to this primary effect and included histological changes (atrophy of the spleen, lymph nodes, and Peyer's patches) and increased risk of infection.

B. Pharmacologic activity

Due to differences between healthy B-cells and malignant cells (e.g. CD20-expression, resistance to ADCC or CDC), the doses administered to healthy monkeys may not be directly relevant to patients with CLL. Assuming cross-species comparison on a mg/kg basis (and assuming a patient

weight of 70 kg), the doses tested across studies in the cynomolgus monkey of 1.25, 6.25, 12.5, 20, and 100 mg/kg are 4%, 22, 44, 70%, and 350% of the prescription label dose, respectively.

In healthy cynomolgus monkeys treated with ofatumumab, doses as low as 1.25 mg/kg were sufficient to deplete circulating CD20⁺ B-cells to ~4 to 10% of baseline at the earliest time point measured (2 days after dosing). Higher doses did not increase the magnitude of B-cell loss but did extend the duration of B-cell depletion (consistent with observed pharmacokinetics); a clear dose-response was apparent.

Recovery of circulating B-cells in cynomolgus monkeys was observed consistently following cessation of dosing. After 2 doses (D1 and D5) of 1.25 to 12.5 mg/kg of ofatumumab, initial recovery was apparent 3½ weeks later, and full recovery was seen between 8 and 17 weeks. In monkeys dosed with 4 weekly doses of 20 mg/kg/dose (equivalent to 70% of the clinical dose), initial signs of B-cell recovery were observed after 4 to 7 weeks, with full recovery by 16 to 20 weeks after cessation of dosing. Monkeys treated with 20 mg/kg for 7 months (weekly for 50 days, followed by 5 monthly maintenance doses) showed initial signs of B-cell recovery 12 weeks after the end of dosing, followed by a rebound (B-cell counts above baseline) and full recovery by 6 months. In monkeys dosed with 100 mg/kg (3.5-fold the clinical doses), 4 weekly doses suppressed B-cells, with initial signs of recovery 9 to 13 weeks after dosing. Additionally, in monkeys treated with 100 mg/kg for 7 months, initial signs of recovery were observed 13-16 weeks and full recovery was apparent 6 months after cessation of dosing.

Monkeys that developed primate anti-human antibodies (PAHA, i.e. antibodies against the fully human antibody ofatumumab) recovered B-cells more quickly than monkeys that did not develop PAHA. Positive anti-ofatumumab antibody responses have not been detected in patients (79/234 negative responses, other patient PAHA results were inconclusive due to assay limitations).

C. Nonclinical safety issues relevant to clinical use

Increased risk of infection

Three mortalities (3/28, or 10.7%) in the 7-month cynomolgus monkey toxicology study were attributed to infection by common bacteria (*Campylobacter jejuni* and *Balantidium coli*), despite supportive care. The earliest death was 44 days after initiation of treatment. This is consistent with expected pharmacology (i.e. B-cell depletion) and is consistent with clinical observations.

Infusion-reaction/cytokine response

Humans appear more sensitive than cynomolgus monkeys to ofatumumab-induced infusion reactions. In clinical trials, infusion related adverse reactions occurred in 44% of patients with the first infusion (300 mg), 29% with the second infusion (2,000 mg), and less frequently during subsequent infusions. Patients will receive pre-medication to protect against infusion reactions, and will also receive a low initial dose (300 mg/kg, 15% of the subsequent doses), as directed in the labeling for ofatumumab.

The nonclinical studies did not pre-medicate or use a lower initial dose. Ofatumumab-treated cynomolgus monkeys only exhibited signs of infusion reactions after repeated dosing; these events resolved without treatment. No clinical observations of infusion reactions were detected in the short-term studies (i.e. 4 weeks or less of dosing). In the 7-month toxicity study, signs were observed in high-dose animals from D78 onward (the 1st monthly maintenance dose) and in low-dose animals from D106 onward (the 2nd monthly dose). The effect manifested as transient increases in heart rate and heart force (variable range, usually beginning during the 30 minute infusion, and resolving within a few hours of the end of infusion) and occurred sporadically for particular animals. Transient trembling was observed in 3/28 animals (11%). An exploratory study in cynomolgus monkeys confirmed that ofatumumab treatment caused transient (15

minutes to 4 hours post-treatment) increases in serum levels of activated complement and interleukin-6 (IL-6), and neutrophil degranulation.

Delayed onset anemia

Anemia is common in patients with advanced CLL, as leukemia cells replace the bone marrow's normal hematopoietic cells. Anemia was observed in >10% of the patients with CLL in clinical trials with ofatumumab.

Healthy cynomolgus monkeys treated with ofatumumab exhibited anemia; however, the mechanism of this nonclinical effect is likely species-specific and not relevant to humans. Two mortalities in the 7-month study cynomolgus monkey toxicity study were attributed to hemolytic anemia (on D139 and D268). Signs of anemia (decreased hemoglobin, red blood cell count, hematocrit and iron; increased reticulocyte count, total bilirubin, and lactate dehydrogenase) were apparent among ofatumumab-treated monkeys from D50 (after 8 weekly doses) onward, with recovery apparent 6-months after cessation of treatment. Anemia was not apparent after short-term nonclinical dosing (4 or 5 weekly doses). The sponsor attributes the onset of anemia to a species-specific humoral immune reaction (i.e. ofatumumab is a human antibody, recognized as foreign by the monkey immune system). Mechanistic studies in ofatumumab-treated monkeys found antibodies (including ofatumumab) and complement binding directly to the surface of red blood cells; this finding had resolved by 6 months post-dosing.

Fetal toxicity – decreased placenta weight, fetal spleen weight, and, fetal thymus weight

Pregnant cynomolgus monkeys were treated weekly x5, from gestation day (GD) 20 to GD50 with 20 or 100 mg/kg of ofatumumab; Caesarean sections were performed on GD100. Ofatumumab was detected in fetal blood samples, and maternal PK indicates that fetuses continued to be exposed to pharmacologically-active levels at GD100. Fetal exposure to ofatumumab caused depletion of fetal circulating B-cells (~10% of controls) and decreased spleen weight (-16% for the low-dose group, -27% for the high-dose group), consistent with expected pharmacology. However, decreased placenta weight (-10% for the low-dose group, -11% for the high-dose group), and decreased thymus weight in the fetuses of high-dose group mothers (-15%) were also observed. The toxicological relevance of the placental and thymus effects are unknown.

Clinical chemistry: Increased lactate dehydrogenase, increased C-reactive protein

Increased lactate dehydrogenase was observed in the 7-month cynomolgus monkey study from D25 onward during treatment and during the recovery period; full recovery was apparent 6-months after the last dose. This increase may reflect anemia (i.e. damage to red blood cells). However, lactate dehydrogenase is commonly increased in patients with hematopoietic malignancies, and it is considered one of the screening tools to detect CLL. Theoretically, treatment with ofatumumab may confound interpretation of lactate dehydrogenase results (i.e. if the treatment-related effect has a mechanism different than the mechanism for CLL).

Increased C-reactive protein (CRP) was observed in the 7-month study: an initial increase was observed (D2 only), followed by a return to baseline and a subsequent increase lasting through recovery. This pattern suggests overlapping mechanisms-of-action. High CRP is an indicator of inflammation; increased CRP has been associated with rheumatoid arthritis and lupus; notably, anti-CD20 therapies (such as ofatumumab) reportedly have activity in these indications. Ofatumumab effects on CRP may be related to the pharmacologic activity. Separately, increased CRP is also associated with inflammation caused by infection; therefore, the CRP changes may be at least partially due to the indirect activity of ofatumumab on the immune system (i.e. subclinical infection secondary to suppression of humoral immune response).

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: STN 125326\0\0

Review number: BLA 1

Sequence number/date/type of submission: Electronic submission. Original application received 1/30/2009 as 125326/0.0. An amendment including nonclinical information was received on 3/17/2009 as 125326/0.9.

[.enx file link is:

\\cbsap58\MeCTD_Submissions\STN125326\125326.enx]

Information to sponsor: No (X)

Sponsor and/or agent:

- **Name:** Glaxo Group Limited d/b/a GlaxoSmithKline.
- **Address:** Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex, UB6 ONN UK
- **US Agent:** Philip A. Witman. 1250 South Collegeville Road, P.O. Box 5089. Collegeville, PA 19426

Manufacturer for drug substance: Lonza Biologics plc, Slough, Berkshire, SL1 4DK United Kingdom.

Reviewer name: Andrew J. McDougal, Ph.D., D.A.B.T.

Division name: Division of Biologic Oncology Products (DBOP)

HFD #: 107

Review completion date: 7/29/2009

Drug:

Trade name: ARZERRA

Generic name: Ofatumumab (HuMax-CD20)

Code names: GSK1841157
HuMax-CD20
2F2

Chemical name: Immunoglobulin (Ig) G1, anti-(human CD20(antigen))
(human monoclonal HuMax-CD20 heavy chain),
disulfide with human monoclonal HuMax-CD20 κ-chain,
dimer

CAS registry number: 679818-59-8

Molecular formula: Product is a biologic. From m3.2.S.1.2, Figures 1 and 2:

Figure 1 Light Chain Sequence of Ofatumumab



b(4)

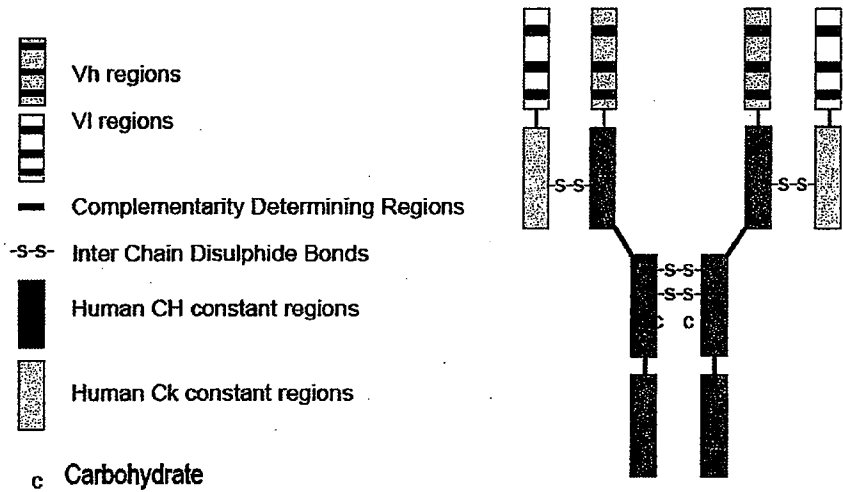
Figure 2 Heavy Chain Sequence of Ofatumumab, Including Leader Sequence (*italic*)



b(4)

Molecular weight: Approximately 149 kDa. Each light chain is approximately 25 kDa, each heavy chain approximately 50 kDa.

Structure: From m3.2.S.1.2:



Relevant INDs/NDAs/BLAs/DMFs:

1. IND 011719
 - a. Predecessor IND to this BLA
 - b. Product: HuMax-CD20

- c. Sponsor: Glaxo Group Ltd DBA GlaxoSmithKline
- d. Responsible division: DBOP
- e. Indication: treatment of relapsed or refractory chronic lymphocytic leukemia (CLL)
- f. NOTE: Genmab A/S was the original sponsor for IND 11719; GSK assumed responsibility for the IND on 4/28/2008.

2. IND

()

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3. IND

- a. Product: HuMax-CD20
- b. Sponsor: Glaxo Group Ltd DBA GlaxoSmithKline
- c. Responsible division: Division of Anesthesia, Analgesia, and Rheumatology Products (DAARP)
- d. Indication: treatment of active rheumatoid arthritis (RA)

b(4)

4. IND

- a
- b
- c
- d

()

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Drug class¹: human monoclonal antibody, immunoglobulin class G1 kappa (IgG1 κ)

Intended clinical population: the treatment of patients with chronic lymphocytic leukemia (CLL) who have disease refractory to alemtuzumab and fludarabine.

Clinical formulation: For infusion. 100 mg (20 mg/ml)² of ofatumumab in / mM citrate buffer (8.55 mg/ml sodium citrate, 0.195 mg/ml citric acid monohydrate), pH 6.5, containing / mM sodium chloride (5.85 mg/ml). Appears as a clear, colorless, aqueous solution. Supplied in Type 1 glass vials sealed with a () coated () rubber stopper secured with an overseal. Prior to administration, the product is diluted into an infusion bag of isotonic 0.9% sodium chloride injection. During administration, the product solution is filtered through an in-line sterile filter.

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Route of administration: Intravenous infusion

Disclaimer: Tabular and graphical information are constructed by the reviewer using information submitted in the BLA, or were copied electronically from the BLA.

¹ The pharmacological class is "CD20-directed cytolytic monoclonal antibody"

² Not including the overage. Each vial is overfilled / ml to allow for extraction of the 5 ml.

b(4)

Studies reviewed³ within this submission:**Table 1. Summary of nonclinical *in vitro* primary pharmacodynamic studies**

Report title	Report #	GLP status ⁴	Test system
Binding characteristics of HuMax-CD20 and HuMab-11B8	GMB 3001-008 v3	No	<i>In vitro</i>
Clustering of CD20 molecules into lipid rafts by CD20-antibodies: HuMax-CD20 and HuMab-11B8	GMB 3001-007 v2	No	<i>In vitro</i>
Complement binding and induction of complement-dependent cytotoxicity by HuMax-CD20 and HuMab-11B8	GMB 3001-003 v3	No	<i>In vitro</i>
Efficient killing of tumor B cells by HuMax-CD20 or HuMab-11B8: antibody-dependent cell-mediated cytotoxicity and the role of whole blood components	GMB 3001-006 v3	No	<i>In vitro</i>
Induction of apoptosis by HuMax-CD20 and HuMab-11B8	GMB 3001-005 v2	No	<i>In vitro</i>
Functional activities of HuMab-2C6: comparison with other CD20-specific antibodies	GMB-3001-023	No	<i>In vitro</i>

Table 2. Listing of nonclinical pharmacokinetic (PK), toxicokinetic (TK), and toxicology *in vivo* studies

Report title	Report #	GLP status
Determination of some circulating and coagulation parameters after administration of HuMax-CD20 to cynomolgus monkeys	GMB-3001-013	No
Pilot and pharmacokinetic study in cynomolgus monkeys using HuMax-20	22804 (663784)	No
HuMax-CD20: pilot study in cynomolgus monkeys	23315 (664395)	No
HuMax-CD20: multiple dose toxicity study in cynomolgus monkeys with a 6 month recovery period	23542 (663805)	Yes
HuMax-CD20: multiple dose intravenous toxicity study in cynomolgus monkeys followed by a 6 month recovery period	25052 (665493)	Yes
Intravenous embryo-fetal developmental study with HuMax-CD20 in the cynomolgus monkey	2148-010	Yes
Cycled repeat dose toxicity study in cynomolgus monkeys with a 4 month recovery period	28366 (509814)	Yes
Immunogenicity: determination of ADA in monkey serum ^a	GMB-3001-021	No

^a This study used serum samples from the chronic toxicity study (#665493).

³ For the nonclinical *in vitro* pharmacology studies in which ofatumumab was one of several antibodies assayed, this review focuses only on the data for ofatumumab; full reviews of the data for other products are not documented in this review.

⁴ GLP is an abbreviation for Good Laboratory Practices.

Table 3. Summary of tissue cross-reactivity studies*

Report title	Report #	GLP status	Test system
Examination of non-human primate cross-reactivity of HuMax-CD20	GMB 3001-002 v3	No	<i>Ex vivo</i> (monkey)
Species cross-reactivity of HuMax-CD20	GMB-3001-024	No	<i>Ex vivo</i> (mouse, rat, rabbit, dog, pig)
HuMax-CD20: an immunohistochemical investigation of cross-reactivity in human tissues	23179 (530757)	Yes	<i>Ex vivo</i> (human)

*The first study is reviewed at section 2.6.2.2.1.7 (pp 23-24 of this review, as a primary pharmacodynamic study); the latter two are reviewed at sections 2.6.6.8.1 (pp 100-103) and 2.6.6.8.2 (p 103) respectively (as special toxicology studies).

Studies not reviewed within this submission:

Table 4. Listing of nonclinical PK, TK, and toxicity *in vivo* studies

Report title	Report #	GLP status
()		

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Table 5. Summary of nonclinical *in vitro* pharmacology studies

Type of study [†]	Report #	GLP status	Test system
()			

b(4)

[†]As provided by the sponsor in 2.6.3 Pharmacology tabulated summary. This column does not list the individual study titles.

Table 6. Summary of nonclinical *in vivo* pharmacodynamic studies

Type of study ¹	Report #	GLP status	Test system
Primary Pharmacodynamics			
()			

b(4)

¹As provided by the sponsor in 2.6.3 Pharmacology tabulated summary. This column does not list the individual study titles.

Table 7. Listing of reference reports

Report title	Report #
()	

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2.6.2 PHARMACOLOGY

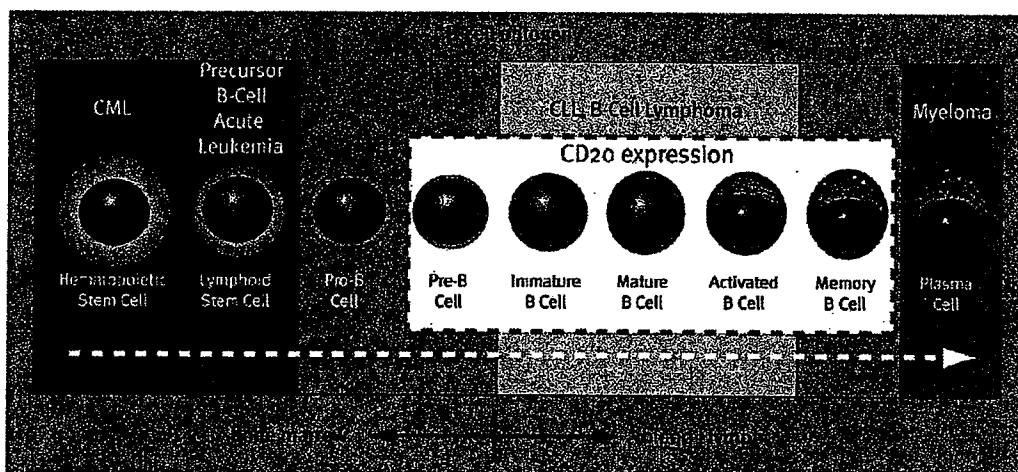
2.6.2.1 Brief summary

Ofatumumab is an immunoglobulin class G1 kappa (IgG1 κ) human monoclonal antibody specific for an epitope on CD20. The product is intended for treatment, by intravenous infusion, of patients with B-cell chronic lymphocytic leukemia (CLL).

CD20 is a non-glycosylated, 297-amino acid transmembrane phosphoprotein (~33 to 37kDa). Reportedly, CD20 spans the membrane four times (two extracellular loops); the large loop is 44 amino acids and is the target of many anti-CD20 antibodies. The shorter loop has approximately 7 extracellular amino acids. CD20 has homology with IgE receptor (Fc*RI) and HTm4, a protein expressed on some lymphoid and myeloid cells. The physiological function of CD20 is unknown. Speculatively, it may be involved in ion channel formation. *In vitro*, CD20 cross-linking results in the rapid formation of lipid rafts in the cell membrane, which putatively helps to regulate signal cascades.

CD20 is expressed from the pre-B cell stage (before IgM cell surface expression), through B-cell maturation, until the plasmacytoid immunoblast phase. CD20 has not been detected on either lymphoid stem cells or plasma cells. Expression of CD20 appears to be limited to B-lineage cells, although literature reports suggest the some CD3⁺ T cells may also express CD20. Several B-cell tumors express CD20, including some B-cell non-Hodgkin lymphoma (NHL), and chronic lymphocytic B-cell lymphoma (CLL). From m2.4:

Figure 1. CD20 Expression During B Cell Ontogeny



The sponsor provided data to the BLA showing that ofatumumab binds human CD20 with high affinity and specificity; the epitope includes both the large and small loops. Binding of ofatumumab to CD20 on the surface of cells induced antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In a mouse xenograft model, ofatumumab exhibited anti-tumor activity against implanted CD20⁺ human tumor cells. In cynomolgus monkeys, administration of ofatumumab resulted in rapid depletion of CD20⁺ B-cells from circulation and lymph nodes. Consistent with the decrease in B-cells, ofatumumab-treated monkeys exhibited a decrease in their immune response to keyhole limpet hemocyanin (KLH) and increased susceptibility to infection.

2.6.2.2 Primary pharmacodynamics

2.6.2.2.1 Mechanism of action:

Nonclinical studies showed that ofatumumab, under the *in vitro* conditions tested, bound with high affinity and specificity to human CD20, inducing ADCC and CDC against CD20⁺ cells. Ofatumumab did not induce apoptosis in CD20⁺ cells under the *in vitro* conditions tested. No CD20 shedding or internalization was observed in the *in vitro* pharmacodynamic studies performed.

Notes on the reliability of the mechanistic studies:

- Nonclinical studies to support the mechanism of action were not performed in compliance with good laboratory practice (GLP) standards, and the predictivity of findings in a limited number of cultured B-cell tumor cell lines for activity in patients with B-cell cancer may be limited.
- The activity assay to support lot release is an antigen binding assay (fluorescence-activated cell sorting [FACS] analysis; m2.3.S).
- The potency assay to support lot release is a CDC assay utilizing an () cell viability assay: the acceptance criteria are () (BLA section m3.2.P.5.4). Notably, none of the nonclinical studies that assessed CDC used the () assay.

b(4)

Review of seven primary PD studies to elucidate the mechanism of action for ofatumumab are documented below (2.6.2.2.1.1 to 2.6.2.2.1.7).

2.6.2.2.1.1

Study title: Binding characteristics of HuMax-CD20 and HuMab-11B8

Key study findings:

- Ofatumumab (HuMax-CD20) bound human CD20 with high affinity and specificity under the *in vitro* conditions tested.
- Mean EC₅₀ = 287 ± 12.7 ng/ml
- Ofatumumab exhibited CDC activity under the *in vitro* conditions tested.
- This study was not conducted in compliance with GLP regulations; evaluation of the study results is limited by a lack of detailed methodology details and test conditions. The predictivity of the *in vitro* study results from cultured cell lines for activity in patients with cancer is unclear.
- This study provides supporting information, but is not sufficiently robust to support a labeling claim comparing ofatumumab to any of the positive control antibodies tested in these experiments.

Study no.: Genmab # 3001-008 v3. GSK # CD2008/01290/00.

Volume #, and page #: Submitted electronically in m4.2.1.1. File name is "gmb3001-008-report.pdf". 29 pp. Report date 7/17/2008.

Conducting laboratory and location: Genmab BV. Jenalaan 18D, 3584 CK Utrecht, The Netherlands.

Date of study initiation: 1/2002.

GLP compliance: No.

QA report: no (X)

Drug, lot #, and % purity: HuMax-CD20, batch #MBR-02-026.
HuMax-CD20-FITC, batch #MK100902
Mouse-anti-human CD20 from // / batch
#700604F.
¹²⁵I-labelled HuMax-CD20. Batch # not reported.
Purities not reported.

b(4)

Methods

- 3 *in vitro* cultured human B-cell lines were used: SU-DHL4 (human anaplastic large cell lymphoma line), EHRB (human Burkitt's lymphoma, B lymphoblast line), and Daudi (human Burkitt's lymphoma, B lymphoblast line).
- Human peripheral blood mononuclear cells (PBMCs) were collected from 3 healthy volunteers and enriched in culture prior to use.
- The antibody HuMab-11B8, mentioned in the study title, is an IgG3 kappa against human CD20. For the purposes of this review, it was considered a positive control for HuMax-CD20.
- Rituximab, a lawfully-marketed anti-CD20 antibody, was also used in this study as a positive control.
- Negative control antibodies of matching isotype were also included in the testing.
- Endpoints:
 - EC₅₀ determination: serial dilutions of FITC-labeled antibody binding to PBMCs measured by flow cytometry.
 - Radiolabel experiment: serial dilutions of ¹²⁵I-labelled antibodies were incubated with hEHRB or Daudi cells for 2 hours; free and bound antibody were separated by centrifugation through phthalate oil and analyzed by a gamma counter. The purpose was to show specificity and to estimate the number of CD20 receptors per cell.
 - Verification that cell-binding is not via Fc-receptors: the radiolabel experiment was repeated with ¹²⁵I-labelled F(ab)₂ fragments.
 - Establishment of dissociation rate: EHRB cells were treated with serial dilutions of ¹²⁵I-labelled antibodies for 2 hours, centrifuged and resuspended, and exposed to unlabeled antibody. At 7 time points over 3 hours, aliquots were centrifuged to separate bound and unbound radiolabel, and analyzed by a gamma counter.
 - Complement dependent cytotoxicity (CDC) assay: Daudi and SU-DHL4 cells were plated, incubated with 10 µg/ml of antibody for 10 minutes, washed, centrifuged, plated, incubated (for 10 minutes, 1 hour or 6 hours), treated with normal human serum for 45 minutes (as a source of human complement), resuspended, and then treated with propidium iodide (live cells exclude the dye; dead cells are positive for propidium iodide). Lysis was analyzed by flow cytometry.

Results

- Ofatumumab mean EC₅₀ for binding to CD20⁺ cells = 287 ± 12.7 ng/ml
- Saturation of binding to CD20 on EHRB and Daudi cells was observed, approximately 2 to 3 x 10⁵ receptors per cell.
- The authors estimate that half of HuMax-CD20 dissociated after 3 hours.

- In the CDC assay, lysis of greater than 90% of the target cells was observed for ofatumumab at all 3 time points. The authors consider this evidence of CDC activity.

2.6.2.2.1.2

Study title: Complement binding and induction of complement-dependent cytotoxicity by HuMax-CD20 and HuMab-11B8

Key study findings:

- Ofatumumab induced CDC in six CD20⁺ cell lines *in vitro*; dose-responses were observed for both antibody concentration and serum concentration (serum being the source of complement).
- Under the *in vitro* conditions tested, ofatumumab appeared to bind 3 complement proteins: C1q, C4c, and C3.
- The authors claim the ofatumumab induced CDC in cells expressing low amounts of CD20, as low as 4500 molecules/cell. This claim could not be evaluated from the data provided.
- This study was not conducted in compliance with the GLP regulations; evaluation of the study results is limited by the limited reporting of methods and results. The predictivity of the *in vitro* study results from a limited number of short-term studies using 3-4 cultured cell lines for activity in patients with cancer is unclear.
- This study provides supporting information, but is not sufficiently robust to support a labeling claim comparing ofatumumab to any of the positive control antibodies tested in these experiments.

Study no.: Genmab #GMB-3001-003 v3. GSK # CD2008/01164/00.

Volume #, and page #: Submitted electronically to m4.2.1.1. File name "gmb3001-003-report.pdf". 60 pp. Report date 7/18/2008.

Conducting laboratory and location: Genmab BV. Jenalaan 18D, 3584 CK Utrecht, The Netherlands.

Date of study initiation: 1/2002

GLP compliance: No.

QA reports: no (X)

Drug, lot #, and % purity: Hybridoma-derived HuMax-CD20, lot #MK100902 2F2
Recombinant HuMax-CD20, lot XX2, L08131/15
FITC-labeled HumaxCD20
Purities not reported.

Methods

- Six cell lines were used: Raji (human B lymphocyte Burkitt's lymphoma), Daudi (human B lymphoblast Burkitt's lymphoma), ARH77 (a human myeloma cell line producing IgG), Su-DHL-4 (human anaplastic large cell lymphoma), DOHH (human B-cell lymphoma), and CEM T (human T lymphoblast).
- Three other antibodies against CD20 were used as positive controls (a mouse anti-human CD20 antibody, rituximab, and HuMab-11B8). The antibody HuMab-11B8, mentioned in the study title, is an IgG3 kappa against human CD20. For

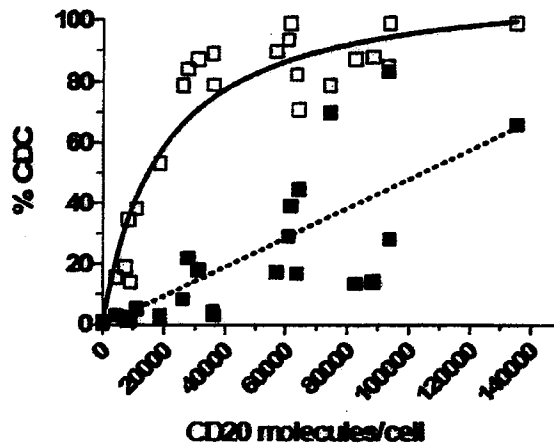
the purposes of this review, it was considered a positive control for HuMax-CD20. Isotypic antibody was used as a negative control.

- CDC assay: cells were incubated with ofatumumab or other antibody at 0.5 to 10 µg/ml, then normal human serum (NHS) was added to induce CDC. At serial time points over 1 hr, cells were resuspended in propidium iodide (PI) solution; cell lysis was determined by measuring the number of PI⁺ cells by flow cytometry.
- Effects of CD55 and CD59 on CDC: fixed doses of either anti-CD55 or anti-CD59 were added to the CDC experiments, to evaluate their potential contribution to CDC.
- Complement binding assay:
 - Fixation of C1q and C4 (complement proteins) to ofatumumab on the surface of CD20⁺ cells was evaluated. Cells were treated with antibody followed by NHS (as in the CDC assay), then pelleted, resuspended, treated with FITC-labeled antibodies against C1q or C4, and analyzed by flow cytometry.
 - ELISA: an ELISA plate was coated with ofatumumab, blocked, incubated with C1q or serum (to measure C3 complement), then treated with rabbit anti-human antibodies against C1q or C3.
- Cell-transfection assay to evaluate the minimum CD20-expression needed to induce CDC: CEM T cells were transfected with a vector expressing CD20. The level of CD20 expression was assayed using a commercially available test kit, then FITC-antibody binding to the cells was assayed by flow-cytometry.

Results

- Ofatumumab rapidly (less than 5 minutes) induced CDC in the 4 cell lines tested (Daudi, Raji, DOHH, and Su-DHL-4).
- Dose-responses were observed for ofatumumab and the positive-control antibodies; higher concentrations induced CDC more rapidly in the cell lines tested (Daudi, DOHH, ARH-77).
- Human serum was necessary for induction of CDC in the cell lines tested (Daudi, ARH-77, Raji), and a dose-response was shown between increasing concentrations of serum, and increasing percent lysis at 10 minutes. Heat-inactivated serum did not induce CDC.
- Under the conditions tested, addition of anti-CD55 and anti-CD59 antibodies increased the rate of lysis in Raji cells by ofatumumab. The authors report that CD55 and CD59 are complement inhibitor molecules.
- A dose-response was observed, between increasing ofatumumab concentration and increasing anti-C1q binding and anti-C4C binding (measured by flow cytometry) in the 4 cell lines tested (Daudi, ARH77, DOHH, Raji), indicating that ofatumumab might bind these two complement proteins via the complement fixing region.
- The ELISA assay detected ofatumumab binding to C1q and C3; a dose-response was apparent (but the authors did not calculate an EC value).
- A dose-response was reported between percent lysis and CD20-expression in transfected CD20 cells. The authors did not provide supporting evidence for the

reported CD20 expression. From report p 58 (open squares are ofatumumab data, black squares are an anti-CD20 positive control):



2.6.2.2.1.3

Study title: Induction of apoptosis by HuMax-CD20 and HuMab-11B8

Key study findings:

- Under the *in vitro* conditions tested, ofatumumab did not induce apoptosis in two CD20-positive cell lines.
- Induction of apoptosis may not be part of the mechanism of action of ofatumumab tumor cell killing *in vivo*.

Study no.: Genmab #GMB-3001-005 v2. GSK # CD2008/01165/00.

Volume #, and page #: Submitted electronically to m4.2.1.1. File name "gmb3001-005-report.pdf". 20 pp. Report date 7/16/2008.

Conducting laboratory and location: Genmab BV. Jenalaan 18D, 3584 CK Utrecht, The Netherlands.

Date of study initiation: 1/2002

GLP compliance: No.

QA reports: no (X)

Drug, lot #, and % purity: HuMax-CD20, clone XX2, lot L08131/15.

Methods

- Two cell lines were used: Raji (human B lymphocyte Burkitt's lymphoma), Daudi (human B lymphoblast Burkitt's lymphoma).
- Three other antibodies against CD20 were used as positive controls (a mouse anti-human CD20 antibody, rituximab, and HuMab-11B8). The antibody HuMab-11B8, mentioned in the study title, is an IgG3 kappa against human CD20. For the purposes of this review, it was considered a positive control for HuMax-CD20. Isotypic antibody was used as a negative control.
- Apoptosis was evaluated using the Annexin-V assay. Cells were incubated with antibody at 0.1, 1, or 10 µg/ml (Raji cells) or 1 µg/ml (Daudi cells) for 24 hours,

washed, treated with annexin-V-FITC, then PI, and the numbers of annexin-V positive cells and PI positive cells were determined by flow cytometry.

- Homotypic aggregation was assessed by plating Daudi cells, incubating with antibody for 4 hours, and assessing cell adhesion by visual microscopy (photographs provided in the report)

Results

- Ofatumumab did not induce apoptosis under the conditions tested.
- The authors conclude that ofatumumab induced less cell aggregation than the two CD20-positive control antibodies tested. However, no difference was apparent between ofatumumab and either of the two positive control antibodies tested; all three appeared to induce aggregation of labeled cells when compared to the negative control.

2.6.2.2.1.4

Study title: Efficient killing of tumor B cells by HuMax-CD20 and HuMab-11B8: antibody dependent cell-mediated cytotoxicity and the role of whole blood components

Key study findings:

- Under the *in vitro* conditions tested, ofatumumab induced ADCC and CDC in several CD20 expressing cell lines.
- The authors report that ofatumumab induced ADCC more efficiently compared to CDC at lower concentrations, but induction of ADCC and CDC was comparable at CD20 receptor saturation.
- This study was not conducted in compliance with the GLP regulations; evaluation of the study results is limited by a lack of detailed methodology and test conditions. The predictivity of the *in vitro* study results from cultured cell lines for activity in patients with cancer is unclear.
- This study provides supporting information, but is not sufficient robust to support a labeling claim comparing ofatumumab to any of the positive control antibodies tested in these experiments.

Study no.: Genmab #GMB-3001-006 v3. GSK # CD2008/01163/01.

Volume #, and page #: Submitted electronically to m4.2.1.1. File name "gmb3001-006-report.pdf". 33 pp. Report date 11/04/2008.

Conducting laboratories and locations:

- Genmab BV. Jenalaan 18D, 3584 CK Utrecht, The Netherlands.

b(4)

Date of study initiation: 1/2002

GLP compliance: No.

QA reports: no (X)

Drug, lot #, and % purity: HuMax-CD20 from clones 4C9-C7-1D12 and SJT26
Recombinant HuMax-CD20, clone XX2, lot # L08131/15.

Methods

- Three tumor B-cell lines were used: Raji (human B lymphocyte Burkitt's lymphoma), Daudi (human B lymphoblast Burkitt's lymphoma), ARH-77 (B plasma cell leukemia, expresses IgG1).
- Human blood was obtained from healthy volunteers and patients treated with granulocyte colony stimulating factor (G-CSF).
 - Human neutrophil cultures, and human PBMC cultures were enriched *in vitro* prior to use as isolated effector cells.
 - The report does not discuss if or how the blood from different sources was used.
- Results of testing in chronic B lymphocytic leukemia (CLL), hairy cell leukemia (HCL), follicular lymphoma, and primary mantle cell lymphoma were reported. However, reporting on the source of the cells (e.g. primary versus cultured cells, tumor cells, reliability of diagnoses) was not provided and no mention of these cell lines was made in the methods. This omission is a study limitation; however, the data are not needed to review the safety of ofatumumab because this study is not pivotal to the safety assessment.
- Three other antibodies against CD20 were used as positive controls (a mouse anti-human CD20 antibody, rituximab, and HuMab-11B8). The antibody HuMab-11B8, mentioned in the study title, is an IgG3 kappa against human CD20. For the purposes of this review, it was considered a positive control for HuMax-CD20. An isotype-matched, irrelevant antibody was used as a negative control.
- ADCC assay: whole blood or isolated effector cells were plated and treated with antibodies. Separately, the tumor B-cell lines were labeled with $^{51}\text{CrO}_4$ and then added to the plates. After 3 hours incubation, samples were centrifuged and ^{51}Cr in the supernatant measured as an indicator of cell lysis.
- Alternative ADCC assay: Daudi cells were labeled with $^{51}\text{CrO}_4$, incubated with antibody, then incubated with PBMCs. After 4 hours, samples were centrifuged and ^{51}Cr in the supernatant measured as an indicator of cell lysis.
- CDC assay: Daudi cells were labeled with $^{51}\text{CrO}_4$, incubated with antibody, then incubated with human serum (as a source of complement). After 1 hour, samples were centrifuged and ^{51}Cr in the supernatant measured as an indicator of cell lysis.
- Flow cytometry assay: FITC-antibody binding to Daudi cells was assessed.

Results: Under the conditions tested,

- Ofatumumab induced ADCC of CD20⁺ tumor cells when incubated with PBMC but not with neutrophils as effector cells
- Ofatumumab induced CDC of CD20⁺ tumor cells when incubated with human serum
- Ofatumumab induced cell lysis when incubated with whole blood (not clear if by ADCC or CDC) and ARH-77 cells, CLL or HCL as tumor targets.
- Ofatumumab induced CDC, but not ADCC, in follicular lymphoma and mantle cell lymphoma.
- Ofatumumab induced ADCC in ARH-77 and Raji cells; a dose-response was apparent.

- The authors report that ofatumumab was more effective at inducing ADCC (inducing higher percentage of lysis at lower concentrations) than CDC in Daudi cells.
- Authors reported that incubation of Daudi cells with 0.1 µg/ml of ofatumumab resulted in 50% saturation of CD20 receptors, and 5 µg/ml resulted in complete saturation of Daudi CD20 receptors.
- The authors compared ofatumumab to the other anti-CD20 antibodies tested. HuMab-11B8 and rituximab induced ADCC, CDC and cell lysis similar to ofatumumab; ofatumumab was more active than either HuMab-11B8 or rituximab in the assays reported.

2.6.2.2.1.5

Study title: Clustering of CD20 molecules into lipid rafts by CD20-antibodies: HuMax-CD20 and HuMab-11B8

Key study findings:

- Ofatumumab induced clustering of CD20 into lipid rafts under the *in vitro* conditions tested.
- The authors propose that clustering into rafts might be part of the CDC-mechanism of action.
- This study was not conducted in compliance with the GLP regulations; evaluation of the study results is limited by a lack of detailed methodology details and test conditions. The predictivity of the *in vitro* study results from cultured cell lines for activity in patients with cancer is unclear.
- This study provides supporting information, but is not sufficient robust to support a labeling claim comparing ofatumumab to any of the positive control antibodies tested in these experiments.

Study no.: Genmab # 3001-007 v2. GSK # CD2008/01161/00.

Volume #, and page #: Submitted electronically in m4.2.1.1. File name is "gmb3001-007-report.pdf". 23 pp. Report date 7/22/2008.

Conducting laboratory and location: Genmab BV. Jenalaan 18D, 3584 CK Utrecht, The Netherlands.

Date of study initiation: 1/2002.

GLP compliance: No.

QA report: no (X)

Drug, lot #, and % purity: HuMax-CD20, clone #4C9-C7-1D12, batch #MBR02-026-06/08.

FITC-conjugated HuMax-CD20 (batch # not reported)

HuMax-CD20-Cy3 conjugate (batch # not reported)

HuMax-CD20-Cy5 conjugate (batch # not reported)

Purities not reported.

Methods

- The Daudi cell line (human B lymphoblast Burkitt's lymphoma) was the only cell line tested.

- The antibody HuMab-11B8, mentioned in the study title, is an IgG3 kappa against human CD20. For the purposes of this review, it was considered a positive control for HuMax-CD20.
- Rituximab, a lawfully-marketed anti-CD20 antibody, was also used in this study as a positive control.
- Isotype-matched, irrelevant antibodies were also included in the testing as negative controls.
- Three techniques were used to investigate the ability of ofatumumab to induce the clustering of CD20 into lipid rafts.
 - Fluorescence resonance energy transfer (FRET)
 - Cy3 interacts with Cy5 to fluoresce.
 - Daudi cells were incubated 30 minutes with equimolar amounts of Cy-3 conjugated antibody and Cy-5 conjugated antibody for 30 minutes. Flow cytometry was used to measure FRET.
 - Higher fluorescence was considered an indicator that multiple CD20 receptors were near to each other, to allow the bound conjugated antibodies to transfer energy and allow fluorescence.
 - Assessment of raft associated antigen by Triton X-100 insolubility
 - Daudi cells were incubated for 15 minutes with FITC-antibody at 37°C, then cooled to 0°C (to maintain antibody-receptor binding). Aliquots were either untreated or treated with 0.5% triton X-100, and evaluated by flow cytometry.
 - The authors report that lipid rafts, held together by the cytoskeleton, will remain insoluble. The authors report this method removes the non-raft lipid and cytoplasm, leaving only insoluble portions of the cell.
- Sucrose gradient fractionation method
 - Daudi cells were incubated for 20 minutes with antibody, at 37°C, then cooled to 0°C, lyzed, pelleted, and fractionated. The fractions were evaluated by Western blot (mouse anti-CD20).

Results

- The authors report that ofatumumab incubation with CD20⁺ cells was associated with evidence of lipid raft formation in each of the three assays performed. Sufficient data were not provided to allow for evaluation of this conclusion.

2.6.2.2.1.6

Study title: Functional activities of HuMab-2C6: comparison with other CD20-specific antibodies

Key study findings:

- As reported previously, ofatumumab showed specific binding to CD20⁺ cells, and induced CDC but not apoptosis.
- This study was not conducted in compliance with the GLP regulations; evaluation of the study results is limited by a lack of detailed methodology and test

conditions. The predictivity of the *in vitro* study results from cultured cell lines for efficacy in patients with cancer is unclear.

- This study provides supporting information, but is not sufficiently robust to support a labeling claim comparing ofatumumab to any of the positive control antibodies tested in these experiments.

Study no.: Genmab # 3001-023. GSK # CD2008/01180/00.

Volume #, and page #: Submitted electronically in m4.2.1.1. File name is "gmb3001-023-report.pdf". 32 pp. Report date 10/02/2008.

Conducting laboratory and location: Genmab BV. Yenalaan 60, 3584 CK Utrecht, The Netherlands.

Date of study initiation: 4/2004.

GLP compliance: No.

QA report: no (X)

Drug, lot #, and % purity: 2F2 (ofatumumab), clone 2F2, lot #L08131/15.
¹²⁵I-labeled ofatumumab
FITC-labeled ofatumumab
Batch #s and purities not reported.

Methods

- Several anti-CD20 antibodies were tested side-by-side.
- Daudi, Raji, Su-DHL-4 cells, and a primary high grade B lymphoma (cell line not named) were used.
- Binding and dissociation experiment: cells were incubated with serial dilutions of ¹²⁵I-labeled antibody with or without unlabeled antibody over 3 hours, centrifuged through phthalate oils to separate bound from free labeled and unlabeled antibody; pellets were assayed by gamma counting.
- Flow cytometry using FITC-labeled antibody
- CDC assay: cells were incubated with antibody, then treated with PI and assayed by flow cytometry.
- Complement split assay: the generation of "complement split products", C4a, C3a, and C5a, were measured using a commercially available test kit.
- Detection of C1s and C3: Cells were incubated with antibody, normal human serum (as a source of complement), washed, incubated with animal anti-human C3 or anti-C1s, and analyzed by flow cytometry.
- Apoptosis assay; cells were incubated with antibody, washed, pelleted and resuspended, treated with annexin-V-FITC and PI, then assayed by flow-cytometry.
- Homotypic aggregation: Cells were incubated with antibody for 4 and 24 hours, and the extent of cellular adhesion was assayed visually by light microscopy.

Results

- Ofatumumab bound Daudi cells; a dose-response was apparent.
- Dissociation was observed for ofatumumab, but the dissociation rate constant was not calculated by the authors.
- Ofatumumab induced CDC in three B-cell lines and a primary high grade B-cell lymphoma cell line; a dose-response was apparent.

- The authors report that ofatumumab induced generation of C4a, C3a and C5a complement “split” products from normal serum, and also induced C3 deposition and C1s binding. The data provided are inadequate to assess this conclusion (i.e. no raw data or error bars presented).
- Ofatumumab induced aggregation of primary high grade B-cell lymphoma cells at 4 and 24 hours. The biological significance of this is unclear.
- Ofatumumab did not induce apoptosis.

2.6.2.2.1.7

Study title: Examination of non-human primate cross-reactivity of HuMax-CD20

Key study findings: The comparability of the target binding site and the comparability of binding to human and monkey CD20 under the conditions tested show that the cynomolgus monkey is a pharmacologically-relevant species for the nonclinical evaluation of ofatumumab.

Study no.: Genmab #GMB-3001-002 v3. GSK # CD2008/01167/00.

Volume #, and page #: Submitted electronically to m4.2.1.1. File name “gmb3001-002-report.pdf”. 24 pp. Report date 8/08/2008.

Conducting laboratory and location: Genmab BV. Jenalaan 18D, 3584 CK Utrecht, The Netherlands.

Date of study initiation: 1/2002

GLP compliance: No.

QA reports: no (X)

Drug, lot #, and % purity: HuMax-CD20-FITC, batch #MK 100902.

Formulation/vehicle: 5 µg/ml

Methods

- Healthy human tonsil tissue, human peripheral blood mononuclear cells (PBMC), rhesus monkey lymph node tissue, rhesus monkey PBMC, cynomolgus monkey tonsil, and cynomolgus monkey PBMCs were used. PBMCs were enriched by cell culture techniques prior to use.
- Three methods were used to evaluate ofatumumab binding to human and monkey CD20:
 - Immunohistochemistry with FITC-conjugated ofatumumab was performed on human and monkey tissue samples
 - Flow cytometry with FITC-conjugated ofatumumab was performed on human and monkey PBMCs.
 - The protein sequence of the second extracellular loop of cynomolgus monkey CD20 (the putative binding site for ofatumumab) was determined, by analyzing and manipulating RNA extracted from cynomolgus monkey blood using a commercially-available kit.

Results:

- Specific binding of ofatumumab to human tonsil, rhesus monkey lymph node, and cynomolgus monkey tonsil was observed.

- Flow cytometry values for binding to PBMC (units not reported, presumably ng/ml):
 - Human $EC_{50} = 287$
 - Rhesus monkey $EC_{50} = 97.35$
 - Cynomolgus monkey $EC_{50} = 139.1$
- The sequence of the cynomolgus monkey second extracellular loop of CD20 was comparable but not identical to human; one amino acid change (at position 158, ALA_{human} to VAL_{cynomolgus}) was detected.

2.6.2.2.2 Drug activity related to proposed indication:

The activity assay to support lot release is an antigen binding assay (FACS analysis) (m2.3.S).

The potency assay to support lot release is a CDC assay using an () cell viability assay: the acceptance criteria are () (BLA m2.3.S and m3.2.P.5.4). Notably, none of the nonclinical studies that assessed CDC used the / () () .

b(4)

2.6.2.3 Secondary pharmacodynamics

A nonclinical study potentially relevant to rheumatoid arthritis were submitted to the BLA (study # GMB-3001-020, m4.2.1.2); its review is not documented here.

2.6.2.4 Safety pharmacology

No basis of concern for safety pharmacology was identified in the nonclinical studies. No stand-alone nonclinical safety pharmacology studies were conducted. Select safety pharmacology endpoints (clinical signs, electrocardiogram [EKG], body temperature) were incorporated into the pivotal GLP 4-week (report #23542) and 7-month (report #25052) nonclinical toxicology studies conducted in male and female cynomolgus monkeys. The doses were 0, 20, or 100 mg/kg of ofatumumab by 30-minute intravenous infusion on D1, 8, 15, 22 (for both the 4-week and 7-month study) and also D29, 36, 43, 50, 78, 106, 134, 162, an 190 (for the 7-month study only).

Neurological effects: Repeated infusions of ofatumumab over 30 minutes were associated with transient tremors in some animals (study #25052); the affected animals also exhibited transient increased heart rate and/or heart force, indicating that the tremors were related to the infusion of a biologic. Notably, animals were not premedicated; the prescription label recommends that patients be premedicated with corticosteroid (prednisolone or equivalent), analgesic (acetaminophen or equivalent) and an antihistamine (cetizine or equivalent).

No other basis of concern for potential neurotoxicity was identified in the nonclinical studies. No stand-alone neurological battery was performed; clinical signs were monitored daily in each of the cynomolgus monkey toxicity studies.

Cardiovascular effects: Infusion of ofatumumab over 30 minutes was associated with transient increased heart rate and/or heart force, and tremors in some animals (study #25052); these effects typically began shortly after infusion started, resolved shortly after treatment was completed, and are attributed to the infusion of a biologic. Notably, animals were not premedicated; the prescription label recommends that patients be premedicated with corticosteroid (prednisolone), analgesic (acetaminophen) and an antihistamine (cetizine or equivalent).

Notably, electrocardiographic measurements made after dosing (at 30 minutes and 24 hours) for the 4-week study (D1, D8, D15, D22) and for the 7-month study (D1, 29, 50, 106 and 190) did not detect treatment-related changes. Ofatumumab was not assessed in the *in vitro* hERG assay.

Pulmonary effects: No stand-alone pulmonary testing was performed. In the cynomolgus monkey toxicology tests, clinical signs were monitored daily; at necropsy lungs were evaluated grossly, weighed, and evaluated histologically. No direct effects of ofatumumab on the pulmonary system were apparent. However, several treated monkeys exhibited signs of infection (frothy trachea, spongy lung), indicative of an indirect effect of treatment.

Renal effects: No treatment-related changes were detected. No stand-alone renal toxicity testing was performed; the cynomolgus monkey toxicology tests included routine clinical chemistry and urinalysis endpoints.

Gastrointestinal effects: No direct treatment-related changes were detected; several treated animals exhibited gastrointestinal effects (diarrhea, loose stool, inflammation) considered indirectly related to treatment and likely secondary to infection, since ofatumumab appeared to increase risk of infection. Evaluation of potential gastrointestinal toxicity was limited to monitoring for clinical signs, abnormal feces, gross pathology, organ weights, and histopathology in the pivotal cynomolgus monkey toxicity studies.

Abuse liability: No nonclinical testing for ofatumumab. The nonclinical testing did not identify any evidence of abuse liability.

2.6.2.5 Pharmacodynamic drug interactions

No nonclinical testing for ofatumumab pharmacodynamic drug interactions. Although analgesia, anesthesia and a mydriatic agent were routinely used (and supportive care including antibiotics, as appropriate) in pivotal cynomolgus monkey toxicity studies, the studies were not designed to detect potential drug interactions and no interactions were apparent.

As a theoretical concern, since ofatumumab and rituximab bind CD20 at different sites on the extracellular membrane portion of the protein, the presence of both biologics in the

body may result in interactions. Theoretically, co-binding of both ofatumumab and rituximab to CD20 proteins expressed on the surface of normal B-cells or tumor cells may affect the mechanisms of action (e.g. ADCC, CDC, or apoptosis), may increase the likelihood of a hypersensitivity-type reaction, or may affect the body's development of antiproduct antibodies.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

The sponsor provided m2.6.3 Pharmacology Tabulated Summary; their table (in 3 sections) is copied here:

Table 1. Pharmacology

Type of Study	Test System	Overview			Test Article: Ofatumumab	
		Method of Administration	GLP	Testing Facility	Report No. (Study No.)	Location Module
Primary Pharmacodynamics						
Development of anti-CD20 mouse hybridoma cell lines	In vitro	NA	No	MED	MWC-015-MDX-1088 (MDX-1088)	m4.2.1.1
Development of anti-CD20 mouse hybridoma cell lines	In vitro	NA	No	GEN	GMB 3001-010 v2	m4.2.1.1
V _H and V _L region sequencing	In vitro	NA	No	GEN	GMB 3001-001 v2	m4.2.1.1
Comparison of hybridoma (2F2H) and transfectoma-derived (2F2T) ofatumumab	In vitro	NA	No	GEN	GMB 3001-004 v3	m4.2.1.1
Binding characteristics of ofatumumab	In vitro	NA	No	GEN	GMB 3001-008 v3	m4.2.1.1
Effects of mutating extracellular CD20 amino acid residues on antibody binding	In vitro	NA	No	GEN	GMB 3001-011 v4	m4.2.1.1
Epitope mapping using the Pepscan method	In vitro	NA	No	GEN	GMB-3001-016	m4.2.1.1
Epibase profiling for immunogenic T cell epitopes	In vitro	NA	No	GEN	GMB-3001-017	m4.2.1.1
Antibody-induced clustering of CD20 into lipid rafts	In vitro	NA	No	GEN	GMB 3001-007 v2	m4.2.1.1
Complement binding and induction of complement-dependent cytotoxicity	In vitro	NA	No	GEN	GMB 3001-003 v3	m4.2.1.1
Induction of antibody-dependent cell-mediated cytotoxicity	In vitro	NA	No	GEN	GMB 3001-006 v3	m4.2.1.1
Induction of apoptosis	In vitro	NA	No	GEN	GMB 3001-005 v2	m4.2.1.1

Table 1. Pharmacology (Continued)

Type of Study	Test System	Overview			Test Article: Ofatumumab	
		Method of Administration	GLP	Testing Facility	Report No. (Study No.)	Location Module
Primary Pharmacodynamics (Continued)						
Generation of complement split products*	In vitro	NA	No	GEN	GMB-3001-023	m4.2.1.1
Effect on survival in a human lymphoma xenograft model	SCID mouse	Intravenous (bolus)	No	GEN	GMB 3001-009 v2	m4.2.1.1
Effect on tumour outgrowth in a human lymphoma xenograft model	SCID mouse	Intraperitoneal	No	GEN	GMB-3001-012 v4	m4.2.1.1
Effect of deglycosylation and changes in galactosylation on complement binding, induction of complement-dependent cytotoxicity, and binding to FcγRIIIa	In vitro	NA	No	GEN	GMB-3001-018	m4.2.1.1
Correlation between galactose content and cell lysis activity	In vitro	NA	No	GEN	GMB-3001-019	m4.2.1.1
Tissue cross-reactivity in primates (rhesus monkey, cynomolgus monkey, human)	SCID mouse	Intraperitoneal	No	GEN	GMB 3001-002 v3	m4.2.1.1
Tissue cross-reactivity in lower species (mouse, rat, rabbit, dog, pig)	In vitro	NA	No	GEN	GMB-3001-024	m4.2.1.1
Secondary Pharmacodynamics						
Effects in an animal model of rheumatoid arthritis	SCID mouse	Intravenous (bolus)	No	GEN	GMB-3001-020 (SR-8888-07-39)	m4.2.1.2

b(4)

Table 1. Pharmacology (Continued)		Overview		Test Article: Ofatumumab		
Type of Study	Test System	Method of Administration	GLP	Testing Facility	Report No. (Study No.)	Location Module
Safety Pharmacology						
4 Week repeat dose toxicity	Monkey (cynomolgus)	Intravenous (infusion)	Yes	INV	23542 (663805)	m4.2.3.2
7 Month repeat dose toxicity	Monkey (cynomolgus)	Intravenous (infusion)	Yes	/	25052 (665493)	m4.2.3.2
Key:		Testing Laboratory:				
NA = Not applicable		GEN = Genmah				
SCID = Severe combined immunodeficiency		C				
a = This report describes studies on 2C6, another anti-CD20 monoclonal. Ofatumumab was a comparator in these studies. The majority of the data for ofatumumab that are discussed in this report are also presented elsewhere [Reports GMB-3001-003 v3, GMB-3001-005, GMB-3001-006 and GMB-3001-008 v3].		MEU = Medarex				

b(4)

2.6.4 PHARMACOKINETICS (PK) / TOXICOKINETICS (TK)

2.6.4.1 Brief summary

- Following intravenous infusion (30 minutes) of ofatumumab to cynomolgus monkeys, plasma concentrations were generally proportional with dose. T_{max} values were equal to, or longer than, the duration of infusion. The volume of distribution at steady state ranged from 19 to 144 ml/kg, consistent with the expectation that ofatumumab would remain primarily in the vasculature (i.e. not distributing widely into tissues).
- The elimination half-life ($T_{1/2}$) means ranged from 0.7 to 16 days.
- Generally, $T_{1/2}$ values for D1 were shorter than subsequent $T_{1/2}$ values, and clearance rates for D1 were lower than subsequent clearance rates.
 - This is consistent with the expected pharmacology: ofatumumab is binding to normal CD20⁺ B-cells in treated monkeys and depleting them from circulation within 2 days of the initial dose. Subsequent doses of ofatumumab interact with fewer receptors and are therefore subject to less receptor-mediated clearance, resulting in longer elimination half-life.
 - The PK/TK in healthy monkeys may not predict the PK/TK in patients with CLL. CLL cells are expected to express fewer CD20 receptors per cell, and to proliferate more rapidly than normal B lineage cells.
- Gender differences in PK/TK were not apparent in treated cynomolgus monkeys.
- Ofatumumab crossed the placental barrier, being detected in cord blood samples of fetuses collected at GD100 (second trimester of pregnancy).
- The nonclinical doses of 20 and 100 mg/kg are nominally 0.7-fold and 3.5-fold, respectively, of the clinical dose (assuming a patient weight of 70 kg).
- As noted by the sponsor (m2.4): the highest dose of ofatumumab evaluated in the pivotal 7 month study, 100 mg/kg, corresponds to a mean $AUC_{0-\infty}$ for the final dosing interval ranging from approximately 810 to 900 mg*h/mL and a mean C_{max} ranging from approximately 3100 to 3500 μ g/mL. "This represents parity and 2-fold exposure, in terms of AUC and C_{max} , respectively, of the human

therapeutic exposure observed at a dose of 2000 mg, the highest clinical dose" (m2.4).

2.6.4.2 Methods of Analysis

The sponsor reports (m2.6.4) that "concentrations of ofatumumab and rituximab in cynomolgus monkey serum and ofatumumab in cynomolgus monkey plasma were determined using validated enzyme linked immunosorbent assay (ELISA) methods with colorimetric or chemiluminescence detection. ..."

- "Sandwich enzyme linked immunosorbent assay for the determination of ofatumumab and rituximab in monkey serum: A sandwich ELISA method was developed and validated for the determination of ofatumumab and rituximab in monkey serum. ... This method used a sheep anti-human IgG antibody conjugated to fluorescein isothiocyanate (FITC) as the capture antibody and a mouse anti-human IgG (Fc specific) antibody conjugated to horseradish peroxidase (HRP) as a detector antibody. The substrate azino-benzothiazoline sulphonic acid (ABTS) was used to generate a colorimetric endpoint with detection at the λ nm wavelength after the reaction was stopped with oxalic acid. Monkey serum was diluted at least 1:200 in assay buffer."
- "Sandwich enzyme linked immunosorbent assay for the determination of ofatumumab in monkey plasma: A sandwich ELISA method was developed and validated for the determination of ofatumumab in monkey plasma ... Monkey plasma samples were diluted 66.7 fold with assay diluent prior to analysis. The assay used a mouse monoclonal anti-idiotypic antibody to capture ofatumumab, and a Fc specific anti-human IgG1 antibody coupled to HRP to detect ofatumumab. SuperSignal ELISA Femto was used to generate a chemiluminescent response."
- "To examine the production of anti-ofatumumab antibodies in the cynomolgus monkey, methods were developed and validated to quantitate antibodies to ofatumumab in serum using ELISA assays. ... One of the methods determined antibodies to the Fc portion of ofatumumab, whilst the other measured antibodies against the whole ofatumumab IgG molecule. ... A validated ELISA method for the detection of anti-rituximab antibodies in cynomolgus monkey serum was also determined. ... A further ELISA assay was validated to quantitate antibodies to ofatumumab to support a repeat dose toxicology study using an alternative route of administration. ..."

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2.6.4.3 Absorption

The six *in vivo* studies reviewed in this review administered ofatumumab by intravenous infusion. One bridging study compared subcutaneous administration with intravenous infusion (study #CD2007/010204/00; submitted to m4.2.3.2); review of this study is not documented in this review, as the study is intended to support another indication. PK of ofatumumab in the cyclic administration study (#28366) was consistent with the other studies; review of the PK for that study is not documented in this review.

- The pilot study (#22804) dosed cynomolgus monkeys (1/sex/dose) with 1.25, 6.25, or 12.5 mg/kg/day of ofatumumab by 30-minute intravenous infusion for 4 consecutive days; animals were then observed for 130 days.
 - After the first dose, systemic exposure increased with dose subproportionally, clearance increased roughly proportionally with increasing dose, and no apparent dose-dependences were observed for elimination half-life ($T_{1/2}$), volume of distribution (V_d) or volume of distribution at steady state (V_{ss}).
 - The $T_{1/2}$ ranged from 15.7 to 28.5 days.
 - Between day 1 and 4, apparent $T_{1/2}$ increased 3.4 to 11-fold, and the V_d increased 1 to 11-fold. These increases are consistent with B-cell depletion (i.e. clearance of the target receptor from circulation).
- The 4-week study (#23542) dosed cynomolgus monkeys (3/sex/dose main group + 2/sex/dose recovery) by 30-minute intravenous infusion with 0, 20, or 100 mg/kg of ofatumumab weekly x4.
 - On D1:
 - T_{max} ranged from 1.3 hours to 2.8 hours
 - Mean $T_{1/2}$ ranged from 68 to 320 hours
 - Mean V_d ranged from 30 to 58 ml/kg
 - Mean V_{ss} ranged from 33 to 57 ml/kg
 - On D22:
 - T_{max} ranged from 0.75 hours to 2.8 hours
 - Mean $T_{1/2}$ ranged from 17 to 295 hours
 - Mean V_d ranged from 18 to 90 ml/kg
 - Mean V_{ss} ranged from 19 to 57 ml/kg
 - Comparing D1 and D22:
 - AUC and mean C_{max} increased for both dose groups and both sexes, indicating that weekly dosing resulted in accumulation of ofatumumab in the monkeys.
 - Clearance was faster in animals positive for anti-drug antibodies (3/12 low-dose animals)
 - The authors conclude, based on the V_d and V_{ss} data, that the majority of ofatumumab was retained in the plasma; only a small amount appeared to distribute beyond circulation. For context, the authors report that the extracellular fluid volume in monkeys is 208 ml/kg.
 - No sex-related differences in ofatumumab TK were apparent.
- The 7-month study (#25052) dosed cynomolgus monkeys (3/sex/dose main group + 4/sex/dose recovery) by 30-minute intravenous infusion with 0, 20, or 100 mg/kg of ofatumumab weekly x8, then monthly x5.
 - After the initial dose:
 - Clearance ranged from 0.24 to 0.3 ml/h/kg; the authors concluded that renal and hepatic blood flows are not limiting factors for clearance.
 - $T_{1/2}$ means ranged from 3.8 to 5.6 days.
 - V_d ranged from 34 to 52 ml/kg, and V_{ss} ranged from 31 to 48 ml/kg. Citing 208 ml/kg as a reference value for extracellular fluid volume in

- monkeys, the authors concluded that ofatumumab did not distribute substantially beyond central circulation.
- At the end of weekly dosing (i.e. D50, after 8 doses):
 - Accumulation was apparent
 - Clearance and Vd increased dramatically at both doses, compared to D1. The study authors consider this to be an artifact of analysis (more time points and more reliable data collected) rather than a true biological difference.
 - $T_{1/2}$ was 11 to 15 days, increased dramatically at both doses compared to D1. This is consistent with the expected pharmacology (i.e. clearance of target CD20⁺ cells from circulation).
 - At the end of monthly dosing (i.e. D190, after 5 monthly doses):
 - Accumulation was not apparent.
 - Clearance and Vd were comparable to D50.
 - $T_{1/2}$ was 11 to 16 days.
 - No gender differences in TK were apparent.
- The embryofetal study (#2148-010) dosed 12 pregnant cynomolgus monkeys/dose group weekly from GD20 to GD50 (i.e. weekly x5) by 30-minute intravenous infusion with 0, 20, or 100 mg/kg of ofatumumab. Fetuses were evaluated on GD100.
 - Weekly dosing caused accumulation, slightly more rapid at the higher dose.
 - Individual $T_{1/2}$ estimates ranged from 98 to 365 hours.

2.6.4.4 Distribution

The sponsor notes (m2.6.4): “As ofatumumab is an antibody, conventional distribution studies have not been performed. However, the potential for transplacental transfer has been examined as part of an embryofetal development study in the cynomolgus monkey. Both ofatumumab and ADAs were detected in fetal cord blood samples taken during the study, confirming the passage of antibodies across the placenta [Study No. 2148-010]”.

As summarized above, the V_d and V_{ss} results from the nonclinical toxicology studies indicate that ofatumumab was retained primarily within the vasculature.

The immunohistochemistry (tissue cross-reactivity) study in human tissues (study #23179) provides distribution information:

- Strong specific membrane staining was observed in human lymphocytes (lymph node, spleen, thymus, tonsil, and mucosa associated lymphoid tissue [MALT] of the small and large intestines).
- Additionally, specific membrane staining was observed in scattered lymphocytes in subepithelial tissues (cervix, endometrium, kidney, prostate, parotid salivary gland, skin, stomach, ureter, and urinary bladder).

The immunohistochemistry (tissue cross-reactivity) study in selected human, rhesus monkey, and cynomolgus monkey tissues (study #GMB-3001-002) provides distribution information:

- Specific binding of ofatumumab to human tonsil, rhesus monkey lymph node, and cynomolgus monkey tonsil was observed.
- Flow cytometry testing with PBMC showed specific binding to CD20⁺ cells.

The nonclinical toxicology studies in cynomolgus monkeys included immunohistochemistry evaluation of ofatumumab distribution *in vivo*. Two sets of slides were made for each tissue, to compare the number of B-cells binding ofatumumab versus the total number of B-cells with CD20 receptors in each tissue.

- The pilot study (22804):
 - Samples of spleen, thymus, Peyer's patches, bone marrow, and mesenteric lymph node were collected and prepared for immunohistochemistry.
 - The authors did not consider the immunohistochemistry analysis of lymph nodes to be robust or useful.
 - Immunohistochemistry at necropsy (bone marrow, lymph node, Peyer's patches, spleen, thymus) did not detect depletion of CD20⁺ cells, suggesting that the 130 days was adequate for full recovery in these animals.
- The 4-week study (#23542):
 - Tissues samples were collected at necropsy from the thymus, spleen, stomach (body and pylorus), pharynx, parotid salivary gland, tonsil, tongue, thyroid, skin and mammary, cerebrum, liver, and ovary for immunohistochemistry.
 - *Ex vivo* treatment of lymphoid tissue in lymphoid organs (spleen, tonsil, lymphoid follicles) in tissues from control and treated animals with ofatumumab resulted in specific staining.
 - Authors reported that staining was lower in tissues from treated main-group animals but not treated recovery animals, suggesting that ofatumumab from the *in vivo* phase remained bound to cell membrane-bound CD20 at D35.
- The 7-month study (#25052):
 - Tissues samples were collected at necropsy from the lymph nodes (mesenteric and submandibular), Peyer's patches, thymus, spleen, and tonsil for immunohistochemistry.
 - Specific ofatumumab staining was observed in the lymphoid follicles of tissues examined (submandibular lymph node, mesenteric lymph node, Peyer's patches, spleen, thymus, tonsil).
 - Results showed persistence of ofatumumab binding in recovery animals (determined by comparing slides pre-treated with ofatumumab versus normally treated slides).

2.6.4.5 Metabolism

The sponsor notes (m2.6.4): "No studies on the metabolism of ofatumumab are presented in this submission. The metabolism of ofatumumab is expected to be similar to that of other antibody molecules, involving proteolysis to small peptides and amino acids with protection from the Fc-Rn receptor prolonging this catabolism."

2.6.4.6 Excretion

The sponsor notes (m2.6.4): "No studies on the excretion of ofatumumab are presented in this submission. A consideration of the molecular weight of ofatumumab (149 kDa) suggests that renal elimination is unlikely to be of importance as the cut off for glomerular filtration is around 20-60 kDa and clearance of the antibody is predicted to be largely mediated by proteolysis."

2.6.4.7 Pharmacokinetic drug interactions

The sponsor notes (m2.6.4): "No studies on the excretion of ofatumumab are presented in this submission. A consideration of the molecular weight of ofatumumab (149 kDa) suggests that renal elimination is unlikely to be of importance as the cut off for glomerular filtration is around 20-60 kDa and clearance of the antibody is predicted to be largely mediated by proteolysis."

2.6.4.8 Other Pharmacokinetic Studies

None reviewed.

Note: One of the *in vivo* mouse tumor model studies (study #GMB3001-012) did include limited PK data; this reviewer did not consider the data essential, and therefore did not document review of the data in this review.

2.6.4.9 Discussion and Conclusions

Conclusion: The PK/TK of ofatumumab in treated cynomolgus monkeys appears consistent with other human antibodies. Ofatumumab was retained mainly in the blood/vascular system; weekly dosing resulted in accumulation while monthly dosing did not. $T_{1/2}$ was approximately 16 days, and PAHA increased elimination.

Discussion note:

Batch analyses were provided for two nonclinical lots. From m3.2.P.5.4:

Batch Analysis Data for Ofatumumab Injection, 20 mg/mL used in Non-Clinical Studies

Batch Number		PD04021	PD06016
Test	Acceptance Criteria ¹		
Appearance (visual)	Clear, colorless liquid. Visible particulates may be present	Complies	Complies
Extractable Volume For volume			
Particles Particulate Test	For information only. Report result.		
Protein concentration.			
pH at			
Identity -	Comparable to ref. std.	Comparable	Comparable
Antigen binding activity (FACS) ²	Report % relative to reference standard		
SDS-PAGE, Reduced	Report as heavy and light chains. Report MW of light and heavy chain bands	light chain = kDa heavy chain = kDa	light chain = kDa heavy chain = kDa
SDS-PAGE, Non-reduced	Report result as % IgG and compare to reference standard	Comparable	Comparable

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Batch Number		PD04021	PD06016
Test	Acceptance Criteria ¹		
Gel permeation HPLC ²		Comparable	Comparable
Sterility		Complies	Complies
	Potency relative to reference standard	NT	

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Note:

1. Specification in place at time of release
2. Data generated by development method, for details see section m3.2.S.4.3 Analytical Development and m3.2.P.5.6 Justification of specification

2.6.4.10 PK/TK tables and figures

Please see the PK/TK tables presented elsewhere in this review.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

The sponsor provided m2.6.5 Pharmacokinetics Tabulated Summary; their 3 tables are copied below.

Table 1. Pharmacokinetics				Overview		Test Article: Ofatumumab			
Type of Study	Species (strain)	No./ Sex/ Group	Method of Administration	Dose (mg/kg)	Duration of Dosing (Sampling Days)	GLP	Testing Facility	Report No. (Study No.)	Location Module
Absorption									
Pilot 4 Day Toxicokinetics	Monkey (cynomolgus)	1	Intravenous (infusion)	1.25, 6.25, 12.5 ^a	4 days (Days -7, 1, 2, 3, 4, 5, 6, 7, 9, 14, 17, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 99, 106, 113, 120, 127 and 134)	No		22804 (663784)	m4.2.3.2
4 Week Toxicokinetics	Monkey (cynomolgus)	3M/3 ^b	Intravenous (infusion)	0, 20, 100	22 days (Days -35, -7, 1, 2, 3, 4, 6, 8, 15, 22, 23, 24, 25, 26, 28, 30, 32, 35, 40, 43, 50, 57, 64, 71, 78, 107, 140, 170, 198 and 217)	Yes		23542 (663805)	m4.2.3.2
7 Month Toxicokinetics	Monkey (cynomolgus)	3M/4 ^R	Intravenous (infusion)	0, 20, 100	7 months (Days 1, 8, 15, 22, 29, 36, 43, 50, 50, 58, 65, 78, 134, 144, 190, 192, 193, 195, 200, 218, 232, 246, 274, 302, 330 and 372)	Yes		25052 (665493)	m4.2.3.2

Key:
 M = Main group animals
 R = Recovery animals
 a = In this study, rituximab was also administered at the same dose levels
 b = 5 animals/sex (3M/2^R) in the control group

Testing Facility:

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Table 1. Pharmacokinetics (Continued)				Overview		Test Article: Ofatumumab			
Type of Study	Species (strain)	No./ Sex/ Group	Method of Administration	Dose (mg/kg)	Duration of Dosing (Sampling Days)	GLP	Testing Facility	Report No. (Study No.)	Location Module
Absorption (Continued)									
Cycled Repeat Dose Toxicokinetics	Monkey (cynomolgus)	3M/3 ^R	Intravenous (infusion)	0, 20, 100	Two cycles (Days -7, 1, 15, 16, 148, 162, 163, 164, 165, 166, 168, 170, 172, 174, 177, 180, 183, 187, 192, 197 and 217)	Yes		28366 (509814)	m4.2.3.2
Embryofetal Development Toxicokinetics	Monkey (cynomolgus)	12F	Intravenous (infusion)	0, 20, 100	Day 20 to Day 50 of gestation (Days 20, 27, 34, 41, 48, 50, 52, 55, 60, 65, 70, 80, 90 and 100 pc)	Yes		NA (2148-010)	m4.2.3.5.2
2 Week Subcutaneous and Intravenous Repeat Dose Bridging Toxicokinetics	Monkey (cynomolgus)	6F	Subcutaneous Intravenous (infusion)	0, 20, 100 100	15 days (Days 1 and 15, weekly until Week 13 then fortnightly until end of recovery period)	Yes	GSK	CD2007/01024/0 0 (G07285)	m4.2.3.2

Key:
 M = Main group animals
 R = Recovery animals
 NA = Not applicable
 pc = post coitum

Testing Facility:
 GSK = GlaxoSmithKline

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Table 1. Pharmacokinetics (Continued)				Overview		Test Article: Ofatumumab			
Type of Study	Species (strain)	No./ Sex/ Group	Method of Administration	Dose (mg/kg)	Duration of Dosing (Sampling Days)	GLP	Testing Facility	Report No. (Study No.)	Location Module
Distribution									
Embryofetal Development (Toxicokinetics in cord blood)	Monkey (cynomolgus)	12F	Intravenous (infusion)	0, 20, 100	Day 20 to Day 50 of gestation (Caesarean Section)	Yes		NA (2148-010)	m4.2.3.5.2

Key:
 M = Main group animals
 R = Recovery animals
 a = In this study, rituximab was also administered at the same dose levels
 c = 5 animals/sex (3M/2^R) in the control group
 NA = Not applicable

Testing Facility:

b(4)

Table 2. Pharmacokinetics - Analytical Methods and Validation Reports

Test Article: Ofatumumab

Method	Species	Matrix (sample size)	Validated Concentration Range	GLP	Report Number	Location (Module)
Assays for Ofatumumab and Rituximab						
ELISA	Monkey	Serum (100 µL; 200-fold dilution)	Concentration range: 1000 to 20000 ng/mL	No	23373	m4.2.2.1
ELISA	Monkey	Plasma (100 µL, 66.7-fold dilution)	Concentration range: 1000 to 25000 ng/mL	No	CD2007/01220/00	m4.2.2.1
Assays for Anti-Ofatumumab antibodies						
ELISA	Monkey	Serum: Anti-ofatumumab antibody	Concentration range: NA	No	23372	m4.2.2.1
ELISA	Monkey	Serum	Concentration range: 2.5 to 100 ng/mL	No	28289	m4.2.2.1
ELISA	Monkey	Serum	Concentration range: 0.98 to 250 ng/mL	No	28459	m4.2.2.1
ECLIA	Monkey	Serum	Concentration range: 4 to 16384 ng/mL	No	CD2007/01212/00 (M07354)	m4.2.2.1
Assays for Anti-Rituximab antibodies						
ELISA	Monkey	Serum	Concentration range: NA	No	23682	m4.2.2.1

Key:
 ELISA = Enzyme-Linked ImmunoSorbant Assay
 CLIA = Chemiluminescent Immunoassay
 ECLIA = Electrochemiluminescent Immunoassay
 NA = Not applicable

Table 3. Pharmacokinetics: Absorption after Repeated Doses

Test Article: Ofatumumab

Species:		Cynomolgus Monkey							
Study Type:		Pilot 4 day toxicokinetics							
Report No. (Study No.):		22804 (683784)							
No. of Animals/Sex:		2							
Vehicle/Formulation:		Phosphate buffered saline							
Method of Administration:		Intravenous infusion (30 minutes)							
Dose (mg/kg):		1.25, 6.25, 12.5							
Sample:		Serum, prior to dosing, immediately post dosing, and on Days 1, 2, 3, 4, 5, 6, 7, 9, 14, 17, 22, 29, 38, 43, 50, 57, 64, 71, 78, 85, 92, 99, 106, 113, 120, 127 and 134							
Analyte:		Ofatumumab ELISA				Rituximab ELISA			
Assay:		ELISA				ELISA			
PK Parameters	Dose (mg/kg)	Male		Female		Male		Female	
		Day 1	Day 4	Day 1	Day 4	Day 1	Day 4	Day 1	Day 4
AUC _{0-∞} (µg·h/mL)	1.25	538	8900	431	6817	1732	14445	1820	10487
	6.25	4647	93898	5012	76556	20824	50837	15577	76295
	12.5	9079	296155	NC	231124	37955	207463	21302	147705
AUC ₀₋₂₄ (µg·h/mL) ^a	1.25	330	8912	275	6583	1000	12344	1077	7670
	6.25	3076	93522	2962	75256	5817	57905	5621	76182
	12.5	4857	295783	5447	230769	12078	207359	10700	147631
C _{max} (µg/mL)	1.25	46	169	44	165	82	141	83	133
	6.25	342	749	0234	572	392	1002	437	1014
	12.5	324	1092	324	1154	904	3063	794	23136
T _{1/2} (h)	1.25	28.5	225	22.2	76.0	19.7	89.6	18.2	59.4
	6.25	15.7	146	18.5	203	45.4	25.0	29.3	33.2
	12.5	23.5	145	NC	195	48.9	27.7	23.3	18.8
CL (mL/h/kg)	1.25	2.32	0.692	2.90	0.706	0.723	0.496	0.687	0.526
	6.25	1.35	1.22	1.25	1.32	0.30	0.474	0.401	0.401
	12.5	1.38	0.710	NC	0.619	0.392	0.238	0.587	0.365
V _d (mL/kg)	1.25	67.9	29.4	71.5	15.7	20.2	10.7	18.1	10.7
	6.25	28.8	20.9	32.6	25.7	20.2	7.43	0.401	0.401
	12.5	44.2	12.6	NC	15.5	22.3	3.69	19.7	4.93
T _{max}	1.25	0.50	1.50	0.52	0.50	1.50	6.50	1.50	0.50
	6.25	1.50	2.50	3.50	2.50	1.50	0.50	5.50	1.50
	12.5	2.50	0.00	3.50	24.00	2.50	6.50	1.50	6.50

Key: M = Main group animals. R = Recovery animals

ELISA = Enzyme-Linked ImmunoSorbant Assay

Table 3. Pharmacokinetics: Absorption after Repeated Doses

Test Article:
Ofatumumab

Species: Study Type: Report No. (Study No.): No. of Animals/Sex: Vehicle/Formulation: Method of Administration: Dose (mg/kg): Sample:		Cynomolgus Monkey 4 week toxicokinetics 23542 (663805) 3M/3F 30 mM sodium citrate/100 mM sodium chloride Intravenous infusion (30 minutes) 20, 100 Serum, prior to dosing, immediately post dose, and on Days 1, 2, 3, 4, 8, 15, 22, 23, 24, 25, 26, 28, 30, 32 and 35, 40, 43, 50, 57, 64, 71, 78, 107, 140, 170, 198 and 217 Ofatumumab ELISA						
PK Parameters	Dose (mg/kg)	Male			Female			
		Day 1	Day 22		Day 1	Day 22		
		M & R	M	R	M & R	M	R	
AUC _{0-∞} (µg.h/mL)	20 100 NA	116657 324771 NA	11826 145530 NA	336406 1754363 NA	55707 470349 NA	162686 989763 NA	249205 2205818 NA	
AUC ₀₋₁₉₈ (µg.h/mL) ^a	20 100 NA	46143 264179 NA	9742 325872 NA	35837 551528 NA	46782 302881 NA	68306 562856 NA	100689 755253 NA	
C _{max} (µg/mL)	20 100 NA	791 4702 NA	1031 10595 NA		604 5570 NA	1246 11535 NA		
T _{1/2} (h)	20 100 NA	320 97.1 NA	37.8 17.6 NA	207 241 NA	68.3 194 NA	206 163 NA	213 295 NA	
CL (mL/h/kg)	20 100 NA	0.203 0.315 NA	3.95 0.368 NA	0.189 0.190 NA	0.367 0.253 NA	0.302 0.182 NA	0.203 0.133 NA	
V _{ss} (mL/kg)	20 100 NA	57.4 38.6 NA	23.9 27.8 NA	22.7 25.3 NA	32.9 39.5 NA	37.0 22.1 NA	24.1 19.0 NA	

Key: M = Main group animals. R = Recovery animals a = 5 animals/sex (3M/2F) in the control group ELISA = Enzyme-Linked Immunosorbent Assay
NA = Not applicable

Table 3 (Continued). Pharmacokinetics: Absorption after Repeated Doses

Test Article: Ofatumumab

Species: Study Type: Report No. (Study No.): No. of Animals/Sex: Vehicle/Formulation: Method of Administration: Dose (mg/kg): Sample:		Cynomolgus Monkey 7 month toxicokinetics 25052 (665493) 3M/4F 30 mM sodium citrate/100 mM sodium chloride Intravenous infusion (30 minutes) 20, 100 Serum, Days 1, 8, 16, 22, 29, 36, 43, 50, 58, 65, 78, 134, 144, 190, 192, 193, 195, 200, 218, 232, 246, 274, 302, 330 and 372 Ofatumumab ELISA									
PK Parameters	Dose (mg/kg)	Male				Female					
		Day 1	Day 58	Day 134	Day 190	Day 1	Day 60	Day 134	Day 190		
		M & R	M & R	M & R	M	M & R	M & R	M & R	M	R	
AUC _{0-∞} (µg.h/mL)	20 100	73950 402347	405835 2065198	140266 560464	NC NC	202732 903407	62783 423185	292676 1458986	93746 421266	NC 112886	128482 809700
AUC ₀₋₁₉₈ (µg.h/mL)	20 100	41299 264893	119208 545047	62492 304533	NC NC	58439 287241	41202 272379	96208 487785	46493 282951	NC 112298	46397 272464
C _{max} (µg/mL)	20 100	531 2699	1239 6382	695 3735	697 3110	462 2676	1114 5414	638 4034	567 3460		
T _{1/2} (h)	20 100	135 127	346 361	198 147	NC NC	202 393	91.9 100	318 261	169 104	NC 22.0	256 275
CL (mL/h/kg) ^a	20 100	0.282 0.251	3.21 3.79	5.86 6.75	5.77 6.31	7.40 7.51	0.326 0.237	3.73 3.78	6.72 5.50	NC 5.39	8.37 6.30
V _{ss} (mL/kg)	20 100	48.0 42.4	23.2 23.9	37.84 35.89	NC NC	38.25 54.0	39.5 30.9	30.9 21.6	44.8 35.2	NC 27.2	45.2 45.0

Key:
M = Main group animals. R = Recovery animals.
ELISA = Enzyme-Linked Immunosorbent Assay
NC = Not calculated
a = The higher clearance values were determined using an AUC_{0-∞}.

Table 3. Pharmacokinetics: Absorption after Repeated Doses

Test Article: Ofatumumab

Species: Study Type: Report No. (Study No.): No. of Animals/Sex: Vehicle/Formulation: Method of Administration: Dose (mg/kg): Sample: Analyte: Assay:	Cynomolgus Monkey								Embryofetal development toxicokinetics		
	Cycled repeat dose toxicokinetics 28366 (509814)								NA (2148-010)		
	3M/3F 130 mM sodium citrate/100 mM sodium chloride, pH 6.5 Intravenous infusion (30 minutes) 20, 100 Serum, Days 1, 15, 16, 148, 162, 163, 164, 165, 166, 168, 170, 172, 174, 177, 180, 183, 187, 192, 197 and 217 Ofatumumab ELISA								12F 30 mM sodium citrate/100 mM sodium chloride solution pH 6.5 Intravenous infusion (30 minutes) 20, 100 Serum, Days 20, 27, 34, 41, 48, 50, 52, 55, 60, 65, 70, 80, 90 and 100 pc Ofatumumab ELISA		
PK Parameters	Dose (mg/kg)	Male			Female			Dose (mg/kg)	Female		
		Day 15	Day 162		Day 16	Day 162			Day 20	Day 48pc	
		M & R	M	R	M & R	M	R				
AUC _{0-∞} (mg·h/mL)	20	20096	1827	4144	66864	6452	134602	20	107300	212800	
	100	482688	192076	257645	590493	456240	273288	100	536600	1646000	
AUC _{0-t} (mg·h/mL)	20	19089	1827	4141	41385	6452	11332	20	55170	87860	
	100	280334	128242	134759	296428	245761	138086	100	309900	622600	
C _{max} (mg/mL)	20	481	504		672	701		20	628	760	
	100	9909	2812		12383	4071		100	3100	5880	
T _v (h)	20	21.6	6.50	17.1	199	10.5	273	NC	NC	NC	
	100	149	96.9	133	210	158	168	NC	NC	NC	
CL (mL/h/kg)	20	1.28	11.4	4.98	0.451	3.15	6.84	NC	NC	NC	
	100	0.287	0.743	0.730	0.250	0.288	0.813	NC	NC	NC	
V _d (mL/kg)	20	40.0	52.7	65.3	57.1	37.7	114	NC	NC	NC	
	100	82.4	74.8	81.2	82.7	60.7	88.2	NC	NC	NC	

Key:

M = Main group animals. R = Recovery animals.

ELISA = Enzyme-Linked Immunosorbent Assay

NC = Not calculated

Table 3 (Continued). Pharmacokinetics: Absorption after Repeated Doses

Test Article: Ofatumumab

Species: Study Type: Report No. (Study No.): No. of Animals/Sex: Vehicle/Formulation: Method of Administration: Dose (mg/kg): Sample: Analyte: Assay:	Cynomolgus Monkey						
	2 week subcutaneous and intravenous repeat dose toxicokinetics CD2007/01024/00 (G07285)						
	6F 50 mM Sodium acetate trihydrate/0.05mM disodium edelate dihydrate (EDTA)/1% L-Arginine free base/51mM sodium chloride/0.02% polysorbate 80, pH 5.5 Subcutaneous, intravenous infusion (30 minutes) 20, 100 (subcutaneous) 100 (intravenous infusion) Days 1, 15. Additional samples taken up to Day 250 Serum ELISA						
PK Parameters	Dose (mg/kg)	Day 1			Female Day 15		Off Dose
AUC _{0-∞} (mg·h/mL)	20 ^a	57900			46400		80000
	100 ^a	257000			27400		753000
	100 ^b	340000			392000		1005000
C _{max} (mg/mL)	20 ^a	218			297		297
	100 ^a	1000			1510		1510
	100 ^b	2760			3270		3270
T _{max}	20	120			60		60
	100	48.0			48		48
	100	0.5			1		1

Key:

ELISA = Enzyme-Linked Immunosorbent Assay

pc = post coitum

a = Subcutaneous

b = Intravenous

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The cynomolgus monkey model was used to predict the effects of ofatumumab in patients. The pivotal nonclinical studies tested only two doses: 20 and 100 mg/kg of ofatumumab. Both of these doses appeared to saturate the receptor. Dose responses were not apparent for magnitude of effect, but were apparent for duration of responses. Treatment-related effects generally appeared to be expected pharmacology, or indirect

effects attributable to the expected pharmacology. A no observed effect level (NOEL) was not identified. The primary pharmacotoxic activity is depletion of B-cells from circulation and lymphoid tissues. Recovery by or before 6-months was consistently apparent.

The high dose, 100 mg/kg, is technically considered severely toxic since it caused transient tremors and mortalities in >10% of treated animals in the 7-month study. However, the effects observed in monkeys are considered clinically monitorable and treatable.

Genetic toxicology: No studies to assess potential genetic toxicity were performed.

Carcinogenicity: No studies to assess potential carcinogenicity were performed.

Reproductive toxicology:

An embryofetal study was performed (dosing GD20-GD50, with saturating levels of ofatumumab remaining in maternal circulation until GD100), with Cesarean section on GD100. No teratogenicity was observed. Ofatumumab depleted fetal B-cells (to ~10% of control values); ofatumumab and PAHA (presumably maternal PAHA) were detected in fetal blood, demonstrating that the antibodies crossed the placental barrier. Treatment was associated with decreased placental weights, decreased fetal spleen weight, and decreased fetal thymus weight. The potential biological significance of these changes is unclear.

No stand-alone studies were performed to assess the effects of ofatumumab on fertility parameters. The standard toxicology studies are only of limited utility to assess potential reproductive toxicity: all ofatumumab-treated males were sexually immature, and only 7 sexually mature females (4/12 in the 4-week study, #23542; 3/12 in the 7-month study, #25052), as evidenced by clinical signs of menstruation, were treated with ofatumumab. However, no evidence of treatment-related reproductive toxicity was apparent in these two nonclinical studies.

Special toxicology: Two studies, a tissue-cross reactivity study in human tissues (#23179) and a screening tissue cross-reactivity study in multiple species (#GMB-3001-024) were reviewed. Ofatumumab is not pharmacologically active in the mouse, rat, rabbit, dog, or pig. Ofatumumab binds human lymphocytes in multiple organs.

2.6.6.2 Single-dose toxicity

No single dose nonclinical toxicity study was submitted to the BLA.

2.6.6.3 Repeat-dose toxicity

Seven repeat dose toxicity studies, each in cynomolgus monkeys, were submitted. The shortest duration of dosing was 4 days (study #663784); the longest duration of dosing administered a total of 13 doses over 7 months (study #25052). Six of these studies are reviewed below.

The clinical dose is a fixed dose, 2000 mg/patient. Assuming cross-species comparison on a mg/kg basis and a patient weight of 70 mg/kg, the nonclinical doses of 1.25, 6.25, 12.5, 20, and 100 mg/kg would be the equivalent of 4%, 22, 44, 70%, and 350% of the prescription label dose, respectively. Assuming a patient weight of 60 kg would result in the nonclinical doses of 1.25, 6.25, 12.5, 20, and 100 mg/kg being the equivalent of 4%, 19, 38, 60%, and 300% of the prescription label dose, respectively.

The route-of-administration bridging study is not reviewed in this review;

b(4)

2.6.6.3.1

Study title: Pilot and pharmacokinetic study in cynomolgus monkeys using HuMax-CD20.

Key study findings: Cynomolgus monkeys were dosed 1/sex/dose level daily for 4 days with 1.25, 6.25, or 12.5 mg/kg/day of ofatumumab, followed for 130 days, and sacrificed on D134. B-cell depletion was evident (monitoring blood and lymph node biopsies), with initial recovery by D29 and full recovery ranging from D60 to D120. No other clearly-treatment related effects were observed. TK analysis observed a rapid increase in elimination half-life and volume of distribution between D1 and D4, consistent with the expected pharmacology (i.e. ofatumumab binding to and B-cells, followed by B-cell depletion from circulation).

Study no.: report #22804. Genmab reference # 03-03-03-Hx-CD20. GSK # CD2008/01295/00.

Volume #, and page #: Submitted electronically in m4.2.3.2. File name "22804-report.pdf". 394 pp.

Conducting laboratory and location: Scotland.

b(4)

Date of study initiation: 2/04/2003

GLP compliance: No. Although the report includes a signed statement of compliance with the OECD principles of GLP, the study does not have a QA statement and it is listed by the sponsor as not GLP (m2.6.7 Toxicology Tabulated Summary).

QA report: no (X)

Drug, lot #, and % purity: HuMax-CD20 (clone 2F2), lot #LO8131/27. Purity not reported (no certificate of analysis). Purity determined by HPLC: 96.5% IgG.

Methods

Doses: 1.25, 6.25, or 12.5 mg/kg/day of ofatumumab (no negative control group)

Species/strain: cynomolgus monkey (*Macaca fascicularis*)

Number/sex/group/schedule: 1/sex/dose level, dosed 4 consecutive days.

Route, formulation, volume, and infusion rate: 30 minute intravenous infusion via the saphenous vein, at constant dose volume of 10 ml/kg.

Satellite groups used for toxicokinetics or recovery: none

Age: Approximately 2 years of age

Weight: 2.2 to 2.8 kg at initiation of dosing

Study design notes:

- Animals were observed for 130 days after dosing, prior to necropsy on D134.
- Rituximab was administered as a positive control, 1/sex/dose at 6.25 or 12.5 mg/kg/day.

Mortality, clinical signs: Observations were made during the day. A detailed physical examination was conducted predose, daily on D1-5, and then weekly, including abdominal palpation, auscultation, rectal temperature, examination of skin, examination of body orifices.

Body temperature: Measured daily from Day -7 to Day 5, and weekly thereafter. Rectal probe thermometer.

Body weights: Measured weekly

Food consumption: Not evaluated.

Ophthalmoscopy: Not evaluated.

EKG: Not evaluated.

Hematology: measured on D2, 8, 30, 45, 60, 134. Endpoints were: hemoglobin, red blood cell count, hematocrit, packed cell volume, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, red cell distribution width, hemoglobin distribution width, platelet distribution width, reticulocytes, white blood cell counts, differential white blood cell counts (neutrophils, lymphocytes, monocytes, eosinophils), and platelets (mean platelet volume)

Clinical chemistry: measured on D2, 8, 30, 45, 60, 134. Endpoints were: urea, sodium, potassium, chloride, total protein, albumin, alkaline phosphatase, globulin, A:G ratio, creatinine, total bilirubin, calcium, inorganic phosphate, C-reactive protein, C3 and C4 complement components

Coagulation: measured on D2, 30, 45, 60, 134. Endpoints were: prothrombin time, activated partial thromboplastin time, and fibrinogen

Urinalysis: overnight collections on D 5/6, 31/32, 60/61, and 127/128. Endpoints were volume, specific gravity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood pigments, and microscopic examination of the spun deposit for epithelial cells, crystals, white blood cells, red blood cells, organisms, casts, abnormal constituents

Gross pathology: Detailed external and internal examination including body orifices, cranial, thoracic, and abdominal organs and tissues.

Organ weights: adrenal gland, brain, heart, kidneys, liver, lungs, ovary, pancreas, pituitary gland, prostate gland, spleen, testes, thyroid gland + parathyroid, uterus + cervix

Histopathology: Adequate Battery: yes (X)

Peer review: no (X)

- Standard tissues examined: abnormal tissues, adrenal, aortic arch, bone marrow (femur), brain, eyes, gall bladder, gastrointestinal tract (stomach, duodenum, ileum, jejunum, cecum, colon, rectum), heart, injection site, kidney, liver, lungs, lymph nodes (mesenteric, submandibular), esophagus, optic nerve, ovary, pancreas, Peyer's patches, pituitary, prostate, sciatic nerve, seminal vesicle, skeletal muscle, skin + mammary gland, spleen, sternum, testes, thymus, thyroid + parathyroid, tongue, trachea, urinary bladder, uterus + cervix, vagina.
- Samples of spleen, thymus, Peyer's patches, bone marrow, and mesenteric lymph node were collected and prepared for immunohistochemistry.

- Two sets of slides were made for each tissue, to compare the number of B-cells binding ofatumumab versus the total number of B-cells with CD20 receptors in each tissue.

Biopsy: Biopsies from the superficial lymph node were taken predose, D8, 30, 50, 75, 96, and 113 as well as at necropsy (D134). Analysis by flow cytometry and immunohistochemistry was conducted.

Toxicokinetics: Blood samples taken:

- Predose (day -7)
- Day 1 and 4: predose, immediately after dosing, and at 1, 2, 3, 5, 6, and 12 hours post-dose
- Day 2 and 3 predose only
- Days 5, 6, 7, 9, 14, 17, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 99, 106, 113, 120, 127 and 134.

Measurement of anti-product antibody formation: blood samples taken predose, D8, 15, 29, 54, 75 and 134

Cytometric analysis: Blood samples taken predose, before dosing on D2, D8, 15, 29, 45, 60, 92, 120 and 134. Markers analyzed by flow cytometry were:

- CD3, CD4, and CD8 for T-cell identification
- CD3, CD16 and CD8 for NK cell identification
- CD3, CD10, CD19 and CD20 for B-cells
- CD3, CD40, CD23, and CD20 for B-cell identification
- CD3 and CD21 for B-cell identification
- Human Ig, CD3, CD19, and CD20 to estimate receptor saturation

Results

Mortality: No unscheduled mortalities reported.

Clinical signs: No apparent treatment-related effects.

Body temperature: No apparent treatment-related effects.

Body weights: No apparent treatment-related effects.

Food consumption: Not evaluated.

Ophthalmoscopy: Not evaluated.

EKG: Not evaluated.

Hematology: No apparent treatment-related findings. This study did not include current controls. Although effects are observed comparing pre-dose and post-dose value (i.e. decreased Hb, RBC, Hct on D2 and D8, increased reticulocytes on D8 only), comparison of the hematology data from this study to other studies in the BLA supports the attribution of the hematological changes to the frequency of blood collection for TK.

Clinical chemistry: An increase in C-reactive protein (CRP) on D2 was apparent for ofatumumab-treated animals. This endpoint appeared to have recovered by D8.

Urinalysis: No apparent treatment-related effects.

Gross pathology: No apparent treatment-related effects.

Organ weights: No apparent treatment-related effects.

Histopathology: Adequate Battery: yes (X), no ()

Peer review: yes (), no (X)

No apparent treatment-related effects.

Toxicokinetics:

- AUC and C_{max} summary after the initial dose (from report p 383):

Gender	Animal	Dose Level (mg/kg/day) [Ratio]	AUC(0-∞) (mg/kg/day) [Ratio]	AUC(0-t) (mg/kg/day) [Ratio]	$C_{max}(obs)$ (ng/ml) [Ratio]
Male	1	1.25 [1]	537836 [1]	329657 [1]	45533 [1]
	2	6.25 [5]	4647369 [8.64]	3076443 [9.33]	341952 [7.51]
	3	12.5 [10]	9079376 [16.88]	4856661 [14.73]	323632 [7.11]
Female	7	1.25 [1]	430996 [1]	274938 [1]	44065 [1]
	8	6.25 [5]	5012321 [11.63]	2962029 [10.77]	234184 [5.31]
	9	12.5 [10]	NC [NC]	5446624 [19.81]	323632 [7.34]

NC indicates estimates not calculable; lambda-z estimation not possible for the profile

- Summary of other calculated TK parameter ranges:

Parameter	After 1 st dose
Clearance (ml/h/kg)	1.3 to 2.9
T_{max} (h)	0.5 to 3.5
$T_{1/2}$ (h)	15.7 to 28.5
V_d (ml/kg)	30.4 to 95.5
V_{ss} (ml/kg)	38.8 to 71.5

- For D1: systemic exposure increased with dose subproportionally, clearance increased roughly proportionally with increasing dose, and no apparent dose-dependences were observed for elimination half-life ($T_{1/2}$), volume of distribution (V_d) or volume of distribution at steady state (V_{ss}).

- AUC and C_{max} summary after the 4th daily dose (from report pp 386-387):

Males:

Animal	Dose Level (mg/kg/day)	Day	AUC(0-∞) (ng/h/ml)	AUC(0-t) (ng/h/ml)	Cmax(obs) (ng/ml)
1	1.25	1	537836	329657	45533
		4	8900425	6911607	169108
2	6.25	1	4647389	3076443	341952
		4	93898489*	93522079	749168
3	12.5	1	9079376	4856661	323632
		4	296155304	295783403	1092032

* indicates estimate derived from a 'J' flagged profile, therefore considered unreliable by the pharmacokineticist

Females:

Animal	Dose Level (mg/kg/day)	Day	AUC(0-∞) (ng/h/ml)	AUC(0-t) (ng/h/ml)	Cmax(obs) (ng/ml)
7	1.25	1	430996	274938	44085
		4	6817237	6593419	164496
8	6.25	1	5012321	2962029	234184
		4	78556310*	75256141	571872
9	12.5	1	NC	5446624	323632
		4	231123818*	230769026	1154336

* indicates estimate derived from a 'J' flagged profile, therefore considered unreliable by the pharmacokineticist

NC indicates estimates not calculable; lambda-z estimation not possible for the profile

- Between day 1 and 4, apparent $T_{1/2}$ increased 3.4 to 11-fold, and the V_d increased 1 to 11-fold. These increases are consistent with B-cell depletion (i.e. clearance of the target receptor from circulation)

Measurement of primate anti-human antibody (PAHA) formation: Both low-dose animals developed anti-product antibody responses (animal #1M beginning at D29, #7F beginning at D54). PAHA was not noted in other ofatumumab-treated animals.

B-cell depletion:

- Depletion of B-cells was shown by flow cytometric analysis of blood and lymph node samples. B-cells were depleted to ~4% of baseline on D2 (the first time point measured after initiation of dosing). Initial recovery was apparent by D29, with full recovery ranging from D60 to D120 for individual animals.
- The study authors concluded that immunohistochemistry of lymph node biopsy samples was not a robust endpoint to detect changes in B-cells or other potential treatment-related effects.

- Immunohistochemistry at necropsy (bone marrow, lymph node, Peyer's patches, spleen, thymus) did not detect depletion of CD20⁺ cells, suggesting that the 130 days was adequate for full recovery in these animals.

2.6.6.3.2

Study title: Determination of some circulating and coagulation parameters after administration of HuMax-CD20 to cynomolgus monkeys.

Key study findings: This study was reportedly the initial study performed with ofatumumab in the cynomolgus monkey model; the purpose was to evaluate "activation and release of inflammatory and coagulation parameters" (report p 10); endpoints were limited to assessment of signs of infusion reaction/cytokine release syndrome. A group of 4 cynomolgus monkeys was dosed with ofatumumab on D1 and D5. Markers of inflammation and coagulation were increased when measured 15-minutes to 4-hours after ofatumumab treatment. The authors consider this evidence of a "so-called cytokine release syndrome" (report pp 5, 7, 17).

Study no.: GMB-3001-013 v2. GSK #CD2008/01294/0-0.

Volume #, and page #: Submitted electronically in m4.2.3.7.7. File name gmb-3001-013-report.pdf". 27 pp.

Conducting laboratory and location: Genmab BV, Jenalaan 18D, 3584 CK, Utrecht, The Netherlands.

Date of study initiation: 1/02/2003

GLP compliance: No.

QA report: no (X)

Drug, lot #, and % purity: HuMax-CD20, clone XX2 (NS/0), batch #L08131/15.

Methods

Doses, numbers, sex:

Animal #, sex	Day 1 dose (mg/kg)	Day 5 dose (mg/kg)
1M	25	50
2F	50	75
3M	75	100
4F	100	150

Species/strain: cynomolgus monkey (*Macaca fascicularis*)

Number/sex/group/schedule: 1/group.

Route, formulation, volume, and infusion rate: Intravenous injection (presumably bolus injection).

Age and weight range: not reported.

Blood sampling times:

- predose, 15 minutes, 30 minutes, 2 hours and 4 hours post-dose.
- Data are only reported for one of the two days of injection (presumably the D1 injections).

Recovery: Animals were kept for a 17-day observation period (no reporting of endpoints monitored, no necropsy endpoints)

Endpoints assessed in serum by ELISA assays:

- C4b/c (activated C4).
 - The authors report that C4 is a complement factor in humans and monkeys.
- C3b/c (activated C3).
 - The authors report that C3 is a complement factor in humans and monkeys.
- Thrombin-anti-thrombin III (TAT) complexes.
 - The authors note that thrombin is a central enzyme in coagulation, and is inhibited by anti-thrombin III, forming stable complexes (TAT) that can be measured in serum.
 - The authors stated that because the enzymatic activity of thrombin in plasma is very short, measurement of TAT complexes was selected over direct measurement of thrombin activity.
- Plasmin- α 2-antiplasmin (PAP) complexes
 - The authors note that plasmin is a central enzyme in coagulation, and is inhibited by α 2-antiplasmin, forming stable complexes (PAP) that can be measured in serum
- Elastase- α 1-antitrypsin complexes (HNE/ATc)
 - The authors report that elastase is one of the degranulation products released from activated neutrophils, and that elastase is rapidly inhibited by α 1-antitrypsin to form stable complexes (HNE/ATc) in plasma.
- Interleukin 6 (IL-6)
- Tumor necrosis factor alpha (TNF α)

Results

- All markers measured except TNF α were increased after treatment
- The data presented are difficult to interpret; the authors report that C3b/c and C4/bc partially recovered by 4 hours.

2.6.6.3.3**Study title:** HuMax-CD20: pilot study in cynomolgus monkeys.**Key study findings:**

- A total of 4 cynomolgus monkeys were dosed by iv infusion on D1 and D5 with doses ranging from 25 to 150 mg/kg of ofatumumab, with necropsy on D22. b(4)
- No adverse effects were apparent.

Study no.: / / report #23315. Genmab reference # 180803-HxCD20. GSK #CD2008/0519/00.**Volume #, and page #:** Submitted electronically in m4.2.3.2. File name "23315-report.pdf". 49 pp.**Conducting laboratory and location:** *LA* b(4)**Date of study initiation:** 8/06/2003

GLP compliance: Yes, the report includes a signed statement of compliance with the OECD principles of GLP. However, the study does not have a QA statement, and is listed by the sponsor as not GLP (m2.6.7 Toxicology Tabulated Summary).

QA report: no (X)

Drug, lot #, and % purity: HuMax-CD20. Lot #LO9136/10. 16 mg/ml. HPLC: 99.28% IgG, 0.57% aggregate, 0.15% fragment.

Methods

Doses, numbers, sex:

Animal #, sex	Day 1 dose (mg/kg)	Day 5 dose (mg/kg)
1M	25	50
3F	50	75
2M	75	100
4F	100	150

Species/strain: cynomolgus monkey (*Macaca fascicularis*)

Route, formulation, volume, and infusion rate: intravenous infusion at a dose volume of 10 mg/kg over a 30 minute infusion period. Diluent was 30 mM sodium citrate/100 mM sodium chloride buffer.

Satellite groups used for toxicokinetics or recovery: None. All animals were necropsied on D22.

Age: 2 years of age

Weight: 2.5 to 3.1 kg at initiation of dosing

Note: Additional blood samples were collected for possible analyses not reported.

Endpoints:

Mortality, clinical signs: "frequent intervals" during the day, including inspection for vomit, blood, and fecal changes.

Body weights: measured predose, and on days 1, 5, 12, 15, and 22.

Food consumption: Not assessed.

Ophthalmoscopy: Not assessed.

EKG: Not assessed.

Hematology: Blood samples collected predose, before dosing on D5, D12, and before necropsy on D22. Endpoints were: hemoglobin, red blood cell count, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, reticulocytes, white cell counts, differential white blood cell count (neutrophils, lymphocytes, monocytes, eosinophils, basophils), platelets, and large unclassified cells

Clinical chemistry: Blood samples collected predose, before dosing on D5, D12, and before necropsy on D22. Endpoints were: urea, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, sodium, potassium, chloride, total protein, albumin, globulin, albumin:globulin ratio, cholesterol, creatinine, total bilirubin, calcium, inorganic phosphate, triglycerides, and C-reactive protein

Coagulation: Blood samples collected predose, before dosing on D5, D12, and before necropsy on D22. Endpoints were: prothrombin time, activated partial thromboplastin time, and fibrinogen.

Urinalysis: Not evaluated.

Gross pathology: Gross necropsy (external including body orifices, internal)

Organ weights: adrenals, brain, heart, kidneys, lungs, ovary, pancreas, pituitary gland, prostate gland, spleen, submaxillary salivary gland, testes + epididymides, thymus, thyroid gland + parathyroid, uterus with cervix.

Histopathology: Samples collected but not evaluated.

Results

Mortality: No unscheduled deaths.

Clinical signs: No apparent treatment-related effects.

Body weights: No apparent treatment-related effects.

Hematology:

- An increase in reticulocyte counts is apparent on D12 only for all treated animals.
- No other apparent treatment-related effects.

Clinical chemistry:

- Total bilirubin decreased dramatically for all animals after treatment (D5-22).
- No apparent treatment-related effects.

Gross pathology: No apparent treatment-related effects.

Organ weights: Animal 4F (dosed at 100 and 150 mg/kg) had high spleen and thyroid weights.

Histopathology: Not evaluated.

Toxicokinetics: Not evaluated.

2.6.6.3.4

Study title: HuMax-CD20: multiple dose intravenous toxicity study in cynomolgus monkeys followed by a 6 month recovery period.

Key study findings: Groups of cynomolgus monkeys (3/sex/dose main-groups, 2/sex/dose recovery groups) were dosed by intravenous infusion with 0, 20, or 100 mg/kg of ofatumumab weekly x4 (on D1, 8, 15, 22).

- NOAEL is the low-dose, 20 mg/kg/week. Authors reported 100 mg/kg/week as the NOAEL.
- LOAEL is the high-dose, 100 mg/kg/week, based on brain histopathology (focal arteritis and perivascular inflammatory cell infiltration) and decreased antibody response to keyhole limpet hemocyanin (KLH) in Freund's Incomplete Adjuvant (FIA).
 - The authors did not comment on the brain lesions, and did not consider the KLH response adverse.
- Clearance of B-cell from circulating blood and (biopsied) lymph nodes was detected by flow cytometry at the first post-dose time point measured (D8).
 - Males appeared to recover faster than females.
 - At the low dose:
 - males exhibited initial signs of recovery 28 days after cessation of treatment, and full recovery 90 days after cessation of treatment.

- females exhibited initial signs of recovery 48 days after cessation of treatment, and full recovery 111 days after cessation of treatment.
 - At the high dose:
 - males exhibited initial signs of recovery 62 days after cessation of treatment, and full recovery 111 days after cessation of treatment.
 - females exhibited initial signs of recovery 90 days after cessation of treatment, and full recovery 127 days after cessation of treatment.
 - Animals with PAHA cleared ofatumumab more quickly, and recovered B-cells faster than other treated animals.
 - Notably, B-cell depletion could not be confirmed via standard hematology endpoints (i.e. total leukocyte counts).
- Pharmacokinetic parameters changed between the first and last dose, consistent with the mode of action (binding to CD20 and clearing CD20⁺ cells from circulation, thereby reducing the pool of target receptors).
 - After the last dose, the elimination half-life ranged from 8.6 to 12.3 days.
 - The V_d and V_{ss} estimates indicate that ofatumumab is retained in the plasma, with only a relatively small proportion distributing beyond central circulation.
- Gross pathology:
 - The authors concluded that no effects were apparent.
 - However, treatment was associated with spongy lung (possibly evidence of increased susceptibility to infection), reddened lymph nodes, and pale foci in the spleen.
 - These effects are not clearly adverse, and are attributed to the pharmacologic effect of ofatumumab.
- Histology:
 - Treatment caused atrophy of the germinal centers or follicles of spleen, mandibular lymph node, mesenteric lymph node, and Peyer's patches. Recovery was evident.
 - Authors concluded that no other effects were treatment-related.
 - However, brain effects (focal arteritis, perivascular inflammatory cell infiltration) were observed in high-dose main-group females. Although these lesions would not be clear evidence of toxicity *per se*, in the context of the clinical findings, they are considered treatment-related and adverse.
 - Additionally, lung effects (alveolar macrophage accumulation, inflammatory cell infiltration, congestion) were apparent in treated main-group and recovery animals, suggesting an increased susceptibility to infection.
- The high-dose, but not the low-dose, inhibited the induction of specific anti-KLH antibodies, a measure of humoral immune function.
- Treatment did not appear to suppress cellular immune function (delayed-type hypersensitivity) under the conditions tested.
- This study has limited value for assessing potential reproductive toxicity.
 - Each of the treated males was sexually immature (i.e. immature testes).

- 1/6 low-dose females and 3/6 high-dose females were sexually mature (i.e. active menstrual cycle).
- No treatment-related effects on reproductive organs were apparent.

Study no.: 23542. () study #663805. Genmab reference #Hx-CD20-N-004.
GSK #CD2008/01520/00.

Volume #, and page #: Submitted electronically in m4.2.3.5.2. File name is "23542-report.pdf". 736 pp. b(4)

Conducting laboratory and location: ()

Date of study initiation: 8/11/2003

GLP compliance: Yes, in compliance with the OECD principles of GLP. Separate from the study report, the sponsor submitted a signed statement from study laboratory (now () stating compliance with 21CFR58, with noted minor differences. The differences are not expected to have impacted the outcome of the study. b(4)

QA report: yes (X)

Drug, lot #, and % purity: HuMax-CD20, 16.6 mg/ml. Lot #s L09136/10 and L09167/15. HPLC testing found 99.28% IgG, 0.57% aggregate, 0.15% fragment.

Methods

Doses: 0, 20, 100 mg/kg weekly #5 (D1, 8, 15, 22)

Species/strain: cynomolgus monkeys (*Macaca fascicularis*) originating from Mauritius and supplied from () b(4)

Number/sex/group or time point (main study): 3/sex/dose. Sacrificed two weeks after the last dose (D35).

Route, formulation, volume, and infusion rate: Intravenous infusion of 30 minutes into the saphenous vein at 10 ml/kg dose volume. Formulation was 30 mM sodium citrate/100 mM sodium chloride diluent, pH 6.5.

Satellite groups used for recovery: 2/sex for the control group, 3/sex/dose for the two treated groups. Sacrificed 6 months after the last dose (D217).

Age: 14-20 months at arrival (acclimated for 9 weeks prior to initiation of dosing).

Weight: 2.0 – 2.9 kg at initiation of dosing.

Observations and times:

Mortality, clinical signs: "regular intervals" during the day, including inspection for vomit, blood, and abnormal feces.

Body weights: Weekly

Food consumption: Not evaluated separately from daily clinical observations

Ophthalmoscopy: predose, D30, "toward the end" of the 6 month recovery period. 1% tropicamide was used as a mydriatic agent.

EKG: Lead II trace was used to evaluate interval data (P-R, QRS, Q-T) and heart rate. Predose, 30 minutes and 24 hours after each dose (on D1, 8, 15, 22).

Body temperature: predose, once daily from D-7 to D29 and weekly thereafter

Hematology: predose, D2, 8, 22, 35, 40, 84, 118, 161, 217. Endpoints were: hemoglobin, red blood cell count, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, reticulocytes, white cell counts, differential white blood cell count (neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unclassified cells), platelets, and mean platelet volume.

Coagulation parameters: predose, D2, 22, 35, 40, 84, 118, 161, 217. Endpoints were: prothrombin time, activated partial thromboplastin time, and fibrinogen.

Clinical chemistry: predose, D2, 8, 22, 35, 40, 84, 118, 161, 217. Endpoints were: urea, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase, sodium, chloride, total protein, albumin, globulin, albumin:globulin ratio, cholesterol, creatinine, total bilirubin, calcium, inorganic phosphate, iron, triglycerides, C-reactive protein, and immunoglobulins (IgG, IgM, IgA)

Urinalysis: predose, D5, 34, 41, 85, 119, 162, 216. Endpoints were: volume, specific gravity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood pigments, and microscopic examination (epithelial cells, crystals, white blood cells, red blood cells, organisms, casts, other abnormalities).

Gross pathology: external, internal including orifices (ears, nostrils, mouth, anus, vulva), cranial, thoracic, and abdominal organs and tissues.

Organ weights (paired organs weighed separately): adrenal, brain, heart, kidney, liver, lung, ovary, pancreas, pituitary, prostate, spleen, testis with epididymis, thymus, thyroid, parathyroid, uterus with cervix.

Histopathology: Adequate Battery: Yes.

Note:

- Spinal cord samples were collected but not analyzed.
- Gall bladder and nasal turbinates were not collected or analyzed.
- Although the lungs were weighed and evaluated, the report does not explicitly list bronchi.

Peer review (yes/no): Unclear. The report was peer reviewed, but the report does not explicitly document peer review of the histology evaluation.

List of tissues: Abnormal tissue, adrenal, aortic arch, brain, eye, gall bladder, gastrointestinal tract (stomach, duodenum, ileum, jejunum, cecum, colon, rectum), heart, injection site, kidney, liver, lung, lymph node (mesenteric, submandibular), esophagus, optic nerve, ovary, pancreas, salivary gland (parotid, submaxillary), Peyer's patches, pituitary, prostate, sciatic nerve, seminal vesicle, skeletal muscle, skin, mammary gland, spleen, sternum including bone marrow, testes, thymus, thyroid and parathyroid, tongue, trachea, urinary bladder, uterus with cervix, vagina.

Toxicokinetics: Blood collected predose, D1 (predose, immediately after infusion, at 0.5, 1.5, 3, 6, and 12 hours postdose), D2 (24 hours after initial dose), D3 (48 hours after initial dose), D4 (72 hours after initial dose), D6 (120 hours after initial dose), D8 and D15 (predose and 1 hour after initiation of infusion), D22 (predose, immediately after infusion, 0.5, 1.5, 3, 6 and 12 hours postdose), D23, 24, 25, 6, 28, 30, 32 and from recovery animals only on D40, 50, 57, 64, 71, 78, 107, 140, 198, and 217

Measurement of anti-product antibodies: Blood collected predose, D29, 35 and from recovery animals only on D40, 70, 100, 130, 160, 190 and 217.

Blood collection for flow cytometry: Blood collected predose, prior to dosing on D1, 8, 15, 22; on D29, 35; and from recovery animals on D50, 70, 84, 112, 133, 149, 182, and at necropsy (D217). Samples evaluated by flow cytometry for CD markers (CD3, CD4, CD8, CD16, CD20, CD21, CD22, CD23, CD40), ofatumumab, and human immunoglobulin (Hulg, another detection method for ofatumumab).

Lymph node biopsy: all animals predose; recovery animals on D30, 58, 84, 112, 149, 182 and at necropsy (D217). Taken from the inguinal and axillary nodes under anesthesia, with pre and post-surgical analgesia. Samples evaluated by flow cytometry for CD markers (CD3, CD4, CD8, CD16, CD20, CD21, CD22, CD23, CD40) and Hulg.

Immunohistochemistry: As an exploratory endpoint, tissues samples were collected at necropsy from the thymus, spleen, stomach (body and pylorus), pharynx, parotid salivary gland, tonsil, tongue, thyroid, skin and mammary, cerebrum, liver, and ovary were collected for immunohistochemistry. Duplicate sections were stained to determine the total number of CD20⁺ B-cells, and also the number of B-cells binding ofatumumab.

Assessment for cellular immune function (delayed-type hypersensitivity):

- Main-group animals were immunized intradermally on D10, 13, and 16 with a combination of 4 antigens (Diphtheria toxoid, Tetanus toxic, *Trichophyton*, *Candida albicans*) in saline. Main-group animals were challenged on D30 with the 4 antigen combination.
- Recovery animals were challenged with the 4 antigen combination on D160, 163, and 166, and challenged on D180.
- Responses to antigens were assessed 24, 48, and 72 hours after each administration descriptively: lesion location, induration size, color, appearance.

Assessment for humoral immune function:

- Main-group animals were immunized by subcutaneous injection of KLH on D4 (0.5 mg of KLH) and D18 (0.3 mg of KLH). Blood samples were collected predose, prior to KLH administration on D4, D7, prior to KLH administration on D18, D21, D30, D35.
- Recovery group animals were immunized by subcutaneous injection of KLH on D32 (0.5 mg of KLH) and on D46, 120, and 214 (0.3 mg of KLH). Blood samples were collected predose, D32, 35, 46, 49, 120, 123, 214, and necropsy (D217).

Results**Mortality:** No unscheduled mortality.**Clinical signs:** No apparent treatment-related effects.**Note:** Signs of menstruation were observed for 2/5 control females (#18, #19), 1/6 low-dose females (#25), and 3/6 high-dose females (#29, 30, 31). These findings are noted as part of the assessment of the usefulness of this study to evaluate potential reproductive toxicity.**Body weights:** No apparent treatment-related effects.**Food consumption:** Not evaluated.**Ophthalmoscopy:** No apparent treatment-related effects.**EKG:** No apparent treatment-related effects.**Body temperature:** No apparent treatment-related effects.**Hematology:** No apparent treatment-related effects.**Clinical chemistry:** No apparent treatment-related effects.**Urinalysis:** No apparent treatment-related effects.**Gross pathology:** No apparent treatment-related effects. The authors attributed observations of skin thickening and redness in some recovery animals to KLH administration, and this reviewer agrees that the findings do not appear related to ofatumumab-treatment.**Table #8. Selected gross findings from the one-month study (#23542):**

Effect	Males			Females		
	0	20 mg/kg	100 mg/kg	0	20 mg/kg	100 mg/kg
Main Group						
Lung	Spongy	0/3	0/3	0/3	1/3	0/3
Lymph node, pancreatic	Reddened	0/3	1/3	0/3	0/3	1/3
Recovery						
Lung	Spongy	0/2	2/3	0/3	0/3	1/3
Lymph node, pancreatic	Reddened	0/2	0/3	1/3	0/3	0/3
Spleen	Pale focus	0/2	1/3	1/3	0/2	0/3

Organ weights: No apparent treatment-related effects.

Histopathology:

- Main-group animals at both doses (20 and 100 mg/kg) exhibited lymphoid organ atrophy (germinal centers and/or follicular atrophy) – spleen, mandibular and mesenteric lymph nodes, Peyer’s patches from the ileum at both doses (20 and 100 mg/kg).
 - Partial recovery – germinal center atrophy observed in the spleen of one low-dose female (#24F) and one high-dose female (#29F).
 - Lymph nodes and Peyer’s patches fully recovered.
- Lung effects: the increased incidences of immune-cell related effects (macrophage accumulation, inflammatory cell infiltration, agonal congestion) is considered an indirect effect of treatment, related to B-cell depletion and increased susceptibility to infection.
- One main-group mid-dose male (#9M) exhibited renal pathology (apparently unrelated to treatment): both kidneys stained markedly positive for fat (oil red O stain) and had remarkable levels of tubular renal fat. The authors note that tubular vacuolation was not apparent.

Table #9. Selected histological findings from the one-month study (#23542):

Effect		Males			Females		
		0	20 mg/kg	100 mg/kg	0	20 mg/kg	100 mg/kg
Main Group							
Lung	Alveolar macrophage accumulation, focal	0/3	0/3	1/3	0/3	0/3	0/3
	Inflammatory cell infiltration, interstitial, focal	0/3	1/3	0/3	0/3	1/3	2/3
	Inflammatory cell foci	0/3	0/3	0/3	0/3	0/3	1/3
	Congestion, agonal	0/3	0/3	0/3	0/3	1/3	0/3
Spleen	Follicular atrophy Mild	0/3	0/3	0/3	0/3	0/3	2/3
	Moderate	0/3	0/3	1/3	0/3	1/3	0/3
	Germinal center atrophy Minimal	0/3	1/3	0/3	0/3	0/3	1/3
	Mild	0/3	0/3	2/3	0/3	2/3	0/3
Lymph node (mandibular)	Germinal center atrophy						
	Minimal	0/3	1/3	2/3	0/3	2/3	3/3
	Mild	0/3	0/3	1/3	0/3	1/3	0/3
Lymph node (mesenteric)	Germinal center atrophy						
	Minimal	0/3	1/3	1/3	1/3	1/3	0/3
	Mild	0/3	0/3	0/3	0/3	2/3	2/3
Peyer's patches	Moderate	0/3	0/3	1/3	0/3	0/3	0/3
	Atrophy, minimal	0/3	1/2	1/2	0/2	0/3	0/2
	Germinal center atrophy, minimal	0/3	0/2	1/2	0/2	1/3	1/2
Brain	Arteritis / periarteritis, focal	0/3	0/3	0/3	0/3	0/3	1/3
	Inflammatory cell infiltration, perivascular	0/3	0/3	0/3	0/3	0/3	2/3
	Inflammatory cell infiltration, meningeal, focal	0/3	0/3	0/3	1/3	0/3	0/3
Recovery							
Lung	Inflammatory cell infiltration, interstitial, focal	0/2	0/3	1/3	1/2	0/3	0/3
	Hemorrhage, agonal	0/2	0/3	0/3	0/2	1/3	0/3
	Congestion, agonal	0/2	2/3	0/3	0/2	0/3	1/3
Spleen	Germinal center atrophy - minimal	0/2	0/2	0/2	0/2	1/3	1/3
	Lymphoid follicular hyperplasia	0/2	1/3	0/3	0/2	1/3	0/3

NOTE: Regarding reproductive organ gross and histological pathology: All males had immature testis. One recovery female (#25, low-dose) had histological evidence of uterine menstruation.

Toxicokinetics:

- On D1:
 - T_{max} ranged from 1.3 hours to 2.8 hours
 - Mean $T_{1/2}$ ranged from 68 to 320 hours
 - Mean V_d ranged from 30 to 58 ml/kg
 - Mean V_{ss} ranged from 33 to 57 ml/kg
- On D22:
 - T_{max} ranged from 0.75 hours to 2.8 hours
 - Mean $T_{1/2}$ ranged from 17 to 295 hours
 - Mean V_d ranged from 18 to 90 ml/kg
 - Mean V_{ss} ranged from 19 to 57 ml/kg
- Comparing D1 and D22:
 - AUC and mean C_{max} increased for both dose groups and both sexes, indicating that weekly dosing resulted in accumulation of ofatumumab in the monkeys.
 - Clearance was faster in animals positive for anti-drug antibodies (3/12 low-dose animals)
- The authors conclude, based on the V_d and V_{ss} data, that the majority of ofatumumab was retained in the plasma; only a small amount appeared to distribute beyond circulation. For context, the authors report that the extracellular fluid volume in monkeys is 208 ml/kg.
- No sex-related differences in ofatumumab TK were apparent.

- From the study report (pp.469-472):

Table A: Geometric Mean (CV%) Parameter Estimates Indicative of Systemic Exposure to HuMax-CD20 Following I.v. Infusion on Day 1 in Male and Female Cynomolgus Monkeys

Sex	Group	Dose (mg/kg/week) [Ratio]	Part	AUC(0-∞) (µg.h/mL) (CV%) [Ratio]	AUC(0-t) (µg.h/mL) (CV%) [Ratio]	AUC(0-168 h) (µg.h/mL) (CV%) [Ratio]	C _{max} (obs) (µg/mL) (CV%) [Ratio]
Male	2	20 [1.0]	Main & Recovery	116657 (91.3) ⁿ⁼³ [1.0]	38110 (51.5) [1.0]	46143 (20.6) [1.0]	791 (21.8) [1.0]
	3	100 [5.0]	Main & Recovery	324771 (26.4) ⁿ⁼³ [2.9]	215517 (52.1) ⁿ⁼⁴ [5.7]	264179 (25.7) ⁿ⁼⁴ [5.7]	4702 (33.3) ⁿ⁼⁴ [5.9]
Female	2	20 [1.0]	Main & Recovery	55707 (27.6) ⁿ⁼³ [1.0]	46782 (16.4) [1.0]	46782 (16.4) [1.0]	604 (30.9) [1.0]
	3	100 [5.0]	Main & Recovery	470349 (79.5) ⁿ⁼⁴ [8.4]	279066 (53.2) [6.0]	302881 (36.4) [6.5]	5570 (11.9) [9.2]

n=6 for Day 1 (Main & Recovery) unless otherwise specified

Table B: Arithmetic Mean (SD) Parameter Estimates Relating to HuMax-CD20 Following I.v. Infusion on Day 1 in Male and Female Cynomolgus Monkeys

Sex	Group	Dose (mg/kg/week)	Part	T _{max} (obs) ^a (h) (Range)	T _{1/2} (h) (SD)	CL (mL/h/kg) (SD)	V _d (mL/kg) (SD)	V _{ss} (mL/kg) (SD)
Male	2	20	Main & Recovery	2.75	320.42 (322.92) ⁿ⁼³	0.2034 (0.1210) ⁿ⁼³	57.93 (11.75) ⁿ⁼³	57.42 (9.573) ⁿ⁼³
	3	100	Main & Recovery	1.25	97.10 (47.55) ⁿ⁼³	0.3147 (0.07798) ⁿ⁼³	40.54 (9.298) ⁿ⁼³	38.63 (7.989) ⁿ⁼³
Female	2	20	Main & Recovery	2.00	68.33 (73.74) ⁿ⁼³	0.3674 (0.09082) ⁿ⁼³	29.78 (24.57) ⁿ⁼³	32.90 (11.20) ⁿ⁼³
	3	100	Main & Recovery	2.00	193.71 (188.97) ⁿ⁼³	0.2530 (0.1672) ⁿ⁼³	43.82 (16.74) ⁿ⁼³	39.51 (14.03) ⁿ⁼³

^a Median (range)
n=6 for Day 1 (Main & Recovery) unless otherwise specified

b(4)

Table C: Geometric Mean (CV%) Parameter Estimates Indicative of Systemic Exposure to HuMax-CD20 Following I.v. Infusion on Days 1 and 22 in Male Cynomolgus Monkeys

Group	Dose (mg/kg/week)	Day	Part	AUC(0-∞) (µg.h/mL) (CV%)	AUC(0-t) (µg.h/mL) (CV%)	AUC(0-168 h) (µg.h/mL) (CV%)	C _{max} (obs) (µg/mL) (CV%)
2	20	1	Main & Recovery	116657 (91.3) ⁿ⁼³	38110 (51.5)	46143 (20.6)	791 (21.8)
			Main	11826 (929.3)	11005 (765.7)	9742 (477.5)	1031 (42.1) ⁿ⁼⁴
		22	Recovery	336406 (11.9) ⁿ⁼²	79852 (2215.7)	35837 (481.4)	
			Main & Recovery	324771 (26.4) ⁿ⁼³	215517 (52.1) ⁿ⁼⁴	264179 (25.7) ⁿ⁼⁴	4702 (33.3) ⁿ⁼⁴
3	100	1	Main & Recovery	145530 (-) ⁿ⁼¹	401489 (108.0)	325672 (80.7)	10595 (33.0) ⁿ⁼⁴
			Main				
		22	Recovery	1754363 (39.6)	1753889 (39.7)	551528 (38.1)	
			Main & Recovery				

n=6 for Day 1 (Main & Recovery), n=3 for Day 22 (both Main and Recovery animals) unless otherwise specified

Table D: Geometric Mean (CV%) Parameter Estimates Indicative of Systemic Exposure to HuMax-CD20 Following I.v. Infusion on Days 1 and 22 in Female Cynomolgus Monkeys

Group	Dose (mg/kg/week)	Day	Part	AUC(0-∞) (µg.h/mL) (CV%)	AUC(0-t) (µg.h/mL) (CV%)	AUC(0-168 h) (µg.h/mL) (CV%)	C _{max} (obs) (µg/mL) (CV%)
2	20	1	Main & Recovery	55707 (27.6) ⁿ⁼³	46782 (16.4)	46782 (16.4)	604 (30.9)
			Main	162666 (21.9) ⁿ⁼⁴	105153 (5.7)	66306 (2.5)	1246 (28.8) ⁿ⁼⁴
		22	Recovery	249205 (80.8)	247651 (80.9)	100669 (25.8)	
			Main & Recovery	470349 (79.5) ⁿ⁼⁴	279066 (53.2)	302881 (36.4)	5570 (11.9)
3	100	1	Main & Recovery	989763 (29.6)	747248 (27.2)	562856 (27.4)	11535 (22.9) ⁿ⁼⁴
			Main				
		22	Recovery	2206818 (25.8)	2172502 (27.6)	755283 (9.3)	
			Main & Recovery				

n=6 for Day 1 (Main & Recovery), n=3 for Day 22 (both Main and Recovery animals) unless otherwise specified

Table E: Arithmetic Mean (SD) Parameter Estimates Relating to HuMax-CD20 Following I.v. Infusion on Days 1 and 22 In Male Cynomolgus Monkeys

Group	Dose (mg/kg/week)	Day	Part	T _{max(obs)} ^a (h) (Range)	T _{1/2el} (h) (SD)	CL (mL/h/kg) (SD)	V _d (mL/kg) (SD)	V _{ss} (mL/kg) (SD)
2	20	1	Main & Recovery	2.75	320.42 (322.92) ⁿ⁼³	0.2034 (0.1219) ⁿ⁼³	57.93 (11.75) ⁿ⁼³	57.42 (9.573) ⁿ⁼³
			Main	0.75	37.84 (52.00)	3.953 (3.345)	62.45 (62.89)	23.94 (8.738)
		Recovery	207.27 (1.96) ⁿ⁼²		0.1992 (0.006346)	59.57 (1.335)	22.68 (1.953)	
		22	Main & Recovery	1.25	97.10 (47.55) ⁿ⁼³	0.3147 (0.07798) ⁿ⁼³	40.54 (9.296) ⁿ⁼³	38.63 (7.989) ⁿ⁼³
Main	2.00		17.89 (-) ⁿ⁼¹	0.3676 (0.2824)	17.59 (-) ⁿ⁼¹	27.84 (-) ⁿ⁼¹		
				Recovery	241.27 (119.15)	0.1897 (0.06975)	64.26 (35.39)	25.35 (6.122)

^a Median (range)
n=6 for Day 1 (Main & Recovery), n=3 for Day 22 (both Main and Recovery animals) unless otherwise specified

b(4)

Table F: Arithmetic Mean (SD) Parameter Estimates Relating to HuMax-CD20 Following I.v. Infusion on Days 1 and 22 In Female Cynomolgus Monkeys

Group	Dose (mg/kg/week)	Day	Part	T _{max(obs)} ^a (h) (Range)	T _{1/2el} (h) (SD)	CL (mL/h/kg) (SD)	V _d (mL/kg) (SD)	V _{ss} (mL/kg) (SD)
2	20	1	Main & Recovery	2.00	66.33 (73.74) ⁿ⁼³	0.3674 (0.09082) ⁿ⁼³	29.78 (24.57) ⁿ⁼³	32.90 (11.20) ⁿ⁼³
			Main	2.75	208.44 (68.21) ⁿ⁼²	0.3017 (0.007578)	89.88 (25.90) ⁿ⁼²	36.98 (2.928) ⁿ⁼²
		Recovery	213.08 (73.18)		0.2031 (0.05451)	62.30 (24.58)	24.14 (8.332)	
		22	Main & Recovery	2.00	193.71 (186.97) ⁿ⁼⁴	0.2530 (0.1672) ⁿ⁼⁴	43.82 (16.74) ⁿ⁼⁴	39.51 (14.03) ⁿ⁼⁴
Main	1.00		163.01 (50.18)	0.1822 (0.06213)	42.13 (13.26)	22.12 (6.379)		
				Recovery	294.70 (62.38)	0.1328 (0.01250)	56.68 (12.42)	18.03 (3.424)

^a Median (range)
n=6 for Day 1 (Main & Recovery), n=3 for Day 22 (both Main and Recovery respectively) unless otherwise specified

b(4)

Anti-product antibody formation:

- The authors reported positive results in 0/10 controls, 3/12 animals at 20 mg/kg and 2/12 animals at 100 mg/kg.
- The authors did specify the criteria for a positive response. The validation for the ELISA method was documented in report #23372 (provided in m4.2.2.1). The authors of the validation report recommended considering a titer greater than 1:1200 or 1:600 as a positive response.
- Using 1:1200 as the threshold, the authors' reported numbers are accurate: 3/12 low-dose (males #8, 9, 11) and 2/12 high-dose (male #17, female #34).
- However, using 1:600 as the threshold, the number of positive responses were: 1/10 controls (male #2), 4/12 low-dose animals (males #6, 8, 9, 11), and 3/12 high-dose animals (males #13 and 17, female #34).

Blood flow cytometry:

- This study did not evaluate the rapidity of B-cell depletion; the first post-dose measurement was D8 (before the second dose). Blood samples were obtained prior to each dose (D1, 8, 15, 22), D29, D35 (main group sacrifice), and in recovery animals on D50, 70, 84, 112, 133, 149, 182, and 217.
- B-cell depletion:
 - At D8, mean CD20⁺ cell counts for both groups were 6-8% of pre-dose levels.
 - For both dose levels, males appeared to recover faster than females (excepting PAHA+ animals).
 - For the low-dose males, initial B-cell recovery was evident at D50 and full recovery was apparent by D112.
 - For low-dose females, initial B-cell recovery was evident at D70 and full recovery was apparent by D133.
 - For high-dose males, recovery was not apparent at D84, and full recovery was apparent between D112 and 133.
 - For high-dose females, recovery was not apparent at D112 and full recovery was apparent by D149.
 - Two low-dose males (#9 and #11) exhibited PAHA and showed B-cell recovery on D22.
 - In addition to CD20, other B-cell markers analyzed were CD21, CD40, and CD23. These other markers gave similar responses to CD20.
- No apparent treatment-related effect on T-cell counts (CD3, CD4, CD8 markers).
- The authors concluded that no effects on NK cell counts (CD3⁺CD16⁺CD8⁺) were apparent, but noted that some animals exhibited marked variations in NK cell counts over time (> 10-fold). Overall, no pattern is apparent.

Lymph node flow cytometry:

- Treatment-related B-cell depletion was observed, and was consistent with the effects seen in blood.
- NK-cell counts in lymph nodes were low; no treatment-related effect was apparent, but the data were of limited usefulness for assessing potential NK-cell effects.

Immunohistochemistry

- *Ex vivo* treatment of lymphoid tissue in lymphoid organs (spleen, tonsil, lymphoid follicles) in tissues from control and treated animals with ofatumumab resulted in specific staining.
- Authors reported that staining was lower in tissues from treated main-group animals but not treated recovery animals, suggesting that ofatumumab from the *in vivo* phase remained bound to cell membrane-bound CD20 at D35.

Assessment of cellular immune function:

- No treatment-related effects were apparent, but the results were inconsistent within groups, indicating that the assay may not be robust enough to detect treatment-related effects.
- Effects ranged from “no abnormality detected” to 10 mm weal with redness (reported as a very mild reaction) in controls and treated animals.

Assessment of humoral immune function:

- Inhibition of specific KLH antibodies was not observed for low-dose animals, but was observed in all high-dose animals.
 - In main-group animals (injected on D4 and D18), inhibition was most severe at the D18, 21 and 30 time points.
 - For recovery animals (injected on D32, 46, 120, and 214), inhibition was apparent at D35, 46, and 49; animals appeared to have recovered by D120.

2.6.6.3.5

Study title: Cycled repeat dose toxicity study in cynomolgus monkeys with a 4 month recovery period.

Key study findings: Groups of 3/sex/dose cynomolgus monkeys were dosed with 0, 20, or 100 mg/kg of ofatumumab by intravenous infusion. They received a total of 4 doses. Each cycle consisted for 2 doses given 2 weeks apart. The second cycle was administered after partial recovery of circulating B-cells.

- Overall, results were consistent with other studies.
- PAHA detected in 15/24 treated animals
- Coombs test positive (indicating binding of IgG or complement to RBCs) was detected as early as D15; the response resolved by 104 days post-treatment.

Study no.: 28366. GSK # CD2008/01522/00. Test facility study # 509814.

Volume #, and page #: Submitted electronically to m4.2.3.2. File name “28366-report.pdf”. 824 pp.

Conducting laboratory and location: ()

b(4)

Date of study initiation: 12/21/2006

NOTE: This study was initiated after initiation of the chronic study (study #25052).

GLP compliance: Yes, compliant with the OECD principles of GLP.

QA report: yes (X)

Drug, lot #, and % purity: ofatumumab (HuMax-CD20), batch # PD06015.

- SDS PAGE reduced: 99.2% IgG as heavy and light chains
- SDS PAGE non-reduced: 96.4% IgG
- Gel permeation HPLC: 98.2% IgG monomer, 1.8% aggregate

Methods

Doses: 0, 20, or 100 mg/kg of ofatumumab on D1, 15, 148, and 162

Species/strain: Cynomolgus monkey (*Macaca fascicularis*) of Mauritian origin

Number/sex/group or time point (main study): 3/sex/dose

Route, formulation, volume, and infusion rate: Intravenous infusion over 30 minutes. Formulated in vehicle (30 mM sodium citrate, 100 mM sodium chloride, pH 6.5).

Satellite groups used for recovery: 3/sex/dose (4 month recovery period).

Age: males 15 to 20 months, females 15 to 23 months

Weight: males 2.1 to 2.3 kg, females 1.9 to 2.2 kg

Unique study design or methodology (if any):

- The objective of this study was to investigate the toxicities observed in the 7-month study.
- Monkeys received two cycles of iv ofatumumab. Each cycle consisted of two iv doses given 14 days apart.
- The protocol specified initiation of the second cycle when at least 75% of treated animals recovered B lymphocyte counts above 50% of their individual predose baseline values.
- The protocol specified sacrifice of the animals when at least 75% of treated animals recovered B lymphocyte counts above 50% of their individual predose baseline values.

Standard endpoints: mortality, clinical signs, body weight, food consumption, ophthalmoscopy, EKG, hematology, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology. TK, PAHA.

Additional endpoints:

- Flow cytometry of whole blood samples.
 - Samples collected predose, prior to dosing on D1 and 15, D45, 73, females only on D86, males only on D94, D113, and prior to dosing on D148 and 162.
 - Markers included CD3, CD4, CD8, CD16, CD20, CD21, CD27, and Hulg
- Lymph node biopsy for flow cytometry and immunohistochemistry
 - From the superficial lymph nodes (inguinal and axillary)
 - Predose, main-group animals on D45, 73, and 122, recovery animals on D46, 74, 123, 192, 221, 252 and 282 (necropsy)
- Direct Coombs test
 - Measured predose, D1, 15, 45, 60, 73, 94, 113, 148, 162, and for recovery animals only on D191, 221, 252, 267, 274, 281
 - Assay for anti-IgG, anti-IgM, anti-IgA, anti-C3c, and anti-C3d
 - A positive result, agglutination of red blood cells, is considered indicative of coating of red blood cells by either immunoglobulin or complement.
- Assessment of humoral function
 - Main-group:
 - Immunization with KLH on D149 and 163.
 - Blood samples collected predose, D152, 166 and 168
 - Recovery animals:
 - Immunization on D250 and 264
 - Blood samples collected D172, 253, 264, 270, 274
- Immunohistochemistry (mesenteric and mandibular lymph nodes, Peyer's patches, spleen, tonsil).

Results

Results were generally consistent with the 7-month study. Only selected endpoints are noted below:

Mortality: No unscheduled mortalities.

Clinical signs:

- During the second cycle of dosing (D148 and 162), signs of infusion reactions were detected:
 - #8M (low-dose): vomited on D148 during dosing
 - #17M (high-dose): after dosing on D162, was “hot to the touch” on D163 and exhibited moderate (D163) and mild (D164-165) bruising and swelling of the ankles and legs.
 - #32F (high-dose): on D148, exhibited irregular breathing during dosing
 - #35F (high-dose): on D148, exhibited increased heart rate 4 minutes after dosing
- Evidence of menstruation was observed in 4/6 controls (#21, 22, 23, 24), 2/6 low-dose females (#28, 29) and 4/6 high-dose females (#32, 34, 35, 36).

Hematology:

- Decreased lymphocyte counts, consistent with expected pharmacology
- Signs of anemia (increased reticulocyte counts; decreased red blood cell counts, hemoglobin, and hematocrit)

Clinical chemistry:

- Increased iron concentration for high-dose females on D30 and 45
- Increased total bilirubin on D45 and 60 for high-dose females

Flow cytometry:

- After the first cycle (dosing on D1 and 15), B-cell depletion was observed with initial signs of recovery from D45 to D113, and full recovery between D94 and D148.
- After the second cycle (dosing on D148 and 162), B-cell depletion was observed, recovery was variable and appears to have been affected by PAHA.

Lymph node biopsy: flow cytometry and immunohistochemistry results reportedly consistent with other studies (full review not documented in this review)

PAHA:

- PAHA detected in 15/24 treated animals
- At 20 mg/kg:
 - 5/6 males and 4/6 females
 - 1/6 positive on D15 onward
 - 9/12 positive from D30 onward
- At 100 mg/kg:
 - 4/6 males and 2/6 females

- No PAHA detected on D15 or 30
- 3 positive PAHA responses detected on D60
- 6/12 positive PAHA responses detected from D113 onward
-

Necropsy: gross pathology, organ weights, histopathology, and immunohistochemistry were reportedly consistent with the other studies (full review not documented in this review)

Direct Coombs test

- Of the endpoints measured, positive responses were only detected for IgG, IgM, and C3d. The positive scores for IgM were transient (only D45-86) and were limited to high-dose females (3/6).
- For IgG and C3d, 4 animals tested positive pre-dose (1 control male, 1 low-dose female, 2 high-dose females)
- Of animals not testing positive pre-dose, responses for IgG and C3d:
 - 0/11 controls tested positive
 - 4/11 low-dose animals (1 male, 3 females) tested positive.
 - The 1 male (#10) beginning on D113
 - The 3 females beginning on D45
 - 4/6 high-dose males and 4/4 high-dose females tested positive
 - First male (#15) beginning on D45
 - The first female (#31) beginning on D15, 3/4 females by D45, all females by D86.
- The study authors noted that the incidence of direct Coombs test positive results was higher among females than males. However, the difference is not of sufficient magnitude to suggest a gender difference, considering the small group sizes and lack of statistical significance.
- Full recovery by D267 (i.e. no responses on D267, 274, 281).

Assessment of humoral function:

- No treatment-related effect on anti-KLH IgM antibodies.
- For the high-dose group, treatment was associated with a 4-to-6 fold decrease in the titer of anti-KLH IgG antibodies after both cycles.
- Authors conclude the T-cell dependent antigen response was affected by treatment.
- Authors conclude that the primary humoral immune function response (mediated via IgM) was not affected by treatment.

2.6.6.3.6

Study title: HuMax-CD20: multiple dose intravenous toxicity study in cynomolgus monkeys followed by a 7 month recovery period.

Key study findings: In this pivotal chronic toxicity study, cynomolgus monkeys (3/sex/dose main group and 4 recovery animals/sex/dose) were treated by intravenous infusion with 0, 20, or 100 mg/kg of ofatumumab. Dosing was weekly x8 (50 days), followed by 5 monthly doses (140 days).

- B-cell depletion during dosing (to ~5 to 10% of baseline in peripheral blood and lymph nodes)
 - The low-dose appeared to exhibit maximal pharmacologic activity
 - The high-dose did not increase the intensity of any of the treatment-related responses, but did delay recovery (consistent with expected TK)
 - Low-dose recovery: to >80% of baseline by 84 days after cessation of treatment, rebound 140-163 days after dosing, full recovery 6 months after dosing
 - High-dose recovery: initial signs of recovery 92-112 days after cessation of dosing, rebound 140-160 days after dosing, full recovery 6 months after dosing.
- Death attributed to infection for 3/28 treated animals (10.7%); considered a secondary effect of treatment (i.e. increased susceptibility)
- Deaths attributed to hemolytic anemia for 2/28 treated animals (7%); this may be a monkey-specific response to treatment.
- Infusion reactions:
 - Not observed with the initial weekly dosing (x8); earlier onset at the high-dose (D78, the 1st monthly dose or D106, the 2nd monthly dose) compared to the low-dose (D134, the 3rd monthly dose)
 - Increased heart rate & increased heart force, as well as trembling (trembling in 3/28 treated animals) shortly after initiation of the 30-minute infusion, resolving within a few hours of the end of infusion
 - Attributed to immune response to the product (i.e. cynomolgus monkey responding to a foreign protein), rather than part of the pharmacological mechanism of action (i.e. anti-CD20)
- Anemia:
 - signs of anemia from D50 into recovery (decreased hemoglobin, red blood cell count, hematocrit and iron; increased reticulocyte count, total bilirubin, lactate dehydrogenase); recovery apparent by D372.
 - Direct Coombs test: treated (but not control) animals were positive (indicating binding of either Ig or complement products to RBCs); a subsequent ELISA indicated ofatumumab was binding directly to the RBC; recovery apparent
- Clinical chemistry:
 - Increased lactate dehydrogenase from D25 onward; recovery apparent.
 - Increased C-reactive protein; recovery apparent.
 - This finding may be an indicator of pharmacologic activity.

- This finding may also be an indicator of inflammation secondary to infection.
- Increased total bilirubin from D75 onward; recovery apparent
- Pathology:
 - lymphoid atrophy, lymphoid depletion, or follicular atrophy in the lymph nodes (submandibular and mesenteric), Peyer's patch (ileum), and spleen
 - Signs of inflammatory cell infiltration (considered a secondary effect related to increased susceptibility to infection)
- Immune suppression:
 - No effect on DCT
 - Treatment-related effect on response to keyhole limpet hemocyanin, indicating that ofatumumab blocked the IgG-dependent humoral response.
- TK:
 - Elimination $T_{1/2}$ was initially 4-6 days, increased to 11 to 15 days with repeated dosing.
 - Immunohistochemistry: verified ofatumumab binding to target tissues (lymph nodes, Peyer's patch, spleen, tonsil) at end of treatment and end of recovery
 - An ELISA detected ofatumumab binding to RBCs for 2/14 of the treated animals.
 - PAHA detected in only one animal; in the context of the other studies, this suggests that the PAHA data from this long term study are unreliable.
- Assessment of reproductive toxicity: No signs of toxicity. 3/14 treated females were sexually mature. All males were sexually immature.

Report no.: 25052. GSK # CD2008/01521/00. / Study # 665493.

Volume #, and page #: Submitted electronically to m4.2.3.2. File name "25052-report.pdf". 919 pp.

Conducting laboratory and location: /

b(4)

Date of study initiation: 1/10/2005.

GLP compliance: Yes, compliant with the OECD principles of GLP. The sponsor also provided a statement from the conducting laboratory, verifying compliance with 21CFR58; minor deviations were noted. These deviations are not expected to have affected the outcome or interpretation of the study.

QA report: yes (X)

Drug, lot #, and % purity: HuMax-CD20. Batch #s PD04021 (total of 825 vials received in three separate shipments).

- SDS PAGE reduced: 98.7% IgG as heavy and light chains
- SDS PAGE non-reduced: 94.5% IgG
- Gel permeation HPLC: 99.2% IgG monomer, 0.8% aggregate.

Vehicle: 30 mM sodium citrate / 100 mM sodium chloride

Methods

Doses:

- 0, 20, 100 mg/kg IV 30 minute infusion

- 8 weekly doses (on D1, D8, D15, D22, D29, D36, D50) followed by 5 monthly doses (on D78, D106, D134, D162, D190).

Species/strain: cynomolgus monkeys (*Macaca fascicularis*) from Mauritius (shipped from the /

b(4)

Number/sex/group or time point (main study): 3/sex/dose

Route, formulation, volume, and infusion rate: intravenous infusion over 30 minutes, via the tail vein or other appropriate vein. Total infusion volume was 10 ml/kg.

Satellite groups used for recovery: 4/sex/dose

Age: 12 to 21 months on arrival (12-22 months at start of treatment)
Weight: Males 2.0 – 2.5 kg at start of treatment
Females 1.8 to 2.3 kg at start of treatment

Observations and times:

Mortality, clinical signs: "regular intervals" during the day, including inspection for vomit, blood, and abnormal feces.

Body weights: Weekly; more frequently if weight loss was noted

Food consumption: Not evaluated separately from daily observations

Ophthalmoscopy: predose, during week 8 and week 23, for recovery animals only during week 38 and either week 52 or 53. 1% tropicamide was used as a mydriatic agent.

Animals were sedated if necessary.

EKG: Lead II trace was used to evaluate interval data (P-R, QRS, Q-T) and heart rate. Predose, 30 minutes and 24 hours after dosing on D1, 29, 50, 106, and 190.

Body temperature: measured predose but not routinely during the study. Body temperature results were reported for female #30, as part of the veterinary assessment prior to premature sacrifice of this animal.

Hematology: predose, D2, 25, 50, 76, 106, 150, 190; recovery animals only on D246, 302, 372. Endpoints were: hemoglobin, red blood cell count, hematocrit, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, reticulocytes, white cell counts, differential white blood cell count (neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unclassified cells), platelets, and mean platelet volume.

Coagulation parameters: blood collected with hematology collections. Endpoints were: prothrombin time, activated partial thromboplastin time, and fibrinogen.

NOTE: The report does not explicitly state whether blood samples for hematology and clinical chemistry were collected prior to dosing, on days where blood collection and dosing coincide (i.e. D50, 106, 190).

Clinical chemistry: blood collected with hematology collections. Endpoints were: urea, glucose, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase, sodium, chloride, total protein, albumin, globulin, A:G ratio, cholesterol, creatinine, total bilirubin, calcium, inorganic phosphate, iron, triglycerides, C-reactive protein, and immunoglobulins (IgG, IgM, IgA).

Urinalysis: predose, D2, 25, 50, 76, 106, 152, 190; recovery animals only on D246, 302, 370. Endpoints were: volume, specific gravity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood pigments, and microscopic examination (epithelial cells, crystals, white blood cells, red blood cells, organisms, casts, other abnormalities).

Gross pathology: external, internal including orifices (ears, nostrils, mouth, anus, vulva), cranial, thoracic, and abdominal organs and tissues.

Organ weights (paired organs weighed separately): adrenal, brain, heart, kidney, liver, lung, ovary, pancreas, pituitary, prostate, spleen, testis with epididymis, thymus, thyroid, parathyroid, uterus with cervix

Histopathology: Adequate Battery: Yes.

Note:

- Spinal cord samples were collected but not analyzed.
- Gall bladder and nasal turbinates were not collected or analyzed.
- Although the lungs were weighed and evaluated, the report does not explicitly list bronchi.

Peer review (yes/no): Yes

List of tissues: Abnormal tissue, adrenal, aortic arch, brain, eye, gall bladder, gastrointestinal tract (stomach, duodenum, ileum, jejunum, cecum, colon, rectum), heart, injection site, kidney, liver, lung, lymph node (mesenteric, submandibular), esophagus, optic nerve, ovary, pancreas, salivary gland (parotid, submaxillary), Peyer's patches, pituitary, prostate, sciatic nerve, seminal vesicle, skeletal muscle, skin, mammary gland, spleen, sternum including bone marrow, testes, thymus, thyroid and parathyroid, tongue, trachea, urinary bladder, uterus with cervix, vagina

Toxicokinetics: Blood collected

- D1 (predose, immediately postdose, 1.5 h, 6 h)
- D8, 15, 22, 29, 36, 43 (predose and immediately postdose)
- D50 (predose, immediately post dose, and 0.5 h, 1.5 h, 3 h, 6 h, 12 h)
 - D51 (24 h postdose from D50)
 - D52 (48 h postdose from D50)
 - D53 (72 h postdose from D50)
 - D55 (120 h postdose from D50)
 - D58 (192 h postdose from D50)
 - D65 (360 h postdose from D50)
- D78 (predose and immediately postdose)
- D134 (predose, immediately post dose, and 0.5 h, 1.5 h, 3 h, 6 h, 12 h)
 - D135 (24 h postdose from D50)
 - D136 (48 h postdose from D50)
 - D137 (72 h postdose from D50)
 - D139 (120 h postdose from D50)
 - D144 (240 h postdose from D50)
- D190 (predose, immediately post dose, and 0.5 h, 1.5 h, 3 h, 6 h, 12, 24 h)
- Recovery animals on D192, 193, 195, 200, 218, 232, 246, 274, 302, 330, 372

PAHA assessment: Blood collected prior to dosing on:

- Prior to first dose
- D15, 29, 43, 50, 64, 78, 106, 134, 162, 190
- From recovery animals only on D218, 246, 274, 302, 330, 372

Blood collection for flow cytometry:

- Predose, prior to dosing on D8, 15, 29; on D50, 57, 64; prior to dosing on D78, 106, 134, 162, 190.
- From recovery controls on D197, 223, 246, 274, 302, 330, 347, 372
- From recovery low-dose animals on D226, 353, 372
- From recovery high-dose animals on D224, 350, 372
- Flow cytometry markers (same as for the 4-week pivotal study and the lymph node biopsy):
 - CD3/CD4/CD8
 - CD3/CD16/CD8
 - CD20/CD21/CD27
 - HuIg/Humax-CD20/CD3

Lymph node biopsy: Collected from superficial (inguinal and axillary) lymph nodes under general anesthesia or at necropsy for evaluation by flow cytometry:

- Main group animals predose, D8, D192 (necropsy)
- Recovery animals predose, D8, once/animal on D223-225, 274, once/animal on D347-253 and D372 (necropsy)
- Biopsies from the inguinal and axillary lymph nodes.

Immunohistochemistry:

- The presence of CD20 antigen and CD20-bound ofatumumab were assayed.
 - Tissue samples were collected at necropsy from the lymph nodes (mesenteric and submandibular), Peyer's patches, thymus, spleen, and tonsil for immunohistochemistry.
 - Duplicate sections were stained to determine the total number of CD20⁺ B-cells, and also the number of B-cells binding ofatumumab.
- Spleen and thymus tissues from animal #33F (low-dose) and #40F (high-dose) were stained to evaluate immune complexes.
 - Evaluation of monkey Ig (using goat anti-monkey IgG, IgA, IgM)
 - Evaluation of bound ofatumumab.

Assessment for cellular immune function (delayed-type hypersensitivity):

- Main-group animals were immunized intradermally on D40, 43, and 46 with a combination of 4 antigens (Diphtheria toxoid, Tetanus toxoid, *Trichophyton*, *Candida albicans*) in saline. Main-group animals were challenged on D60 with the 4 antigen combination.
 - These dates are later than the 4-week study (immunized D10, 13, 16; challenge on D30)

- Recovery animals were challenged with the 4 antigen combination on D349, D352, D355 with challenge on D369
 - These dates are later than the 4-week study (immunized on D160, 163, and 166, and challenged on D180).
- Responses to antigens were assessed 24, 48, and 72 hours after each administration descriptively: lesion location, induration size, color, appearance.

Assessment for humoral immune function:

- Main-group animals were immunized by subcutaneous injection of KLH in FIA on D4 and D180. Blood samples were collected predose, D18, D180, and D183.
 - These dates are later than the 4-week study (dosed on D4 and D18; blood collected predose, prior to KLH on D4, D7, prior to KLH on D18, D21, D30, D35).
- Recovery group animals were immunized by subcutaneous injection of KLH on D32 (0.5 mg of KLH) and on D46, D109, D193, D274, D369. Blood collected predose, D46, D60, D109, D112, D193, D196, SD274, D277, D369, D372
 - These dates are later than the 4-week study (dosed on D46, 120, and 214; blood samples were collected predose, D32, 35, 46, 49, 120, 123, 214, and at necropsy on D217).

Direct Coombs test:

- Blood samples collected on D150, D175, and from recovery animals on D372.
- Blood samples from animals #7 (control) and #39 (high-dose) only on D122 and 148.
- Assay to detect antibodies that had coated RBC *in vivo*.

Antibody elution & ELISA test:

- To investigate the presence of ofatumumab and other antibodies on the cell surface of RBCs.
 - RBCs were washed
 - Cells were incubated at low pH, to dissociated antibody from antigen
 - Eluate was recovered, pH adjusted to 7.0, and analyzed by ELISA for ofatumumab
- Initially performed on 4 animals
 - Animals 4 and 25 (negative Coombs results) on D216
 - Animals #20 and 42 (positive Coombs) on D218
- Testing expanded to all surviving recovery animals:
 - Recovery controls on D223
 - Low-dose recovery animals on D226
 - High-dose recovery animals on D224

Campylobacter investigation:

- Serum samples collected to analyze for antibodies against *Campylobacter jejuni* using a commercially available immunoblot test kit.
- Samples collected from animals #7M (control), #30F (low-dose), #33F (low-

dose), #20M (high-dose) and #39F (high-dose).

Note: Bone marrow smears were collected at necropsy, but were not evaluated microscopically. This is a study limitation.

Results

Mortality: No control animals, 3 low-dose, and 2-high dose animals.

- 3 deaths attributed to infection (33F, 30F, 20M), which is indirectly attributed to treatment and is considered relevant to patient safety.
- 2 deaths attributed to hemolytic anemia (#40F, 32F), which is indirectly attributed to treatment; the relevance to humans is unclear. Neither animal showed B-cell recovery prior to death.
- #33F (low-dose) was found dead on D44 (a day after the 7th dose)
 - This reviewer attributes the death to infection, based on pathology findings (spongy lung, lung edema and hemorrhage, froth-filled trachea) and positive testing for *C. jejuni*.
 - This death is therefore considered an indirect effect of treatment (i.e. inhibition of plasma humoral immune response), and is considered relevant to human health.
 - No clinical signs prior to death
 - Study authors considered the histology to have developed terminally, and to not be directly treatment-related (splenic necrosis, perivascular edema in the lungs). Immunohistochemistry was performed on tissues (kidney, lung, spleen, liver, brain, skeletal muscle) to determine the presence of immune complexes; no immune complex deposition was detected.
- #30F (low-dose) on D64 (14 days after the 8th dose)
 - Based on histological evidence of infection (inflammation of the ileum and large intestines) and positive tests for *Campylobacter jejuni* (large intestine) and *Balantidium coli* (cecum and colon), the study authors attributed the cause of death to opportunistic infection.
 - Initial clinical signs on D20: sitting quiet in hunched in the cage in the morning, appeared bright and alert in the afternoon
 - After the 4th dose (D22), from D25 onward: intermittent quiet, hunched, liquid feces, low body temperature. Animal was given a 4-week recovery period, then redosed.
 - Signs on D64: slow, hunched, thin, liquid feces, weight loss. Euthanized on D64.
 - Flow cytometry data showed this animal had very low CD20⁺ cells on D64 compared to other treated animals.
- #40F (high-dose) on D139 (5 days after the 11th dose)
 - Based on hematology, pathology (acute vasculitis of the brain and liver, hematopoiesis in the spleen and liver), the study authors attributed the cause of death to severe anemia.
 - No concerning clinical signs prior to the day of sacrifice.
 - D139 signs: unsteady, progressing to semi-conscious state. Very pale, very

- heart rate, weak pulse, low body temperature, poorly responsive. Euthanized.
- Flow cytometry data showed this animal had CD20⁺ cell counts comparable to other treated animals on D134.
- Recovery #32F (low-dose) on D268
 - Based on the hematology data, the study authors attributed the cause of death to severe anemia.
 - No daily clinical signs indicating health issues.
 - D249: hematology results indicated deteriorating anemia.
 - Grade 2 systolic murmur noted.
 - Enlarged spleen, puffiness to lower eyelids
 - D267: confirmatory hematology blood work collected
 - Severe anemia, low platelets.
 - Fast heart rate,
 - Heart murmur 'pounding', breathing fast, pale gums
 - D268: Euthanasia based on hematology results.
 - Flow cytometry data showed this animal had CD20⁺ cell counts comparable to other recovery animals on D246; additionally, terminally-collected blood (D268) did not show signs of recovery.
- Recovery #20M (high-dose) on D276
 - The study authors attributed the cause of death to opportunistic infection.
 - D90 onward: intermittent dehydration (slight to severe) and liquid feces, suspected *Campylobacter* infection. Supported with fluids, dietary supplement, and medicines (Zithromax® [azithromycin], Zantac®).
 - D172: *Campylobacter* infection confirmed by rectal swab.
 - D276: Animal dehydrated, deteriorating. Euthanized on humane grounds.
 - Flow cytometry data showed this animal had CD20⁺ cell counts comparable to other treated animals on D134.

Clinical signs: The authors noted the clinical signs in the 5 premature decedents, and also noted "subjective signs" of increased heart force and heart rate among animals treated with ofatumumab from D134 (the 3rd monthly dose) onward for low-dose animals and D78 (the 1st monthly dose) or D106 (the 2nd monthly dose) onward for high-dose animals. The authors considered these signs incidental. This reviewer disagrees – the transient clinical signs of increased heart force, increased heart rate, and trembling are clearly treatment-related. The effects are detailed in table 10:

Table 10: Selected clinical observations from the 7-month study (#25052)

Dose group (mg/kg)	Animal #	Study day	Time (minutes)	Observation
20	12M	134	Predose	NAD
			DD + 5	Increased heart rate
			PD + 23	Slightly increased heart rate
			PD + 90	NAD
			134-137	
20	14M	134	Predose	NAD
			DD + 9	Increased heart rate
			PD + 8	NAD
20	31F	134	Predose	NAD
			DD + 1	Increased heart force and heart rate

20	35F	134	PD + 9	NAD		
			Predose	NAD		
			DD + 7	Increased heart force		
			PD + 8	Slightly increased heart force		
100	15M	106	PD + 86	NAD		
			Predose	NAD		
			DD + 11	Increased heart force and heart rate, trembling		
			PD + 30	Increased heart force and heart rate		
		134	PD + 376	NAD		
			Predose	NAD		
			DD + 3	Increased heart force		
			DD + 25	Increased heart force and heart rate		
		162	PD + 10	Increased heart force		
			PD + 86	NAD		
			Predose	NAD		
			DD + 9	Increased heart rate		
		190	PD + 3	Increased heart rate		
			PD + 134	Increased heart rate		
			PD + 254	NAD		
			Predose	NAD		
100	16M	106	DD + 4	Increased heart force		
			PD + 268	NAD		
			Predose	NAD		
			DD + 2	Increased heart force and heart rate		
		162	PD + 31	Increased heart force and heart rate		
			PD + 352	NAD		
			Predose	Liquid feces		
		106	DD + 12	Increased heart rate		
			PD + 128	NAD		
			Predose	NAD		
100	17M	106	DD + 6	Increased heart force and heart rate, trembling		
			PD + 29	Increased heart force and heart rate		
			PD + 371	NAD		
			Predose	NAD		
		134	DD + 3	Increased heart force and heart rate		
			DD + 23	NAD		
			Predose	NAD		
		162	DD + 29	Increased heart rate		
			PD + 135	NAD		
			Predose	NAD		
		100	18M	106	DD + 3	Increased heart force and heart rate
					DD + 4	Increased heart force and heart rate, trembling
					PD + 31	Increased heart force and heart rate
					PD + 360	NAD
134	Predose			NAD		
	DD + 1			Increased heart force and heart rate		
	PD + 13			Increased heart force and heart rate		
	PD + 82			NAD		
190	Predose			NAD		
	DD + 11			Slightly increased heart rate		
	PD + 14			Increased heart rate		
	PD + 82			NAD		
100	19M			106	Predose	NAD

			DD + 1	Increased heart force and heart rate
			PD + 29	Increased heart force and heart rate
			PD + 343	NAD
		134	Predose (1 minute prior)	Increased heart force and heart rate
			PD + 31	Increased heart rate
			PD + 80	NAD
		162	Predose	NAD
			DD + 2	Increased heart rate
			PD + 119	NAD
		190	Predose	NAD
			DD + 7	Increased heart force
			PD + 8	Increased heart force
			PD + 249	NAD
100	20M	106	Predose	Bright and active, slightly dehydrated
			DD + 1	Increased heart force and heart rate
			DD + 3	Increased heart force and heart rate, liquid feces
			PD + 29	Increased heart force and heart rate, liquid feces
			PD + 338	NAD
		162	PD + 115	Liquid feces
			PD + 223	Liquid feces, slightly dehydrated
		190	Predose	NAD
			PD + 1	Liquid feces
			PD + 331	Liquid feces
		276	Not dosed	Dehydrated, bright & active, pale gums, sitting quietly, premature sacrificed.
100	21M	106	Predose	NAD
			DD + 2	Increased heart force and heart rate
			PD + 29	Increased heart force and heart rate
			PD + 332	NAD
		134	Predose	NAD
			DD + 3	Increased heart force and heart rate
			DD + 15	Increased heart rate
			PD + 9	Increased heart rate
			PD + 76	NAD
		190	Predose	NAD
			DD + 2	Increased heart force
			PD + 8	Increased heart force
			PD + 243	NAD
100	36F	106	Predose	NAD
			DD + 1	Increased heart force and heart rate
			PD + 30	Increased heart force and heart rate
			PD + 325	NAD
		134	Predose	NAD
			DD + 2	Increased heart force and heart rate
			PD + 9	Increased heart force
			PD + 74	NAD
		162	Predose	NAD
			DD + 6	Increased heart rate
			PD + 107	NAD
		190	Predose	NAD

			DD + 19	Increased heart force
			PD + 13	Increased heart force
			PD + 232	NAD
100	37F	106	Predose	NAD
			DD + 1	Increased heart force and heart rate
			PD + 29	Increased heart force and heart rate
			PD + 319	NAD
		134	Predose	NAD
			DD + 2	Increased heart rate
			PD + 12	Increased heart rate
			PD + 72	NAD
		162	Predose	NAD
			DD + 13	Increased heart rate
			PD + 104	NAD
100	38F	106	Predose	NAD
			DD + 5	Increased heart force and heart rate
			PD + 35	Increased heart force and heart rate
			PD + 315	NAD
		162	Predose	NAD
			DD + 26	Increased heart rate
			PD + 99	NAD
100	39F	78	Predose	NAD
			DD + 6	Increased heart force and heart rate
			PD + 311	NAD
		106	Predose	NAD
			DD + 5	Increased heart rate
			PD + 28	NAD
		134	Predose	NAD
			DD + 5	Increased heart rate
			PD + 11	Increased heart rate
			PD + 244	NAD
		162	Predose	NAD
			DD + 26	Increased heart force
			PD + 8	Increased heart force
			PD + 223	NAD
100	40F	106	Predose	NAD
			DD + 5	Increased heart force and heart rate
			PD + 30	Increased heart force and heart rate
			PD + 306	NAD
		134	Predose	NAD
			DD + 1	Increased heart force and heart rate, pale gums
			PD + 30	Increased heart force and heart rate
			PD + 68	NAD
		139	Not dosed	AM: unsteady on return to cage PM: found on cage floor, sacrificed.
100	41F	106	Predose	NAD
			PD + 29	Increased heart force and heart rate
			PD + 299	NAD
100	42F	106	Predose	NAD
			DD + 1	Increased heart force and heart rate
			PD + 32	Increased heart force and heart rate
			PD + 297	NAD
		162	Predose	NAD

			DD + 6	Increased heart rate
			PD + 211	NAD

NAD: no abnormalities detected

DD: during dosing

PD: post dosing

NOTE: One animal, #19M (high-dose) exhibited increased heart force and heart rate prior to dosing (on D134); the other animals exhibited increased heart force and heart rate after initiation of dosing. Presumably the pre-dose clinical signs for #19M on D134 were not treatment-related.

NOTE: Clinical signs of menstruation⁵ were observed for:

- 3 controls
 - #24F (D64 only)
 - #25F (D76, 143-156, 329-334)
 - #29 (D147-152)
- 1 low-dose
 - #34F (D25-27, 57-60, 99-103, 144-146, 179-180, 240-245, 271-274, 333)
- 2 high-dose
 - #36 (D183-187, 369-372)
 - #37 (D174-178)

Body weights: Excepting premature-decedents (several of which lost weight), no treatment-related effects were apparent.

Food consumption: Not reported.

Ophthalmoscopy: No treatment-related effects were apparent.

EKG: No treatment-related effects were apparent at the time points measured. However, see clinical signs above regarding clinically-apparent cardiac effects.

Hematology:

- The study authors noted reduced hemoglobin (Hb), red blood cell counts (RBC), and hematocrit (Hct), as well as increased reticulocyte counts (reti) for 'the majority of treated animals' in both treatment-groups during treatment (D50 onward) and during recovery phase. Low-dose animals recovered by D372.
 - These effects indicate treatment-related anemia.
 - In the context of the Coombs data (reviewed below) and clinical chemistry data (increased total bilirubin, decreased iron), these data suggest that ofatumumab may induce a humoral immune response in monkeys, indirectly causing hemolytic anemia.

⁵ Noted as part of the evaluation of the sensitivity of this study to detect potential reproductive toxicity.

- Since the cynomolgus immune response to ofatumumab is not expected to be predictive of the human response to ofatumumab, the biological relevance of the observed anemia to patients is unclear.
- Additionally, treatment is associated with a transient decrease in lymphocyte counts (lymph), apparent on D2 and D25 and recovering thereafter despite continued treatment.
 - These effects were not noted by the study authors, but were noted in another nonclinical study (#28366).
 - Since ofatumumab depletes B lymphocytes, this effect is consistent with expected pharmacology.
- Sporadic, statistically significant differences occurred for PT, APTT, and other endpoints; no pattern was apparent and therefore this reviewer considers these changes to be incidental to treatment.

Table #11: Selected hematology group mean values from the 7-month study (#25052)

Effect		Males			Females		
		0	20 mg/kg	100 mg/kg	0	20 mg/kg	100 mg/kg
Hb	D50	13.1	12.6	12.4	13.0	16.6	12.3
	D76	13.5	12.3	12.3	13.1	12.5	12.5
	D106	13.8	13.0	12.0 ^a	13.4	12.7	12.8
	D150	12.6	11.9	10.8 ^a	12.6	12.0	11.0
	D190	13.4	12.2	11.6	13.9	12.0 ^a	12.3 ^a
	D246 (recovery)	13.3	12.5	10.3	13.8	9.3	12.2
	D302 (recovery)	12.9	13.9	11.6	13.9	12.8	13.6
	D372 (recovery)	13.9	13.2	12.7	13.4	13.6	13.8
RBC	D50	6.30	6.74	6.22	6.70	6.57	6.15
	D76	6.52	6.42	6.25	6.80	6.33	6.31
	D106	6.70	6.93	6.05 ^a	7.04	6.51	6.45
	D150	6.07	6.45	5.49	6.53	6.25	5.63
	D190	6.70	6.45	5.80	7.47	5.96 ^a	6.01 ^a
	D246 (recovery)	6.48	6.66	5.03	7.24	4.39 ^a	5.95
	D302 (recovery)	6.23	7.29 ^a	5.58	7.05	6.27	6.67
	D372 (recovery)	6.75	7.04	6.36	6.89	7.22	7.03
Hct	D50	0.439	0.454	0.413	0.447	0.441	0.417
	D76	0.456	0.424	0.419	0.452	0.432	0.441
	D106	0.458	0.456	0.401 ^{ab}	0.460	0.441	0.442
	D150	0.419	0.417	0.367 ^{ab}	0.426	0.420	0.384
	D190	0.467	0.433	0.390	0.494	0.417 ^a	0.413 ^{ab}
	D246 (recovery)	0.449	0.438	0.346	0.470	0.344	0.418
	D302 (recovery)	0.452	0.477	0.399	0.473	0.445	0.475
	D372 (recovery)	0.471	0.463	0.441	0.471	0.479	0.471
Reti	D50	0.8	0.8	1.4	0.7	1.2	1.4
	D76	0.5	1.0	1.5	0.7	1.9	1.6
	D106	0.5	0.8	1.5 ^{ab}	0.6	1.8	1.9

	D150	0.6	0.9	2.1**	0.6	1.9	2.3*
	D190	0.6	1.4	2.7*	0.6	3.1**	3.0**
	D246 (recovery)	0.8	1.1	4.7	0.6	11.2**	1.4
	D302 (recovery)	1.1	0.6	1.9	0.6	1.2	0.8
	D372 (recovery)	0.3	0.5	0.5	0.4	0.4	0.4
Lymph	Predose	3.43	3.14	3.01	2.24	2.80	2.75
	D2	4.11	2.37**	2.01**	2.95	2.07**	1.90***
	D25	5.15	2.26***	3.08*	3.44	2.47	3.20
	D50	3.69	3.06	3.73	3.42	2.99	3.75
	D76	3.32	3.25	3.05	3.01	2.70	2.71
	D106	3.40	3.44	4.64	2.67	2.86	2.77
	D150	7.15	2.67***	5.35	7.07	2.20	3.51***
	D190	4.18	3.21	4.38	2.93	2.83	3.57
	D246 (recovery)	5.19	3.23	4.23	3.87	2.98	3.42
	D302 (recovery)	5.49	6.17	4.37	4.46	5.24	4.47
	D372 (recovery)	8.47	6.85	8.26	8.97	7.49	5.46*

Means only presented.

*Statistically significant <0.05.

**Statistically significant <0.01.

***Statistically significant <0.001.

Clinical chemistry:

- Increased lactate dehydrogenase (LDHIL)
 - Both dose-groups from D25 onward; the effect appeared progressive during treatment and the intensity peaked during recovery.
 - Full recovery was apparent by the end of recovery
 - More pronounced in male groups compared to female groups.
 - Note: The increases in LDHIL may reflect anemia (i.e. damage to blood cells).
- Increased total bilirubin (T.bil)
 - Both dose groups on D76 and D150 only
 - Full recovery was apparent by the end of recovery
 - Note: The increases in bilirubin may be evidence of hemolytic anemia.
- Decreased serum iron (Fe)
 - Effect immediately apparent (on D2), followed by apparent recovery (D25-106), then another decrease. This pattern suggests different mechanisms of action.
 - Note: Decreased Fe may be evidence of anemia.
 - This effect was not noted by the study authors, but was detected by another nonclinical study with ofatumumab (#28366) and therefore closely examined for the animals of this particular study.

- Increased C-reactive protein (CRP)
 - Like the change in Fe, CRP levels changed immediately after treatment (D2), followed by apparent recovery, then another increase lasting through recovery. This pattern suggests different mechanisms of action.
 - Notes:
 - CRP is produced by the liver, and high CRP is an indicator of inflammation. Increased CRP has been associated with rheumatoid arthritis and lupus; notably, anti-CD20 therapies (such as ofatumumab) reportedly have activity in these indications. Although this reviewer would have expected, *a priori*, a decrease in CRP due to ofatumumab, ofatumumab effects on CRP may be pharmacological.
 - However, increased CRP is also associated with inflammation caused by infection; therefore, the CRP changes may be at least partially due to the indirect activity of ofatumumab on the immune system.
 - This increase was not noted by the study authors, but was noted as an apparently treatment-related effect in another nonclinical study with ofatumumab (study #22804).

Table #12: Selected hematology group mean values from the 7-month study (#25052)

Effect		Males			Females		
		0	20 mg/kg	100 mg/kg	0	20 mg/kg	100 mg/kg
LDHIL	Predose	634	522	476	378	403	441
	D2	520	574	593	653	449	627
	D25	371	445	555**	404	338	449
	D50	405	424	419	351	303	475
	D76	379	415	438	366	351	359
	D106	367	404	520	323	389	389
	D150	372	483	699**	334	494	414
	D190	388	439	724*	344	507	487
	D246 (recovery)	395	421	1251**	309	695	465
	D302 (recovery)	391	393	912	315	357	341
D372 (recovery)	284	320	341	323	312	276	
T.bil	D50	3.8	3.8	2.8	4.1	4.5	3.9
	D76	4.3	4.3	6.3	4.5	7.2	7.0
	D106	5.0	5.0	5.5	4.4	9.4	7.1
	D150	1.7	8.3**	3.9**	1.9	12.2**	3.3*
	D190	3.2	3.0	4.7	4.7	6.3	8.9
	D246 (recovery)	3.4	2.9	6.0	2.4	6.3*	4.9
	D302 (recovery)	1.3	1.7	3.1	2.5	3.3	2.5
	D372 (recovery)	1.8	1.9	1.6	2.0	3.2	3.1
Fe	Predose	19.6	21.2	18.9	14.0	17.9	19.3
	D2	18.2	11.7**	14.0*	13.6	10.7	13.4
	D25	18.8	16.2	17.9	16.6	14.0	17.6

	D50	20.8	17.1	20.1	20.0	16.7	21.7
	D76	17.1	21.8	16.3	14.2	17.7	10.9
	D106	25.5	22.7	24.8	24.3	16.3*	21.2
	D150	31.6	14.8***	30.3	36.7	17.1***	25.5*
	D190	24.1	19.3	28.5	19.7	25.1	22.8
	D246 (recovery)	29.8	15.5	33.7	29.6	44.0	24.6
	D302 (recovery)	23.0	28.3	21.7	17.7	29.9	22.5
	D372 (recovery)	35.0	26.5	25.0	40.2	38.8	29.0
CRP	Predose	0.8	0.2	0.4	1.0	0.3	0.9
	D2	0.5	1.3**	1.3**	1.6	1.0	2.1
	D25	1.2	0.2	0.8	0.4	0.5	1.5
	D50	0.2	0.3	0.3	0.3	0.4	0.5
	D76	0.2	0.3	0.6	0.2	0.3	0.7**
	D106	0.2	0.2	0.4	0.7	0.4	0.5
	D150	0.1	0.4*	0.3	0.2	0.9	0.4
	D190	0.2	0.5	0.3	0.4	0.7	0.5
	D246 (recovery)	0.4	0.5	0.7	0.3	2.6	0.3
	D302 (recovery)	0.3	0.3	0.9	0.2	0.7	0.2
	D372 (recovery)	0.2	1.1	2.3	0.3	0.2	0.2

Means only presented.

*Statistically significant <0.05.

**Statistically significant <0.01.

***Statistically significant <0.001.

Urinalysis: No treatment-related effects were apparent.

Gross pathology:

- The authors noted treatment-related effects in the premature decedents, but no other apparent treatment-related effects.
 - 33F (found dead D44)
 - Reddening of the skin (particularly ventral and dorsal thorax)
 - Reddening of internal organs, darkened adrenal gland
 - Spongy lungs, froth-filled trachea
 - 30F (premature sacrifice D64)
 - Poor general body condition
 - 40F (premature sacrifice D139)
 - Pallor
 - Enlarged spleen
 - Enlarged liver with prominent lobulation
 - Many pinpoint dark foci on the brain
 - Pale kidneys
 - 32F (premature sacrifice D268)
 - Enlarged spleen
 - Pale kidneys and intestine
 - Dark, enlarged adrenal glands

- 20M (premature sacrifice D276)
 - No remarkable gross pathology
- This reviewer considers the evidence of lung and trachea gross pathology to be indirect evidence of treatment-related effects (i.e. response to infection). The brain pathology observed in one treated animal is not clearly treatment-related, but may be indicative of response analogous to the encephalopathy observed in some patients treated with anti-CD20 therapy.

Table #13. Selected gross findings from the 7-month study (#25052):

Effect	Males			Females		
	0	20 mg/kg	100 mg/kg	0	20 mg/kg	100 mg/kg
Main Group						
Lung	Dark focus	0/3	0/3	1/3	0/3	0/3
	Spongy	0/3	0/3	1/3	0/3	0/3
	Pale focus	0/3	0/3	0/3	0/3	0/3
Recovery						
Lung	Spongy	0/3	0/3	0/3	0/3	2/3
	Adhesion(s)	0/3	0/3	1/3	0/3	1/3
Trachea	Froth filled	0/3	0/3	1/3	0/3	2/3
Brain	Dark focus	0/3	0/3	0/3	0/3	0/3

- Premature decedents:
 - 33F: gross evaluation findings included spongy lung, all lobes, froth filled trachea; histology detected moderate widespread perivascular edema and hemorrhage of the lung.
 - 32F: gross evaluation findings included spongy lung, all lobes; adhesions of the right lob (to the thoracic cavity), but no correlating lung histopathology; froth filled trachea.
 - 40F: gross evaluation findings included: many pinpoint dark foci on the brain; histology detected mild acute widespread vasculitis in the brain.
- Scheduled necropsies:
 - 16M (high-dose main group): gross evaluation findings included spongy lung, all lobes; many dark foci on all lobes of the lung (1x1 mm); histology detected agonal congestion / hemorrhage. This male was euthanized as scheduled (D192).
 - 21M (high-dose recovery group): gross evaluation findings included froth filled trachea.

Organ weights: No treatment-related effects were apparent.

Histopathology:

- Premature decedents (selected lesions):
 - 33F - lung edema and hemorrhage, spleen necrosis. The authors (report p 72) note that this animal exhibited ADA, but no evidence of immune complex deposition was observed.
 - 30F - gastrointestinal inflammation (ileum, cecum, colon, rectum)

- 40F - widespread vasculitis of the brain and liver
- 20M – interstitial nephritis
- Main-group animals:
 - Treatment caused minimal-to-moderate lymphoid atrophy, lymphoid depletion, or follicular atrophy in the lymph nodes (submandibular and mesenteric), Peyer’s patches (ileum), and spleen.
 - Signs of inflammatory cell infiltration:
 - Authors noted effects in kidneys, brain, sciatic nerve
 - This reviewer also notes effects in eye, lungs, trachea
 - It is unclear whether this is a direct effect of treatment, or an indirect effect (i.e. treatment may have increased susceptibility to infection,)
- Recovery-group animals:
 - Study authors report no changes attributed to treatment.
 - This reviewer notes evidence of rebound effects:
 - Signs of continued inflammatory cell infiltration (eye, brain), consistent with the hypothesis that this effect is indirect and due to increased susceptibility to infection
 - Rebound lymphoid cell growth in the spleen, Peyer’s patches, and sternum
- Note: All males exhibited immature testes. One female (high-dose) exhibited immature vagina.

Table #14: Selected histopathology findings from the 7-month study (#25052)

Effect		Males			Females		
		0	20 mg/kg	100 mg/kg	0	20 mg/kg	100 mg/kg
Main Group							
Lung	Focal alveolitis	0/3	0/3	1/3	0/3	1/2	0/3
	Inflammatory cell infiltration, interstitial, focal	0/3	1/3	0/3	0/3	0/2	0/3
	Inflammatory cell infiltration, peribronchial	0/3	0/3	0/3	0/3	1/2	0/3
	Agonal congestion / hemorrhage	0/3	0/3	1/3	0/3	0/2	0/3
Trachea	Inflammation	0/3	0/3	0/3	0/3	1/2	1/3
Eye	Inflammatory cell infiltration, ciliary body, focal	0/3	2/3	1/3	0/3	0/2	1/3
Brain	Inflammatory cell infiltration, meningeal, perivascular, widespread, multifocal	0/3	0/3	0/3	0/3	0/2	1/3

	Lymphocytic infiltration, meningeal, perivascular	0/3	2/3	0/3	0/3	0/2	0/3
Sciatic nerve	Inflammatory cell infiltrate, perivascular	0/3	2/3	1/3	0/3	0/2	1/3
Kidney	Inflammation, multifocal	0/3	0/3	0/3	0/3	0/3	2/3
Mandibular lymph node	Lymphoid depletion						
	Minimal	0/3	2/3	0/3	0/3	0/2	1/3
	Mild	0/3	0/3	0/3	0/3	1/2	1/3
	Moderate	0/3	0/3	0/3	0/3	0/2	1/3
Mesenteric lymph node	Lymphoid depletion						
	Minimal	0/3	1/3	1/3	0/3	0/2	0/3
	Mild	0/3	2/3	2/3	0/3	2/2	0/3
	Moderate	0/3	0/3	0/3	0/3	0/2	3/3
Peyer's patches	Lymphoid atrophy						
	Minimal	0/3	1/3	1/3	0/3	0/2	0/3
	Mild	0/3	1/3	2/3	0/3	0/2	1/3
	Moderate	0/3	0/3	0/3	0/3	1/2	1/3
Spleen	Lymphoid atrophy						
	Mild	0/3	1/3	1/3	0/3	0/2	1/3
	Moderate	0/3	1/3	2/3	0/3	1/2	1/3
	Follicular atrophy						
	Mild	0/3	1/3	0/3	0/3	1/2	1/3
Sternum	Lymphoid foci	1/3	0/3	0/3	0/3	0/2	1/3
Recovery							
Eye	Inflammatory cell infiltration, ciliary body, focal	0/4	0/4	2/3	0/4	0/2	0/3
Brain	Inflammatory cell infiltration, choroid plexus, focal	0/4	0/4	1/3	0/4	0/2	0/3
	Lymphocytic infiltration, meningeal; perivascular, focal	0/4	0/4	1/3	0/4	0/2	0/3
Peyer's patches	Lymphoid hyperplasia, with inflammation	0/3	1/4	0/2	0/3	0/2	0/2
Spleen	Lymphoid hyperplasia, focal	0/4	1/4	1/2	1/3	1/2	1/3
Sternum	Lymphoid foci	0/4	1/4	0/3	0/4	1/2	1/3

Toxicokinetics:

- T_{max} ranged from the end of infusion to 48 h after dosing; the authors did not attempt to explain this phenomenon. This reviewer speculates that it may be due to initial sequestration of ofatumumab as it binds CD20⁺ cells in lymphoid organs.

- After the initial dose:
 - Clearance ranged from 0.24 to 0.3 ml/h/kg; the authors concluded that renal and hepatic blood flows are not limiting factors for clearance.
 - $T_{1/2}$ means ranged from 3.8 to 5.6 days.
 - V_d ranged from 34 to 52 ml/kg, and V_{ss} ranged from 31 to 48 ml/kg. Citing 208 ml/kg as a reference value for extracellular fluid volume in monkeys, the authors concluded that ofatumumab did not distribute substantially beyond central circulation.
- At the end of weekly dosing (i.e. D50, after 8 doses):
 - Accumulation was apparent
 - Clearance and V_d increased dramatically at both doses, compared to D1. The study authors consider this to be an artifact of analysis (more time points and more reliable data collected) rather than a true biological difference.
 - $T_{1/2}$ was 11 to 15 days, increased dramatically at both doses compared to D1. This is consistent with the expected pharmacology (i.e. clearance of target CD20⁺ cells from circulation).
- At the end of monthly dosing (i.e. D190, after 5 monthly doses):
 - Accumulation was not apparent.
 - Clearance and V_d were comparable to D50.
 - $T_{1/2}$ was 11 to 16 days.
- No gender differences in TK were apparent.
- **NOTE:** The study authors note (report p 34), "Following review of the bioanalytical phase data by the Sponsor, questions were raised over the acceptability of a number of original serum concentration values. Data were reviewed and new serum concentration data tables compiled. As a result, toxicokinetic evaluation of the serum concentration data was repeated by [redacted]. The decision to re-analyze the data, and to censor outliers, appears reasonable." b(4)

- From report p 866:

Table A: Geometric Mean (CV%) Parameter Estimates Indicative of Systemic Exposure to HuMax-CD20 Following i.v. Infusion on Day 1 in Male and Female Cynomolgus Monkeys

Sex	Dose (mg/kg) [Ratio]	Part	AUC(0-∞) (µg.h/mL) (CV%) [Ratio]	AUC(0-t) (µg.h/mL) (CV%) [Ratio]	AUC(0-6.5 h) (µg.h/mL) (CV%) [Ratio]	AUC(0-168 h) (µg.h/mL) (CV%) [Ratio]	C _{max} (obs) (µg/mL) (CV%) [Ratio]
Male	20 [1.0]	Main & Recovery	73950 ^{ns} (35.4) [1.0]	41375 (24.9) [1.0]	2096 (37.0) [1.0]	41299 (24.9) [1.0]	531 (21.9) [1.0]
	100 [5.0]	Main & Recovery	402347 ^{ns} (15.7) [5.4]	265410 (19.3) [6.4]	13948 (6.5) [6.7]	264893 (19.3) [6.4]	2699 (9.9) [5.1]
Female	20 [1.0]	Main & Recovery	62783 ^{ns} (25.9) [1.0]	41293 (25.7) [1.0]	1699 (104.9) [1.0]	41202 (25.7) [1.0]	462 (49.6) [1.0]
	100 [5.0]	Main & Recovery	423185 ^{ns} (5.1) [6.7]	117697 (294.1) [2.9]	12557 (56.6) [7.4]	272379 ^{ns} (12.2) [6.6]	2676 (36.1) [5.8]

n=7 for Day 1 (Main & Recovery) unless otherwise stated^a

Table B: Arithmetic Mean (SD) Parameter Estimates Relating to HuMax-CD20 Following i.v. Infusion on Day 1 in Male and Female Cynomolgus Monkeys

Sex	Dose (mg/kg)	Part	T _{max} (obs) ^a (h) (Range)	T _{1/2} el (h) (SD)	K _{el} (1/h) (SD)	CL (mL/h/kg) (SD)	V _d (mL/kg) (SD)	V _{ss} (mL/kg) (SD)
Male	20	Main & Recovery	0.50	134.81 ^{ns} (40.65)	0.005489 ^{ns} (0.001403)	0.2819 ^{ns} (0.07992)	52.00 ^{ns} (11.33)	47.96 ^{ns} (12.55)
	100	Main & Recovery	2.00	128.68 ^{ns} (9.95)	0.005501 ^{ns} (0.0004631)	0.2508 ^{ns} (0.03572)	45.71 ^{ns} (6.623)	42.43 ^{ns} (7.019)
Female	20	Main & Recovery	0.50	91.92 ^{ns} (10.07)	0.007606 ^{ns} (0.0008055)	0.3261 ^{ns} (0.07815)	42.89 ^{ns} (9.313)	39.46 ^{ns} (11.83)
	100	Main & Recovery	0.50	100.47 ^{ns} (9.20)	0.006938 ^{ns} (0.0006307)	0.2365 ^{ns} (0.01213)	34.27 ^{ns} (3.382)	30.95 ^{ns} (2.534)

^a Median(range)
n=6 for Day 15 (Main & Recovery) unless otherwise specified^a

b(4)

Table C: Geometric Mean (CV%) Parameter Estimates Indicative of Systemic Exposure to HuMax-CD20 Following i.v. Infusion on Days 1, 50, 134 and 190 in Male Cynomolgus Monkeys

Dose (mg/kg)	Day	Part	AUC(0-∞) (µg.h/mL) (CV%)	AUC(0-t) (µg.h/mL) (CV%)	AUC(0-6.5 h) (µg.h/mL) (CV%)	AUC(0-168 h) (µg.h/mL) (CV%)	C _{max} (obs) (µg/mL) (CV%)	
20	1	Main & Recovery	73950 ^{ns} (35.4)	41375 (24.9)	2098 (37.0)	41290 (24.9)	531 (21.9)	
		Main & Recovery	405835 (20.5)	304128 (14.7)	6473 (31.7)	119208 (15.2)	1239 (27.4)	
	50	Main & Recovery	140268 ^{ns} (30.5)	81235 (18.5)	3443 (15.7)	62492 (18.5)	695 (21.5)	
		Main	-	1088 ^{ns} (5.1)	3467 ^{ns} -	-	-	
	190	Recovery	202732 (55.9)	189796 (56.3)	2841 (35.7)	58439 (5.8)	697 ^{ns} (10.0)	
		Main & Recovery	402347 ^{ns} (15.7)	265410 (19.3)	13948 (6.5)	284893 (19.3)	2699 (9.9)	
	100	1	Main & Recovery	2065198 ^{ns} (20.1)	1277280 (26.7)	27499 (32.9)	545047 (18.2)	6382 (15.7)
			Main & Recovery	560464 ^{ns} (36.9)	276325 (42.8)	16024 (34.3)	304533 ^{ns} (22.5)	3735 (26.9)
50		Main	-	54892 ^{ns} (25.9)	16244 ^{ns} (27.3)	-	-	
		Recovery	903407 (32.3)	765584 (24.4)	13464 (18.0)	287241 (16.4)	3110 ^{ns} (29.9)	
134		Main & Recovery	402347 ^{ns} (15.7)	265410 (19.3)	13948 (6.5)	284893 (19.3)	2699 (9.9)	
		Main & Recovery	2065198 ^{ns} (20.1)	1277280 (26.7)	27499 (32.9)	545047 (18.2)	6382 (15.7)	
190		Main & Recovery	560464 ^{ns} (36.9)	276325 (42.8)	16024 (34.3)	304533 ^{ns} (22.5)	3735 (26.9)	
		Main	-	54892 ^{ns} (25.9)	16244 ^{ns} (27.3)	-	-	

n=7 for Day 1, 50 and 134 (Main & Recovery), n=4 for Day 190 (both Main and Recovery animals) unless otherwise specified

Table D: Geometric Mean (CV%) Parameter Estimates Indicative of Systemic Exposure to HuMax-CD20 Following i.v. Infusion on Days 1, 50, 134 and 190 in Female Cynomolgus Monkeys

Dose (mg/kg)	Day	Part	AUC(0-∞) (µg.h/mL) (CV%)	AUC(0-t) (µg.h/mL) (CV%)	AUC(0-6.5 h) (µg.h/mL) (CV%)	AUC(0-168 h) (µg.h/mL) (CV%)	C _{max} (obs) (µg/mL) (CV%)	
20	1	Main & Recovery	62783 ^{ns} (25.9)	41293 (25.7)	1699 (104.9)	41202 (25.7)	462 (49.6)	
		Main & Recovery	292676 ^{ns} (23.4)	225584 ^{ns} (24.6)	5642 ^{ns} (37.4)	98208 ^{ns} (29.4)	1114 ^{ns} (36.6)	
	50	Main & Recovery	93746 ^{ns} (66.1)	25392 ^{ns} (253.6)	3000 ^{ns} (14.6)	48493 ^{ns} (27.2)	638 ^{ns} (20.2)	
		Main	-	1072 ^{ns} (13.0)	-	-	-	
	190	Recovery	128482 ^{ns} (15.2)	120394 ^{ns} (12.3)	2404 ^{ns} (14.2)	46397 ^{ns} (5.5)	567 ^{ns} (28.3)	
		Main & Recovery	423185 ^{ns} (5.1)	117697 (264.1)	12557 (56.6)	272379 ^{ns} (12.2)	2676 (36.1)	
	100	1	Main & Recovery	1458986 ^{ns} (44.8)	1204139 (51.3)	28832 (17.7)	487786 (26.0)	5414 (18.8)
			Main & Recovery	421266 ^{ns} (25.2)	243873 ^{ns} (45.5)	18862 ^{ns} (29.5)	282951 ^{ns} (26.3)	4034 ^{ns} (21.1)
50		Main	-	59183 ^{ns} (4.4)	19789 ^{ns} (19.4)	112298 ^{ns} (-)	3460 ^{ns} (19.3)	
		Recovery	809700 ^{ns} (7.7)	757219 ^{ns} (6.4)	15928 ^{ns} (10.1)	272464 ^{ns} (5.5)	-	
134		Main & Recovery	421266 ^{ns} (25.2)	243873 ^{ns} (45.5)	18862 ^{ns} (29.5)	282951 ^{ns} (26.3)	4034 ^{ns} (21.1)	
		Main	-	59183 ^{ns} (4.4)	19789 ^{ns} (19.4)	112298 ^{ns} (-)	3460 ^{ns} (19.3)	
190		Recovery	809700 ^{ns} (7.7)	757219 ^{ns} (6.4)	15928 ^{ns} (10.1)	272464 ^{ns} (5.5)	-	
		Main & Recovery	1458986 ^{ns} (44.8)	1204139 (51.3)	28832 (17.7)	487786 (26.0)	5414 (18.8)	

n=7 for Day 1, 50 and 134 (Main & Recovery), n=4 for Day 190 (both Main and Recovery animals) unless otherwise specified

Table E: Arithmetic Mean (SD) Parameter Estimates Relating to HuMax-CD20 Following i.v. Infusion on Days 1, 50, 134 and 190 in Male Cynomolgus Monkeys

Dose (mg/kg)	Day	Part	Tmax(obs) ^A (h) (Range)	T _{1/2} (h) (SD)	Kel (1/h) (SD)	CL (mL/h/kg) (SD)	Vd (mL/kg) (SD)	Vss (mL/kg) (SD)
20	1	Main & Recovery	0.50	134.81 ^{med} (40.65)	0.005489 ^{med} (0.001403)	0.2819 ^{med} (0.07992)	52.00 ^{med} (11.33)	47.96 ^{med} (12.55)
		Main & Recovery	2.00	346.41 (72.18)	0.002081 (0.0004526)	3.210 (0.9073)	1595 (579.6)	23.20 (3.501)
	134	Main & Recovery	2.00	197.61 ^{med} (53.69)	0.003745 ^{med} (0.001124)	5.875 (1.017)	1696 ^{med} (418.5)	37.64 ^{med} (9.736)
		Main	0.50	-	-	5.769 ^{med}	-	-
	190	Recovery	-	202.02 (190.90)	0.006354 (0.005342)	7.399 (2.956)	1838 (1547)	38.25 (13.65)
		Main & Recovery	2.00	126.66 ^{med} (9.95)	0.005501 ^{med} (0.0004631)	0.2508 ^{med} (0.03572)	45.71 ^{med} (6.623)	42.43 ^{med} (7.019)
100	50	Main & Recovery	0.50	360.93 ^{med} (54.69)	0.001948 ^{med} (0.0002714)	3.785 (1.064)	2474 ^{med} (379.1)	23.90 ^{med} (2.606)
		Main & Recovery	1.00	146.99 ^{med} (49.12)	0.005140 ^{med} (0.001759)	6.574 (2.492)	1374 ^{med} (788.6)	35.89 ^{med} (2.650)
	134	Main & Recovery	1.00	-	-	6.311 ^{med} (1.781)	-	-
		Main	6.50	-	-	-	-	-
	190	Recovery	-	392.69 (109.37)	0.001901 (0.0006601)	7.514 (1.275)	4310 (1654)	54.01 (6.065)
		Main & Recovery	1.00	-	-	-	-	-

^A Median (range)
n=7 for Days 1, 50, 134 (Main & Recovery), n=4 for Day 190 (both Main and Recovery animals) unless otherwise specified

b(4)

Table F: Arithmetic Mean (SD) Parameter Estimates Relating to HuMax-CD20 Following i.v. Infusion on Days 1, 50, 134 and 190 in Female Cynomolgus Monkeys

Dose (mg/kg)	Day	Part	Tmax(obs) ^A (h) (Range)	T _{1/2} el (h) (SD)	Kel (1/h) (SD)	CL (mL/h/kg) (SD)	Vd (mL/kg) (SD)	Vss (mL/kg) (SD)
20	1	Main & Recovery	0.50	91.92 ^{med} (10.07)	0.007608 ^{med} (0.0008055)	0.3261 ^{med} (0.07815)	42.89 ^{med} (9.313)	39.46 ^{med} (11.83)
		Main & Recovery	3.50	318.36 ^{med} (62.56)	0.002243 ^{med} (0.0004236)	3.726 ^{med} (1.263)	1763 ^{med} (846.2)	30.88 ^{med} (11.61)
	134	Main & Recovery	2.00	169.30 ^{med} (85.80)	0.005857 ^{med} (0.004884)	6.723 ^{med} (0.9836)	1647 ^{med} (807.1)	44.87 ^{med} (11.44)
		Main	3.50	-	-	-	-	-
	190	Main	3.50	-	-	-	-	-
		Recovery	-	255.79 ^{med} (42.84)	0.002761 ^{med} (0.0004601)	8.373 ^{med} (1.173)	3115 ^{med} (867.9)	45.23 ^{med} (6.095)
100	1	Main & Recovery	0.50	100.47 ^{med} (9.20)	0.006938 ^{med} (0.0006307)	0.2365 ^{med} (0.01213)	34.27 ^{med} (3.382)	30.95 ^{med} (2.534)
		Main & Recovery	0.50	280.71 ^{med} (48.55)	0.002736 ^{med} (0.0005213)	3.776 ^{med} (0.8454)	1487 ^{med} (452.2)	21.56 ^{med} (5.283)
	134	Main & Recovery	3.50	103.78 ^{med} (48.26)	0.007610 ^{med} (0.002585)	5.503 ^{med} (1.774)	769.2 ^{med} (517.8)	35.21 ^{med} (15.53)
		Main	5.00 ^{med}	21.97 ^{med}	0.03154 ^{med}	5.388 ^{med} (1.030)	139.3 ^{med}	27.21 ^{med}
	190	Main	5.00 ^{med}	-	-	-	-	-
		Recovery	-	275.43 ^{med} (50.24)	0.002581 ^{med} (0.0005259)	6.300 ^{med} (0.8476)	2523 ^{med} (844.4)	44.99 ^{med} (5.153)

^A Median (range)
n=7 for Day 1, 50 and 134 (Main & Recovery), n=4 for Day 190 (both Main and Recovery respectively) unless otherwise specified

b(4)

b(4)

Anti-product antibody formation:

- Animal 33F (low-dose) has a weakly-positive result on D15, and increasingly stronger responses when measured on D29 and D43. This animal was found dead on D44.
- **Note:** The association of rapid PAHA response with death is concerning.
 - Relevance to humans not established. Ofatumumab is a human monoclonal antibody; the immune response of cynomolgus monkeys to a foreign-species antibody is not predictive for the human immune response to a human antibody.
 - However, the pharmacological mechanism of action of ofatumumab is depletion of CD20⁺ cells (including CLL and also normal B-cells). Speculatively, ofatumumab would prevent a PAHA response (i.e. depleting the CD20-expressing B-cell lineages before they could mount an anti-ofatumumab response). The potential interplay between the pharmacological mechanism of action of ofatumumab, anti-product antibody, and death in animal #33F is unclear.
- The initial PAHA assay was performed at 8
- Based on the unexpected lack of PAHA, the detection assay was repeated under a separate protocol (not-GLP) by a separate laboratory (C, D), with no difference in results.

b(4)

- The biological relevance of these data are unclear, since PAHA were detected in ofatumumab-treated animals in the shorter studies (#22804, #23542).
 - The muted PAHA may reflect the pharmacology of ofatumumab (i.e. the change may reflect the longer duration of B-cell depletion).
 - The longer sample storage time may have affected the samples.

Blood flow cytometry:

- This study did not evaluate the rapidity of B-cell depletion after dosing; the first post-dose measurement was day 8 (before the second dose).
- B-cell depletion:
 - CD20⁺ B-cells depleted at D8 to D192 (main group sacrifice), to approximately 5 to 10% of pre-dose levels.
 - The study authors define memory B-cells as CD27⁺ CD21⁺ CD20⁺ and non-memory B-cells CD27⁻ CD21⁺ CD20⁺. Similar patterns of depletion were noted for both B-cell subpopulations.
- Recovery:
 - The low-dose males and females exhibited similar results: after the last dose (D190), initial signs of B-cell recovery were observed 56 days later (D246, 20% of baseline), with >80% recovery by after 84 days (D274) followed by a rebound effect at 140-163 days post dose (D330 and 353, i.e. ~50% more B-cells than baseline), and full recovery 6-months after the last dose (D372).
 - High-dose animals after the last dose (D190) exhibited initial signs of B-cell recovery 94 (females) to 112 days later (males), followed by a rebound effect (1.5- to 2-fold over baseline on D330 and 350), with full recovery 6 months after the last dose (D372).
- No treatment-related T cell changes were apparent.
- NK cells: sporadic decreases were observed among treated animals; the study authors attributed this to individual variability. No clear treatment-related effect is apparent.

Lymph node flow cytometry:

- Biopsies were collected predose, D8, 192, 224, 274, 350, and 372.
- B-cells:
 - Treated animals exhibited marked B-cell depletion (D8 to D192), to approximately 5% of predose levels.
 - One treated animal (low-dose male #8M) exhibited recovery at the end of dosing (D192); the other animals showed initial recovery by 84 days after cessation of dosing (D274) and full recovery from 84 to 160 days (D274 to D350).
- T cells:
 - A slight increase in the percentage of T cell counts were observed at D192 and D225 (but not at D274). The study authors considered this an indirect effect of treatment caused by "the loss of a compartment cell population (B-cells) within the lymph node".
- The number of NK cells within the lymph node was low; no treatment-related effects were apparent.

Immunohistochemistry:

- Specific staining of ofatumumab was observed in the lymphoid follicles tissues examined (submandibular lymph node, mesenteric lymph node, Peyer's patches, spleen, thymus, tonsil)
- Results showed persistence of ofatumumab binding in recovery animals (determined by comparing slides pre-treated with ofatumumab versus normally treated slides)
- The study authors did not consider the additional exploratory work done to evaluate the presence of immune complexes for animals #33F and 40F to be informative.

Direct Coombs test:

- Background notes:
 - This endpoint was initially included to investigate the premature sacrifice of animal #40F, which had evidence of hemolytic crisis at time of death.
 - The assay was designed to attempt to detect human Ig (i.e. ofatumumab) or monkey complement cleavage products (C3b or C3d) on RBCs.
- Positive responses were observed for most treated animals:
 - D122 to D150 measurements: positive results for:
 - Controls – 0/14
 - Low dose – 4/7 males, 3/5 females
 - High-dose – 5/7 males, 6/7 females
 - D176 measurements: positive results for:
 - Controls – 0/14
 - Low-dose – 4/7 males, 4/5 females
 - High-dose – 4/7 males, 5/7 females
 - D372 measurement: no positive responses
- Antibody elution and ELISA verified that HuMax-CD20 was binding to RBC in 2/14 treated animals tested (42F on D218, 20M on D218 and D224).
- This relevance of this assay to humans is unclear, since the cynomolgus immune response to ofatumumab is not expected to predict the potential human response.

Other assays: p 63

- Assessment of delayed-type hypersensitivity:
 - No treatment-related effects were apparent.
 - Intra-group variability was relatively high in all treatment groups (controls and ofatumumab-treated)
- Assessment of humoral immune function (KLH):
 - The authors note that the humoral response is driven initially by IgM (in the first week after immunization) and then increasingly by IgG antibodies.
 - For the main-groups (immunized on D4 and D180), the response appeared muted in the high-dose animals at each of the post-immunization time points measured (D18, 180, and 183), but not in low-dose animals.
 - For the recovery groups (immunized on D32, 46, 109, 193, 274, and 369), responses appeared decreased in both treatment groups for all post-immunization time points measured (D18, 456, 60, 109, 112, 193, 196, 274, 277, 369, 372).

- *Campylobacter jejuni* immunoblot (from report p 763):

Animal Number	Serum Sample	Antibody	Result
7M	Day -7 (Pretrial)	IgA	Positive
		IgG	Positive
7M	Day 1	IgA	Positive
		IgG	Positive
20M	Day 276	IgA	Possible Positive
		IgG	Positive
30F	Day 22	IgA	Negative
		IgG	Positive
30F	Off Dose Period	IgA	Positive
		IgG	Positive
30F	Day 64	IgA	Positive
		IgG	Positive
33F	Day 43	IgA	Positive
		IgG	Positive
39F	Day 22	IgA	Possible Positive
		IgG	Positive
39F	Off Dose Period	IgA	Negative
		IgG	Positive

The authors considered these immunoblot data, in the context of the clinical signs, to indicate infection by *C. jejuni*.

2.6.6.4 Genetic toxicology

No testing for genetic toxicity was performed for ofatumumab. As the sponsor notes in Module 2.4.3., "ofatumumab is not expected to interact directly with DNA or other chromosomal material, therefore standard genotoxicity studies are not considered appropriate..."

Ofatumumab is not pharmacologically active in the traditional models for short-term genetic toxicology testing (standard cell lines, standard rodent species). Therefore, genetic toxicology testing using standard short-term models would have been infeasible.

2.6.6.5 Carcinogenicity

No nonclinical testing to assess carcinogenic potential was performed for ofatumumab. The sponsor notes in Module 2.4.3 that a rodent bioassay was not considered feasible, since ofatumumab was found not to be pharmacologically active in rodent species.

No treatment-related evidence of neoplastic or pre-neoplastic changes was observed in the GLP 7-month repeat-dose toxicity study in cynomolgus monkeys (report # 25052). Groups of 7 male and 7 female monkeys were dosed with 0, 20, or 100 mg/kg of

ofatumumab on D1, 8, 15, 22, 29, 36, 43, 50, 79, 106, 134, 162, and 190 (i.e. weekly x7 followed by monthly); 3/sex/group were sacrificed on D192 and the remaining 4/sex/group were maintained as recovery animals until D372.

2.6.6.6 Reproductive and developmental toxicology

Comment: The sponsor reported (m1.9.1 Request for Waiver of Pediatric Studies) that it does not expect persons under 20 to be diagnosed with CLL; the median age at diagnosis is 72 years, and the disease is extremely rare in patients younger than 40 years of age. This information was considered in assessing the need for and the relevance of nonclinical reproductive and developmental toxicology data to support the treatment of patients with CLL.

An embryofetal development toxicity study (study # 2148-010) was performed for ofatumumab. No stand-alone nonclinical fertility assay was performed for ofatumumab. No nonclinical studies to assess early embryonic development or postnatal development have been performed for ofatumumab.

No evidence of treatment-related reproductive toxicity was observed in cynomolgus monkeys treated with ofatumumab in the 4-week study (study #23542), which treated 4 sexually mature females (and 8 immature females, 12 immature males) or in the 7-month study (report # 25052), which treated 3 sexually mature females (and 11 immature females, 14 immature males). Fertility was not assessed; evaluation of reproductive toxicity for these assays is limited to clinical signs (e.g. evidence of menstruation), reproductive organ weight, gross reproductive organ pathology, and reproductive organ histopathology.

Comment: There is no legislative pathway by which data for other approved biologics products may be used in support of findings of safety or efficacy for products regulated under BLA. However, safety concerns raised by any relevant data (including data for marketed biologics) should be considered. This reviewer conducted a literature search for relevant information, and did not find a basis for regulatory concern:

- Rituximab is a prescription pharmaceutical that targets CD20. A 2009 paper, Rey et al.⁶ identified eight published case studies^{7, 8, 9, 10, 11, 12, 13, 14} of pregnant patients

⁶ Rey J., Coso D., Roger V., Bouayed N., Belmecheri N., Ivanov V., Gastaut J.A., Bouabdallah R. 2009. Rituximab combined with chemotherapy for lymphoma during pregnancy. *Leuk. Res.* 2009 33:3 (e8-e9)

⁷ Perg D, G. Koren and M. Lishner. 2007. The treatment of Hodgkin's and non-Hodgkin's lymphoma in pregnancy, *Haematologica* 92:1230-1237.

⁸ Herold M, S. Schnohr and H. Bittrich. 2001. Efficacy and safety of a combined rituximab chemotherapy during pregnancy, *J Clin Oncol* 19:3439.

⁹ Kimby E, A. Sverrisdottir and G. Eliander. 2004. Safety of rituximab therapy during the first trimester of pregnancy: a case history, *Eur J Haematol* 72:292-295.

¹⁰ Ojeda-Urbe M, C. Gilliot, G. Jung, B. Drenou and A. Brunot. 2006. Administration of rituximab during the first trimester of pregnancy without consequences for the newborn, *J Perinatol* 26:252-255.

¹¹ Decker M, C. Rothermundt, G. Holländer, A. Tichelli and C. Rochlitz. 2006. Rituximab plus CHOP for treatment of diffuse large B-cell lymphoma during second trimester of pregnancy, *Lancet Oncol* 7:693-694.

treated with rituximab. Multiple publications¹⁵ address the topic, although it is not clear whether they are based on these published papers or additional, unpublished, experiences. Maternal treatment with rituximab is associated with decreased B-cell counts in offspring, but treatment has not been associated with adverse outcomes. However, follow-up appears limited. Overall, the developmental toxicity data for rituximab do not raise a safety concern for the potential developmental toxicity of ofatumumab.

- One laboratory^{16, 17} has published several papers regarding B-cell depletion in fetal sheep using an anti-IgM antibody. The relevance of these data to ofatumumab is unclear; they do not raise concerns of regulatory significance.
- Several reports¹⁸ from the 1970s chronicle treatment of pregnant women with cytotoxic chemotherapy to deplete maternal bone marrow; outcomes ranged from reportedly healthy births to loss of pregnancy and infant deaths. Because ofatumumab is highly specific for CD20 by design, the developmental toxicity of cytotoxic bone marrow suppression therapy is not considered relevant to ofatumumab.

Study title: Intravenous embryo-fetal development study with HuMax-CD20 in the cynomolgus monkey.

Key study findings: Treatment weekly x5 with 20 and 100 mg/kg of ofatumumab caused nearly complete depletion of B-cells in dams, with slight signs of recovery by GD100. Treatment also caused substantial depletion of neonatal B-cell counts (measured in cord blood and from spleen samples at necropsy on GD100), and ofatumumab was detected in cord-blood at necropsy on GD100.

- Treatment was not associated with external, visceral, or skeletal abnormalities in offspring. Treatment was associated with decreased spleen weight (-15.7% for the low-dose group, -27% for the high-dose group); since the spleen histopathology evaluation did not detect changes, these decrease in spleen weight are considered a secondary effect of the pharmacology of ofatumumab, and not

¹² Friedrichs B, M. Tiemann, H. Salwender, K. Verpoort, M.K. Wenger and N. Schmitz. 2006. The effects of rituximab treatment during pregnancy on a neonate, *Haematologica* 91:1426-1427.

¹³ Temprano TK, R. Bandlamudi and T.L. Moore. 2005. Antirheumatic drugs in pregnancy and lactation, *Semin Arthritis Rheum* 35:112-121.

¹⁴ Klink DT, van Elburg RM, Schreurs MW, van Well GT. 2008. Rituximab administration in third trimester of pregnancy suppresses neonatal B-cell development. *Clin Dev Immunol*. 2008:271363

¹⁵ For example: Keeling S.O. and Oswald A.E. 2009. Pregnancy and rheumatic disease: "By the book" or "by the doc". *Clinical Rheumatology* 2009 28:1(1-9).

¹⁶ Press CM, Reynolds JD, McClure SJ, Landsverk T. 1998. Development of accessory cells in B-cell compartments is retarded in B-cell-depleted fetal sheep. *Dev Immunol*. 6(3-4):223-231.

¹⁷ Press CM, McCullagh P, Landsverk T. 2001. Effect of early fetal splenectomy on prenatal B-cell development in sheep. *Immunology*. 102(2):131-136.

¹⁸ For example: Doney KC, Kraemer KG, Shepard TH. 1979. Combination chemotherapy for acute myelocytic leukemia during pregnancy: three case reports. *Cancer Treat Rep*. 63(3):369-371.

clear evidence of toxicity. Notably, maternal ADA formation was associated with normal spleen weight at the mid-dose but not the high-dose.

- Three treated dams (two mid-dose, and one high-dose) developed antidrug antibodies, and these were detectable in the cord-blood samples taken on GD100. Two high-dose animals tested Coombs positive (indicative of autoimmune antibodies against RBCs) without evidence of anemia. The high-dose ADA⁺ animal was one of the two Coombs positive animals. Ofatumumab was not detectable in the cord blood of the offspring of the three ADA⁺ animals, and these offspring had B-cell counts at or near control levels.
- Treatment was also associated with decreases in absolute placenta, and fetal thymus weight. These changes were not statistically significant but magnitude of the decrease was more than 10% compared to concurrent controls. Fetal body weight did not appear to vary with treatment. These organs were examined visually but not by histology. Therefore, the biologic and toxicological significance of these changes in organ weights are unclear.

Study no.: Covance study # 2148-010

GlaxoSmithKline study #CD2008/01523/00.

Volume #, and page #: Submitted electronically in m4.2.3.5.2. File name is "2148-010-report.pdf". 494 pp.

Report date and status: 10/27/2008. Listed as a revised final report.

Conducting laboratory and location:

Date of study initiation: April 2007. In-life start date 4/26/2007. In-life end date 10/02/2007.

GLP compliance: Yes, signed. The body of the report lists compliance with GLP as outline in German Chemical law annex 1 to 19a, OECD principles of GLP ENV/MC/CHEM(98)17. BLA amendment #0008 (3/17/2008) includes a signed statement of compliance with 21CFR58, and documenting deviations.

QA reports: yes (X), signed.

Drug, lot #, and % purity: HuMax-CD20 (ofatumumab). Batch #PD06015.

Control lot information: Vehicle (30 mM sodium citrate + 100 mM sodium chloride) was used as the negative control. Batch # SJT26.

Methods

Doses: 0, 20, or 100 mg/kg weekly x5

Species/strain: *Macaca fascicularis* (cynomolgus monkey), purpose-bred, Asian origin. Weight range 2.6 to 3.9 kg. All animals were at least 3 years old.

Number/sex/group: 12 females/dose.

Route, formulation, volume, and infusion rate: 30 minute iv infusion

Satellite groups used for toxicokinetics: None.

Study design:

Pregnant female monkeys were dosed weekly from GD 20 to GD50.

Fetuses were delivered by cesarean section and sacrificed on GD100 ± 1.

Placentae were examined, and fetal blood was collected from the umbilical cord.

b(4)

Parameters and endpoints evaluated:

- Maternal endpoints:
 - Clinical signs twice daily (behavior, appearance, and feces).
 - Daily morbidity and mortality check at the beginning and end of working hours.
 - Observations for aberrant signs for the first hour after each dose administration.
 - Weekly detailed fur inspection.
 - Ultrasonic examination on post-coitum day (pcD) 18, 19 and 20 to identify pregnant dams.
 - Ultrasonic examination to verify continuation of pregnancy on GD30±1, 44±1, 58±1, 72±1 and 86±1
 - Vaginal smears from gD20 to pcD100±1.
 - Estimated food intake twice daily (GD20 to end of study), classified as normal or reduced.
 - Body weights (GD19, 26, 33, 40, 47, 54, 61, 68, 75, 82, 89, 96, and 100±1).
 - Hematology blood samples on GD34, 50, 75 and 100±1 (reticulocyte counts, red blood cell counts, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, differential white cell counts [neutrophils, monocytes, eosinophils, lymphocytes, basophils]).
 - Toxicokinetic blood samples on pcD20, 27, 34, and 41 (predose, and at 1.5, 6, and 12 hours post-dosing), on pcD48 (predose, and at 1.5, 6, 12 and 24 hours post-dose), and after treatment on GD50, 52, 55, 60, 65, 70, 80, 90 and 100±1.
 - Anti-product antibody blood samples on GD20, 34, and 48 (during treatment) and also GD60, 80, and 100±1.
 - Direct Coombs test blood samples predose, GD34, 50, 75 and 100±1.
 - Lymphocyte subset analysis blood samples predose, GD25 and also GD50±1, 60±1, 70±1, 80±1, 90±1 and 100±1. Levels of CD3⁺, CD4⁺, CD8⁺, CD16⁺, NKp45, CD20⁺, CD21⁺, and CD40⁺ cells.
- Fetal endpoints
 - Weight
 - Sex
 - Fetal distance measurements: coccyx to cranium, tip of nose to os occipitale, os frontale to os occipitale, eye to eye, width of head
 - External abnormalities. Visual (by eye or stereomicroscopic) evaluation of external defects: body form, symmetry of head, facial form, formation of lower jaw, eyebrows, eyes, eyelids, hair on head, nipple formation, anus, fingers, toes, nails of finger and toes, ears, tails, upper and lower extremities, external genitals, palpation of the vertebral column, umbilical cord, palate

- Visceral abnormalities. Visual inspection: stomach, cecum, small intestine, large intestine, testes, epididymides and vas deferens, ovaries and uterus, ureters, kidneys and adrenals, urinary bladder, liver, spleen and gallbladder, diaphragm, thymus, thyroid, heart, aortic arch, cardiac septum and cardia auricles, lungs, esophagus and trachea
- Skeletal abnormalities
- Selected organ weights (adrenal, brain, eyes, heart, kidney, liver including gallbladder, lungs, ovaries, spleen, testes, thymus, uterus)
- Selected histology: spleen, mesenteric lymph node, ileum with Peyer's patches, thymus
- Placenta weight and gross appearance
- Fetal blood samples (umbilical cord) for product, anti-product antibodies, and lymphocyte subset analysis
- Spleen sample for lymphocyte subset analysis, to determine T-cells (CD3, CD4, CD8, CD45), B-cells (CD3, CD20, CD21, CD40, CD45) and NK-cells (CD3, CD16, NKp46, CD8, CD45)

Results

Mortality (dams): No unscheduled deaths.

Clinical signs (dams): No apparent treatment-related effects.

Body weight (dams): No apparent treatment-related effects.

Food consumption (dams): No apparent treatment-related effects.

Hematology: No apparent treatment-related effects.

Clinical chemistry: Not evaluated.

Immunophenotyping:

B-cells (calculated as CD20⁺/CD40⁺ cells and separately as GD21⁺ cells) were completely depleted in both treatment groups on GD25, 50, and 60 (low levels detectable in some animals; less than 1% of controls or predose values). The study authors did not consider the appearance of detectable B-cells to be evidence of recovery. This reviewer considers the results to show initial recovery (although still less than 5% of controls or predose values) beginning on GD70 for the 20 mg/kg group, and GD90 for the 100 mg/kg group.

The depletion of B-cells appeared to cause changes in the relative counts of NK and T cells. Although no changes were apparent for absolute NK cell counts (measured as CD16⁺NKp46⁺ cells) or absolute T cell counts (considered separately as CD3⁺ T cells, CD4⁺ helper T cells, and CD8⁺ cytotoxic T cells), statistically significant increases in relative NK cell percentages were observed for the high-dose group (no changes at

GD25, increased at GD60, 70, 80, and 100) and statistically significant increases in relative T cell percentages were observed for both treatment groups from GD25 onward. These relative changes are not considered toxicologically or pharmacologically significant.

Direct Coombs test

Positive results were observed in 2/12 high-dose dams (#13686 and #13415): the animals had negative results on GD34, and positive results on GD50, 75 and 100. Neither animal exhibited signs of anemia (measured on GD34, 50, 75, 100). One of the two affected animals (#13415) also exhibited ADA. The study authors did not comment on whether they considered the positive results to be treatment-related.

Comment: No Coombs testing was performed for the neonates. If the direct Coombs test had been performed with fetal RBCs, it would be useful in assessing potential autoimmune hemolysis. However, this test was only performed for the dams.

Comment: The study report did not explicitly state the purpose of including this maternal endpoint. The direct Coombs test is a direct antiglobulin test: red blood cells (RBC) are mixed with Coombs reagent; agglutination indicates the presence of antibodies on the surface of the RBCs. A positive result is indicative of IgG autoantibody against either a RBC protein, or an antigen (such as complement) bound to RBCs. The direct Coombs test is less sensitive than the indirect Coombs test; both tests are typically performed with neonatal erythrocytes.

Comment: Theoretically, these results suggest concern for patients (adults and developing) at increased risk of autoimmune hemolysis from diseases such as systemic lupus erythematosus and mis-matched transfusions. These results also suggest a theoretical concern for the fetus/newborn, for risk of erythroblastosis fetalis type incompatibility (e.g. Rhesus factor disease).

Toxicokinetics:

- From p 11 of the study report:

Table 2: Geometric mean (CV %) toxicokinetic parameters indicative of systemic exposure to HuMax-CD20 following single and repeated infusion administration (0.5 h duration) of 20 and 100 mg/kg Humax-CD20 to cynomolgus monkeys

Study Day (post coitum)	Dose (mg/kg) [Ratio] ^a	n	AUC ₀₋₇₂ (µg·h/mL) (CV%) [Ratio] ^b	AUC ₀₋₂₄ (µg·h/mL) (CV%) [Ratio] ^c	C _{max} (µg/mL) (CV%) [Ratio] ^d
20	20 [1]	9 ^{c,d}	55170 (13) [1]	107300 (34.5) [1]	628 (17.4) [1]
	100 [5]	12	309800 (13.0) [5.6]	536600 (18.8) [5]	3100 (13.0) [4.9]
48	20 [1]	7 ^a	87860 (32.6) [1]	212800 (37.4) [1]	760 (29.3) [1]
	100 [5]	11 ^b	622600 (20.3) [7.1]	1646000 (26.0) [7.7]	5680 (17.3) [7.5]

Note: ^aFold increase in dose or parameter in relation to the lowest dose level

^a= Two animals at 20 mg/kg were excluded due to antidrug antibody response. No samples were available for analysis from 2 animals

^b= One animal at 100 mg/kg were excluded due to antidrug antibody response

^c= One animal at 20 mg/kg was excluded due to a sampling error

^d= Two animals at 20 mg/kg were not evaluable for TK parameter estimation purposes

- This data shows evidence of accumulation, slightly more rapid at the higher dose.
- Individual T_{1/2} estimates ranged from 98 to 365 hours.
- In the cord-blood samples (measured at GD100), the mean concentration of ofatumumab was 8625 ± 3926 ng/ml for the 20 mg/kg/week group, and 66467 ± 22854 ng/ml for the 100 mg/kg/week group.
- Notably, no ofatumumab was detectable in the cord-blood from the offspring of the three treated animals that developed ADA.

Anti-drug antibody (ADA) formation

- Adults: 0/12 controls, 2/12 dams (#13234 and #13513) at 20 mg/kg and 1/12 dams (#13415) at 100 mg/kg tested positive.
 - Both animals in the 20 mg/kg/week group were negative on GD20 and GD34, subsequently positive (measured on GD48, 60, 80 and 100).
 - The single animal at 100 mg/kg/week tested negative on GD20, 34, 48 and 60, and positive on GD80 and GD100.
 - The fetal cord blood samples for the offspring of these three animals also tested positive for ADA.
- The authors note that the presence of product may have masked the true immune response; therefore, the reported incidences may be biased low. In the 3 animals exhibiting ADA, ofatumumab was not detected in the cord blood samples. In the animals without detectable ADA, ofatumumab was detected in cord blood samples.
- This test indicates that maternal ADA appears to have crossed the placental barrier by GD100.

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):

- Prenatal loss:
 - 0/12 controls
 - 2/12 (16.7%) for the 20 mg/kg/group.
 - Abortion for #13183 confirmed on GD43 (embryo not obtained). Heavy bleeding was observed on GD42 and GD43.
 - Embryonic death for #13294 on GD41; dead embryo was removed by early Cesarean section. Heavy bleeding was reported for GD41.
 - The sexes of the losses were not reported.
 - 0/12 for the 100 mg/kg group
 - No treatment-related effect is apparent for prenatal loss: the increased incidence of prenatal loss for the low-dose group is within expected historical range, and no dose-response was apparent.

Offspring (malformations, variations, etc.):

- Control group had 4 male and 8 female neonates (12 total). Low-dose group had 5 male and 5 female neonates (10 total). High-dose group had 5 male and 7 female neonates (12 total).
- No changes in the incidences of abnormalities (external, visceral, skeletal) were apparent.
- No apparent effect on fetal body weight (means were within 2% for the three groups).
- **Decreased mean spleen weight: -15.7% and -27.0% compared to controls for the low- and high-dose groups, respectively.** Statistically significant for the high-dose group only.
 - The authors concluded that the spleen weights were within the lower limit of historical control data, but that “a compound related effect could not be excluded.”
 - Authors (report p 31) report the reference spleen weight data as 236.63 mg $\pm$ 72.50 mg.
 - The decrease in spleen weight is consistent with the pharmacologic activity of ofatumumab, and these changes are considered treatment-related. In the absence of histopathological evidence of changes to the spleen, this effect is not clearly adverse.
 - Notably, spleen weight was normal for the two mid-dose offspring of ADA⁺ dams. However, the offspring of the single, high-dose ADA⁺ dam had a low spleen weight, comparable to other offspring from the same treatment group.
- Notable differences in magnitude were observed (not statistically significant) that appear treatment-related:
 - **Decreased mean placenta weight: -9.7% and -11.1% compared to controls for the low- and high-dose groups, respectively.** Not statistically significant.

- Decreased mean thymus weight for high-dose animals compared to controls: -14.7%

Table 15: Selected organ weights

Organ	Control	20 mg/kg/week	100 mg/kg/week
Placenta (g)	58.8 ± 9.2	53.1 ± 8.4	52.3 ± 14.1
Fetus weight (g)	105.7 ± 10.3	107.9 ± 11.8	103.7 ± 18.4
Fetal spleen (mg)	228.7 ± 43.8	192.7 ± 51.9	167.0 ± 65.0
Fetal thymus (mg)	213.8 ± 55.3	229.1 ± 62.7	182.3 ± 76.1

Values expressed as means ± standard deviation, with the range in parentheses

- B-cell depletion was observed in both treatment groups.

Table 16: Selected fetal immunophenotyping B-cells results

Parameter	Controls	20 mg/kg/week	100 mg/kg/week
Blood			
Absolute CD20 ⁺ CD40 ⁺ B-cells	0.59 ± 0.39	0.06 ± 0.15*	0.09 ± 0.25*
Relative CD20 ⁺ CD40 ⁺ B-cells	19.2 ± 9.4	1.8 ± 3.9*	2.0 ± 5.8*
Absolute CD21 ⁺ B-cells	0.52 ± 0.36	0.06 ± 0.13*	0.09 ± 0.24*
Relative CD21 ⁺ B-cells	17.1 ± 8.6	1.6 ± 3.4*	2.1 ± 5.41*
Spleen			
Relative CD20 ⁺ CD40 ⁺ B-cells	33.3 ± 11.0	5.9 ± 9.6*	3.4 ± 7.8*
Relative CD20 ⁺ CD40 ⁺ B-cells	25.6 ± 9.0	4.9 ± 7.2*	3.8 ± 6.7*

Data expressed as mean + standard deviation.

*Statistically significant, p < 0.001.

- Notably, the two mid-dose animals born to ADA⁺ dams had dramatically higher B-cell counts compared to offspring from other treated animals.
 - One offspring from a mid-dose, ADA⁺ dam had B-cell counts approximately half of controls.
 - The other mid-dose offspring and the single high-dose offspring from ADA⁺ dams had B-cell counts at necropsy approximately equal to controls.
- No apparent changes were noted in histopathology of the spleen or the other organs evaluated.

b(4)

2.6.6.7 Local tolerance

No stand-alone local nonclinical tolerance tests were conducted. The nonclinical toxicity studies included evaluations of the injection site (observations and histopathology); no treatment-related effects were apparent.

2.6.6.8 Special toxicology studies

Summary: Three tissue cross-reactivity studies for ofatumumab were submitted to the BLA. Ofatumumab binding was consistent with the expected anatomical locations of B-cells, and no off-target binding was apparent under the conditions tested. Ofatumumab bound human and cynomolgus monkey B-cells, but did not bind B-cells in pig, dog, rabbit, rat, or mouse spleen samples. In the human tissue cross-reactivity study, ofatumumab binding appeared limited to B-cells and no off-target binding was apparent.

The non-human primate tissue cross-reactivity study (study #GMB-3001-002) is fully reviewed in section 2.6.2.2 of this review.

Notably, the GLP toxicology studies included immunohistochemistry of selected (lymphoid and negative control) tissues for ofatumumab.

2.6.6.8.1

Study title: HuMax-CD20: an immunohistochemical investigation of cross-reactivity in human tissues.

Key study findings: Strong, specific membrane staining was observed in human lymphocytes (lymph node, spleen, thymus, tonsil, mucosa associated lymphoid tissue [MALT] of the small and large intestines). Additionally, specific membrane staining was observed in scattered lymphocytes in subepithelial tissues (cervix, endometrium, kidney, prostate, parotid salivary gland, skin, stomach, ureter, and urinary bladder).

Study no.: () report # 23179. () project # 530757. GSK study # CD2008/01311/00.

Volume #, and page #: Submitted electronically in m4.2.3.7.7. File name "23179-report.pdf". 50 pp.

Conducting laboratory and location: ()

Date of study initiation: 2003

GLP compliance: A signed statement of compliance with the OECD principles of GLP as set forth by the United Kingdom Department of Health was provided. Compliance with 21CFR58 was not explicitly documented.

QA reports: yes (X)

Drug, lot #, and % purity: Fluorescein isothiocyanate (FITC)-labeled HuMax-CD20 (HuMax-CD20-FITC, 2F2/XX2 LO8131/15), batch # HuMax-CD20 FITC 20030526. Purity not quantified (banding pattern verified by native agarose gel, non-reduced SDS PAGE, and reduced SDS-PAGE).

b(4)

Formulation/vehicle: 17 vials of 0.5 ml of 1.103 mg/ml of HuMax-CD20-FITC. Formulated in 50 mM Tris, 100 mM sodium chloride buffer, pH 8.0 with addition of 0.01% NaN₃ and 0.1% bovine serum albumin.

Methods

Concentrations tested: 0.5, 5, and 20 µg/ml. Titration at 0, 0.5, 1, 5, 10, 20 and 50 µg/ml.

Study design:

- Negative control antibody: nonspecific purified Ig-FITC conjugate at 5.0 µg/ml
- Positive tissue control: human tonsil
- Negative tissue control: human heart muscle
- The immunohistochemical staining method used rabbit anti-FITC as the secondary antibody, biotinylated goat anti-rabbit antibody as the tertiary antibody, and a streptavidin-biotin enzyme complex system for visualization.
- Cryosectioned human donor tissues, generally from 3 donors, were evaluated from: adrenal gland, blood cells, bone marrow, brain (cerebrum, cerebellum), breast, eye, fallopian tube, gastro-intestinal tract (stomach, small intestine, large intestine), heart, kidney, liver, lung, lymph node, ovary, pancreas, parathyroid gland, peripheral nerve, pituitary gland, placenta, prostate, salivary gland, parotid, skin, spinal cord, spleen, striated muscle, testis, thymus, thyroid gland, tonsil, urinary bladder, ureter, uterus (cervix, endometrium, vascular endothelium).

Results:

Table 17: selected data

Tissue	0.5 µg/ml	5 µg/ml	20 µg/ml
Adrenal Gland	0/3	0/3	0/3
Blood Cells: leukocyte staining			
Minimal	0/3	1/3	0/3
Mild	1/3	0/3	0/3
Moderate	1/3	0/3	2/3
Bone Marrow: leukocyte staining	1/3	1/3	0/3
Brain (Cerebrum)	0/3	0/3	0/3
Brain (Cerebellum): mononuclear cell staining	0/3	1/3	1/3
Breast	0/3	0/3	0/3
Eye	0/3	0/3	0/3
Fallopian Tube	0/3	0/3	0/3
Gastro-intestinal Tract (Stomach): few lymphocyte staining	2/3	2/3	1/3
Gastro-intestinal Tract (Small Intestine): diffuse lymphocyte staining in Peyer's patches	1/3	1/3	1/3

Gastro-intestinal Tract (Large Intestine): diffuse lymphocyte staining in Peyer's patches	1/3	1/3	0/3
Heart	0/3	0/3	0/3
Kidney: lymphocyte staining	1/3	1/3	1/3
Liver	0/3	0/3	0/3
Lung: lymphoid staining	2/3	2/3	1/3
Lymph Node: lymphoid staining			
Diffuse	3/3	3/3	2/3
Scattered	0/3	0/3	1/3
Paracorticoid	1/3	1/3	1/3
Medulla	1/3	1/3	1/3
Ovary	0/3	0/3	0/3
Pancreas	0/3	0/3	0/3
Parathyroid Gland	0/3	0/3	0/3
Peripheral Nerve	0/3	0/3	0/3
Pituitary Gland	0/3	0/3	0/3
Placenta	0/3	0/3	0/3
Prostate: lymphocyte staining			
Numerous	1/3	1/3	1/3
Scattered	2/3	2/3	2/3
Salivary Gland: lymphocyte staining			
Numerous	1/3	1/3	1/3
Scattered	1/3	0/3	1/3
Few	0/3	1/3	0/3
(Parotid)			
Skin: scattered lymphocyte staining	2/3	2/3	2/3
Spinal Cord	0/3	0/3	0/3
Spleen: diffuse lymphocyte staining	3/3	3/3	3/3
Striated Muscle	0/3	0/3	0/3
Testis	0/3	0/3	0/3
Thymus: diffuse lymphocyte staining	3/3	3/3	3/3
Thyroid Gland	0/3	0/3	0/3
Tonsil: Numerous lymphocytes	3/3	3/3	3/3
Urinary Bladder: lymphocyte staining			
Scattered	0/3	0/3	1/3
Few	1/3	1/3	0/3

Ureter: scattered lymphocyte staining	1/3	1/3	1/3
Uterus (Cervix): lymphocyte staining			
Diffuse	0/3	0/3	1/3
Scattered	1/3	1/3	0/3
Uterus (Endometrium): diffuse lymphocyte staining	1/3	1/3	0/3

2.6.6.8.2

Study title: Species cross-reactivity of HuMax-CD20

Key study findings: No tissue cross-reactivity was observed for ofatumumab under the conditions tested for pig, dog, rabbit, rat, or mouse spleen.

Study no.: Genmab #GMB3001-024. GSK #CD2008/01175/00.

Volume #, and page #: Submitted electronically to m4.2.1.1. File name "gmb3001-024-report.pdf". 24 pp. Report date 9/19/2008.

Conducting laboratory and location: Genmab BV. Jenalaan 18D, 3584 CK Utrecht, The Netherlands.

Date of study initiation: 5/2008.

GLP compliance: No.

QA reports: no (X)

Drug, lot #, and % purity: HuMax-CD20, lot 3105ARS. Purity not reported.

Formulation/vehicle: 32.13 mg/ml

Methods

Concentrations tested: 0.1, 1.0, and 3.0 µg/ml

Study design:

- Spleen tissue samples from pig, dog, rabbit, mouse, and rat (strains not reported) were used.
- Human tonsil tissue was used as a positive tissue control.
- Anti-KLH was used as a negative antibody control, and anti-Ki67 was used as an antibody positive control.

Results:

- Specific ofatumumab staining was observed in the germinal centers of the human tonsil.
- No specific ofatumumab staining was observed in the animal tissues.
- The positive and negative controls gave expected results.
- Rodents, rabbit, pig, or dog are not expected to be pharmacologically-relevant nonclinical models for ofatumumab.

2.6.6.9 Discussion and Conclusions

Conclusions

Ofatumumab caused B-cell depletion

- In healthy cynomolgus monkeys treated with ofatumumab, doses as low as 1.25 mg/kg were sufficient to deplete circulating CD20⁺ B-cells to ~4% of baseline at the earliest time point measured (2 days after dosing). Subsequent studies observed depletion of CD20⁺ B-cells to ~5 to 10% of baseline.
- Increasing the dose did not increase the magnitude of effect; higher doses (e.g. 6.25, 12.5, 20, 100 mg/kg) did not appear to increase the potency of this pharmacologic effect, but the duration of response was longer (consistent with expected pharmacokinetics).
- Due to differences between healthy and malignant cells (e.g. CD20-expression, resistance to ADCC or CDC), these dosing data may not be directly relevant to patients with CLL. However, as a convenient point of reference: the doses tested in the cynomolgus monkey of 1.25, 20, and 100 mg/kg are 4%, 60%, and 300% of the prescription label dose, respectively, when compared on a mg/kg basis. Assuming comparability on a mg/kg basis (and assuming a patient weight of 70 kg): the doses tested in the cynomolgus monkey across studies of 1.25, 6.25, 12.5, 20, and 100 mg/kg are 4%, 22, 44, 70%, and 350% of the prescription label dose, respectively.
- Several of the nonclinical toxicology studies (studies #23542, 28366, 25052) include periodic biopsies of lymph nodes; B-cell depletion in the lymph nodes reflected the depletion observed in circulation.
- In the embryofetal study, fetuses whose mothers were treated on gestation days (GD) 50-100 had low B-cell counts at GD100, ~10% of control counts.
- Ofatumumab causes histological changes: lymphoid atrophy, lymphoid depletion, and follicular atrophy of lymph nodes, Peyer's patches, and spleen.
- These changes are consistent with the pharmacological activity of ofatumumab.
- For patients with CLL, enlarged lymph nodes, liver, and spleen are considered symptoms of the disease (m2.6.1). Therefore, the inverse effects observed in healthy ofatumumab-treated monkeys (i.e. spleen weight loss, lymphoid depletion of the lymph nodes, Peyer's path and spleen) are not considered predictive of adverse effects of ofatumumab when administered to patients with CLL.

B-cell recovery occurred after ofatumumab treatment

- Recovery of circulating B-cells was observed consistently, following cessation of dosing.
- After 2 doses (D1 and D5) of 1.25 to 12.5 mg/kg of ofatumumab, initial recovery was apparent 3 ½ weeks later, and full recovery was seen between 8 and 17 weeks (study #22804).
- After 4 weekly doses (study #23542):
 - For the low-dose (20 mg/kg, equivalent to 60% of the clinical dose), initial signs of B-cell recovery were observed after 4 to 7 weeks, and full recovery was observed in 16 to 20 weeks.

- For the high dose (100 mg/kg, 3-fold the clinical dose), initial signs of B-cell recovery were observed after 9 to 13 weeks, and full recovery was observed in 20 weeks.
- Following treatment in the 7-month study (weekly dosing for 50 days, followed by 5 monthly doses; study #25052):
 - For the low-dose group (20 mg/kg), initial signs of recovery were observed 12 weeks after the end of dosing, followed by a rebound (B-cell counts above baseline).
 - For the high-dose group (100 mg/kg), initial signs of recovery were observed 13-16 weeks after the end of dosing, followed by a rebound period.
 - Full recovery was observed 6 months after the end of dosing at both dose levels.
- Monkeys that developed primate anti-human antibodies (PAHA, i.e. antibodies against the fully human antibody ofatumumab) recovered B-cells more quickly than monkeys that did not develop PAHA.
- Histological evaluation of ofatumumab-treated recovery monkeys found partial or complete recovery of lymphoid tissue atrophy.
- After chronic treatment, a rebound effect was seen in treated monkeys for circulating B-cells and rebound lymphoid cell growth (spleen, Peyer's patches, sternum). This is not considered adverse for this patient population (i.e. patients with CLL); however, no judgment was made for this review regarding whether or not healthy B-cell rebound would be adverse in patients with other indications (such as rheumatoid arthritis).

Ofatumumab increased risk of infection

- Three mortalities in the 7-month study (#25052) were attributed to infection
 - 3/28 treated animals (10.7%)
 - Earliest death was 44 days after initiating treatment
 - The basis for attribution to infection included:
 - Daily observations (i.e. soft feces, decreased activity, heart rate, body temperature, response to antibiotic therapy and supportive care,)
 - Gross pathology and histopathology at necropsy (i.e. spongy lung, froth-filled trachea, inflamed gastrointestinal tissues)
 - Analysis of serum samples for antibodies against *Campylobacter jejuni*
 - Direct tests for *Campylobacter jejuni* and *Balantidium coli*
- In ofatumumab-treated animals immunized against KLH to assess immune function, treatment was associated with a reduced humoral immune response (studies #23542, 28366, 25052):
 - No change observed at the low-dose (60% of the clinical dose), but reduction was seen among high-dose animals (3-fold the clinical dose).
 - After a short duration of dosing (4-weeks), recovery was seen 14 weeks later (study #23542).
 - After chronic dosing (7-months), recovery was not seen as late as 6-months after cessation of dosing (study #25052).

- The decrease in Ig-mediated immunity is consistent with the pharmacology of ofatumumab (i.e. B-cell depletion).
- Histopathologic evaluation of ofatumumab-treated animals detected increased incidences of inflammatory cell infiltrates into various organs; this may be due to some direct aspect of the pharmacology of ofatumumab, but may also be due to increased incidence of subclinical infection.
- Infection is considered clinically monitorable and treatable.
 - This mortality value could be considered a benchmark, given the inherent differences between the model (young healthy monkeys, not pretreated with other chemotherapeutic agents) and patients with CLL who have undergone previous chemotherapies.

Ofatumumab caused infusion-reaction/cytokine response

- Infusion reactions were not seen initially (e.g. one month of dosing), but were seen after 11 weeks, indicating that an immune response developed in the monkeys.
- In the 7-month study (#25052), signs were observed in high-dose animals from D78 onward (the 1st monthly dose) and in low-dose animals from D106 onward (the 2nd monthly dose).
 - Signs were transient (variable range, usually beginning during the 30 minute infusion, and resolving within a few hours of the end of infusion) and occurred sporadically for particular animals
 - 3/28 treated animals exhibited trembling
 - Most treated animals exhibited increased heart rate and increased heart force
 - Notably, monkeys were not pre-treated (i.e. no corticosteroid, antihistamine or anti-inflammatory drugs).
- No clinical observations of infusion reactions were detected in the pilot studies or the 4-week study.
- As supporting evidence, animals treated once with 25-100 mg/kg of ofatumumab exhibited increased levels of activated complement, IL-6, and neutrophil degranulation 15 minutes to 4 hours after treatment (study #23315). No observational data were reported for these animals.
- The monkey's immune response to ofatumumab is unlikely to predict human responses.
- Infusion reaction is clinically monitorable and treatable.
- Based on the effects reported for the clinical trials, the cynomolgus monkey model appears to have underpredicted this risk.
- The monkeys were not pre-treated, and patients will receive pretreatment (corticosteroid, analgesic, antihistamine).

Ofatumumab-treatment was associated with delayed onset anemia

- Anemia was not seen initially (i.e. after 1 month of dosing), but was seen after 7 weeks of treatment, suggesting that the cause may be immune-mediated.
- Recovery of anemia was apparent after cessation of treatment.

- Two mortalities in the 7-month study (#25052) were attributed to hemolytic anemia (on D139 and D268).
- The sponsor suggests this was caused by a humoral immune reaction, and may be a species-specific response (i.e. ofatumumab is a human antibody, and therefore is recognized as foreign by the monkey immune system)
- In the 7-month study, signs of anemia (decreased hemoglobin, red blood cell count, hematocrit and iron; increased reticulocyte count, total bilirubin, and lactate dehydrogenase) were apparent from D50 (after 8 weekly doses) onward, with recovery apparent 6-months after cessation of treatment.
- The results of the other studies are inconsistent for hematology endpoints:
 - Signs of anemia were also reported in treated animals in the cyclic study (first cycle on D1 and 15, second cycle on D148 and 162) immediately after each cycle (study #28366).
 - However, evidence of anemia was not observed in the 4-week study (#23542) or in treated mothers in the embryofetal study (#2148-010), including the two pregnant monkeys that were Coombs test positive.
- Coombs test:
 - Coombs is a direct antiglobulin test: RBCs from the monkeys are mixed with Coombs reagent; agglutination indicates the presence of antibodies or complement on the surface of the RBCs.
 - For the 7-month study (#25052), the assay was used to explore the apparent hemolytic anemia observed. Positive responses were detected for most treated animals when assayed D122-176, and no animals were positive at the end of recovery (D372), indicating recovery. Results of an ELISA assay suggest the ofatumumab may have bound directly to the RBCs of 2 of the Coombs-positive animals.
 - In the subsequent cyclic study (#28366), positive responses were observed as early as D45 for IgG and C3d (a complement protein), and recovery was apparent by D267.
 - In the embryofetal study (#2148-010), only 2/12 treated dams were positive, beginning on gestation day (GD) 50.

Ofatumumab affected fetuses of treated pregnant cynomolgus monkeys

- The embryofetal study treated pregnant cynomolgus monkeys weekly x5 (from GD20 to GD100) with 20 or 100 mg/kg of ofatumumab; Caesarean sections were performed on GD100. Ofatumumab was detected in fetal blood samples, and maternal PK indicates that fetuses were exposed to pharmacologically-active levels from GD50 to GD100. Fetal exposure to ofatumumab caused:
 - Depletion of fetal circulating B-cells (~10% of controls),
 - Decreased spleen weight (-16% for the low-dose group, -27% for the high-dose group)
 - Decreased placenta weight (-10% for the low-dose group, -11% for the high-dose group)
 - Decreased thymus weight for the fetuses of high-dose group mothers (-15% as compared to the control group values)

- Histology was not performed; these weight decreases are presumed to be adverse.

Ofatumumab-treatment increased serum lactate dehydrogenase

- In the 7-month study, lactate dehydrogenase was increased from D25 onward during treatment and during the recovery period; full recovery was apparent 6-months after the last dose.
- This endpoint may reflect anemia (i.e. damage to red blood cells).
- Notably, lactate dehydrogenase is commonly increased in patients with hematopoietic malignancies, and it is considered one of the screening tools to detect CLL.
 - Therefore, the increase in healthy animals/subjects may reflect the pharmacologic activity of ofatumumab.
 - Theoretically, treatment with ofatumumab may confound interpretation of lactate dehydrogenase results (if the treatment-related effect has a mechanism different than the mechanism for CLL)

Ofatumumab-treatment increased serum C-reactive protein

- In the 7-month study, an initial increase (D2) was observed, followed by a return to baseline and a subsequent increase lasting through recovery. This pattern suggests overlapping mechanisms-of-action.
- High CRP is an indicator of inflammation; increased CRP has been associated with rheumatoid arthritis and lupus; notably, anti-CD20 therapies (such as ofatumumab) reportedly have activity in these indications. Although this reviewer would have expected, *a priori*, a decrease in CRP due to ofatumumab, ofatumumab effects on CRP may be related to the pharmacologic activity.
- However, increased CRP is also associated with inflammation caused by infection; therefore, the CRP changes may be at least partially due to the indirect activity of ofatumumab on the immune system i.e. subclinical infection secondary to suppression of humoral immune response.

Points of Discussion/Consideration

- Difference in filtration of aggregates:
 - The clinical protocols (and the prescription label) provide for a filtration step to further remove ofatumumab aggregates. No such step was reported for the nonclinical studies, or for the comparability testing (m3.2.S.4).
 - The sponsor notes (m2.4) that “the primary product-related impurity is an aggregate of ofatumumab, which has been fully characterized and has a limit of $\leq 5\%$ in the drug product specification; levels of aggregate ofatumumab present in drug batches used in the nonclinical toxicology and clinical testing were within this limit, thus supporting the proposed specification.”
 - The sponsor also noted (m2.4) that comparability of the nonclinical and clinical batches, and proposed commercial product, was demonstrated by

- “extensive biochemical analysis, glycan profiling, and a potency assay which measured induction of” CDC.
- The presence of aggregates was documented in the in the nonclinical study reports.
 - The nonclinical GLP toxicology studies provide certificates of analysis for the ofatumumab tested. Different methods were used to assess purity. Purity of the test article was at least 92% (and perhaps as high as >99%).
 - Aggregates were present in the test substance. For the 4-week study (#23542), the 7-month study (#25052), and the embryofetal study (#2148-010), the IgG was 98.2 to 99.2% monomer, 0.57% to 1.8% aggregate. Only the 4-week study (#23542) reported the presence of fragments (0.15%).
 - Based on the lack of severe toxicity in the treated animals, the difference in filtration is not considered a patient safety issue. Theoretically, the presence of aggregates may have contributed to the development of PAHA and positive Coombs results observed (i.e. aggregates being more likely to induce these responses compared to soluble ofatumumab).
 - Lack of postnatal recovery data for animals exposed during gestation
 - Because fetuses were evaluated on GD100, no postnatal B-cell recovery data are available. These data may be important for maximizing the benefit of early childhood immunizations.
 - No data are available regarding the distribution of ofatumumab into milk, or the gastrointestinal absorption of ofatumumab from milk.
 - Apparent inconsistency of the PAHA assay in the 7-month study
 - Only 1/28 treated animals in the 7-month study tested positive for PAHA.
 - In contrast, PAHA were detected in 2/6 treated monkeys in the pilot PK study (study #22804), 5/24 treated monkeys in the 4-week study (#23542), 15/24 treated animals in the cyclic study (#28366) and 3/24 treated mothers in the embryofetal study (#2148-010).
 - These results suggest that the sensitivity of the assay was decreased by the presence of excess ofatumumab in the 7-month study compared to the shorter term studies.
 - Alternative possibilities, considered less likely, include that the longer storage of samples affected the results, or that the PAHA response changed over time.
 - Nonclinical data do not support the dose-selection for the prescription dose
 - The doses of 20 and 100 mg/kg used in the pivotal cynomolgus monkey studies appeared to saturate the CD20 receptors; the magnitude of the pharmacological response did not increase with increasing dose (although the PK, levels of circulating ofatumumab, and the time to recovery of B-cells, were dose-proportional).
 - In the pilot study (#22804), doses as low as 1.25 mg/kg of ofatumumab appeared to saturate the CD20 receptors.

- However, depletion of normal B-cells from healthy monkeys may not adequately predict the effect of ofatumumab on CLL in patients.
- Does ofatumumab bind to a receptor on cynomolgus monkey RBCs?
 - Data in favor of this conclusion:
 - The Coombs data indicate IgG binding to RBCs (not necessarily ofatumumab IgG).
 - An ELISA conducted as part of the 7-month study indicated that ofatumumab bound RBCs for 2 of the Coombs-positive animals.
 - Data weighing against this conclusion:
 - The sponsor did not conduct a full, stand-alone tissue cross-reactivity study using monkey tissues. However, immunohistochemistry was performed on select tissues (lymphoid organs and negative control tissues) collected at necropsy in the GLP monkey toxicology studies, and ofatumumab-binding to RBCs was not reported.
 - The sponsor conducted multiple flow cytometry assays using blood and blood cells; ofatumumab-binding to RBCs was not reported (but may have been attributed to background).
 - Theoretically, RBCs may express low-levels of CD20, or express another protein with homology to CD20, such that low-levels of ofatumumab bind directly to RBCs.
 - The sponsor reported that CD20 has homology with IgE receptor (Fc*RI; reportedly expressed on basophils, mast cells, eosinophils, and some antigen-presenting cells) and HTmM4 (reportedly expressed on lymphoid and myeloid cells).
 - The potential significance of this finding to the safe use of ofatumumab in humans is unclear.
 - Based on the current understanding of the observed toxicities of ofatumumab, no further nonclinical testing to elucidate this issue is recommended.

2.6.6.10 Tables and Figures

Please see the other sections for tables and figures related to this review.

2.6.7 TOXICOLOGY TABULATED SUMMARY

The sponsor provided m2.6.7 Toxicology Tabulated Summary, which consists of 11 tables spanning 52 pages. Copying their summary into this review is impractical. As a brief summary, tables 1 and 4 are copied here:

Table 1. Toxicology Test Article: Ofatumumab

Type of Study	Species (strain)	Animals/Sex/Group	Method of Administration	Dose (mg/kg)	No. of Doses (Duration of Dosing)	GLP	Testing Facility	Report No. (Study No.)	Location Module
Repeat Dose Toxicity									
Pilot 4 day repeat dose	Monkey (cynomolgus)	1	Intravenous (infusion)	1.25, 6.25, 12.5 ^a	4 doses (4 days)	No	7	22804 (663784)	m4.2.3.2
Pilot dose-ranging	Monkey (cynomolgus)	1M	Intravenous (infusion)	25/50 ^b	2 doses (5 days)	No		23315 (664395)	m4.2.3.2
		1F		75/100 ^c					
		1M		100/150 ^d					
		1F							
4 Week repeat dose	Monkey (cynomolgus)	3 ^M /3 ^F ^e	Intravenous (infusion)	0, 20, 100	4 doses (22 days)	Yes		23542 (663805)	m4.2.3.2
7 Month repeat dose	Monkey (cynomolgus)	3 ^M /4 ^R	Intravenous (infusion)	0, 20, 100	8 weekly doses (50 days) followed by 5 monthly doses (140 days)	Yes		25052 (665493)	m4.2.3.2
Cycled repeat dose	Monkey (cynomolgus)	3 ^M /3 ^R	Intravenous (infusion)	0, 20, 100	4 doses (2 cycles of 2 doses given 2 weeks apart) ^f	Yes	L	28366 (509814)	m4.2.3.2
2 Week subcutaneous and intravenous repeat dose bridging	Monkey (cynomolgus)	3M/3F ^g	Subcutaneous	0, 20, 100	2 doses (15 days)	Yes	GSK	CD2007/01024/00 (G07285)	m4.2.3.2
			Intravenous (infusion)	100					

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Table 1 (Continued). Toxicology Test Article: Ofatumumab

Type of Study	Species (strain)	Animals/Sex/Group	Method of Administration	Dose (mg/kg) or Concentration	No. of Doses (Duration of Dosing)	GLP	Testing Facility	Report No. (Study No.)	Location Module
Reproductive and Developmental Toxicity									
Embryofetal development	Monkey (cynomolgus)	12F	Intravenous (infusion)	0, 20, 100	5 doses (once weekly from Day 20 to Day 50 of gestation)	Yes	7	NA (2148-010)	m4.2.3.5.2
Other Toxicity Studies									
Immunotoxicity									
Determination of inflammatory and coagulation parameters	Monkey (cynomolgus)	1M	Intravenous (infusion)	25	Single dose	No	GEN	GMB-3001-013 v2	m4.2.3.7.7
		1F		50					
		1M		75					
		1F		100					
Other Studies									
Immunohistochemistry									
Immunohistochemical cross-reactivity	Human tissues	NA	In vitro	0.5, 5.0, 20.0 µg/mL	NA	Yes	L	23179 (630757)	m4.2.3.7.7
Immunogenicity									
Detection of ADA in monkey serum ^f	Monkey (cynomolgus)	3 ^M /4 ^R	Intravenous (infusion)	0, 20, 100	8 weekly doses (50 days) followed by 5 monthly doses (140 days)	No	L	GMB3001-021 (NA)	m4.2.3.7.1

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Key: NA = Not applicable M = Main group animals R = Recovery animals
 a = In this study, rituximab was also administered at the same dose levels
 b = Day 1 dose/Day 5 dose
 c = 5 animals/sex (3^M/2^F) in the control group
 d = The second cycle commenced when at least 75% of the animals attained B cell counts of at least 50%
 e = 4 control females (2^M/2^F)
 f = Serum samples used in this assay were from the 7 month repeat dose study
 g = Serum samples used in this assay were collected from the pilot dose-ranging toxicity study, following dosing on Day 1.

Testing Laboratory:
 GEN = Genmab

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Table 4. Toxicology: Drug Substance

Test Article: Ofatumumab

Batch No.	Purity* (% IgG)	Specified Impurity			M3.2.S.4.4 ^d Reference	Report No. (Study No.)	Type of Study
		≤100 ng /mg	≤5 ng/mg	≤5 pg/mg			
Proposed Specification:	≥95%						
L08131/27	✓	NT	NT	NT	Table 8	22804 (663784)	IV pilot 4 day repeat dose toxicity
L09136/10	}	NT	NT	NT	Table 8	23315 (664395) 23542 (663805)	IV pilot dose-ranging IV 4 week repeat dose
L09167/15		NT	NT	NT	Table 8	23542 (663805)	IV 4 week repeat dose
22461		ND ^b /NT ^c	✓	✓	Table 9	25052 (665493)	IV 7 month repeat dose
37717		ND ^b /NT ^c	✓	✓	Table 4	28366 (509814) NA (2148-010)	IV cycled repeat dose IV embryofetal development
48885		✓	✓	<0.4	Table 10	CD2007/01024/00 (G07285)	2 dose SC and IV
L08131/15		NT	NT	NT	Table 8	23179 (530757)	Immunohistochemical investigation of cross-reactivity in human tissues

Key:

NT = Not tested; ND = None detected

IV = intravenous; SC = subcutaneous

a = SDS-PAGE (reduced)

b = (Western blot)

c = (ELISA)

d = Full batch analysis data may be found in m3.2.S.4.4, Batch Analysis.

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OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

- The cynomolgus monkey is a pharmacologically-relevant model in which to assess the activity of ofatumumab. In the nonclinical monkey studies:
 - Ofatumumab depleted B-cells in peripheral blood and lymph nodes.
 - Recovery was observed by 6-months after cessation of dosing.
 - Ofatumumab appeared to increase the risk of infection and risk of death from infection.
 - Ofatumumab inhibited the humoral immune response to KLH; the cellular immune response appeared unaffected.
- The sponsor tested an adequate range of doses in the cynomolgus monkey model to predict effects in humans.
 - Ofatumumab will be administered to patients by intravenous infusion at an initial dose of 300 mg/patient, followed by 7 weekly doses of 2000 mg/patient, then 4 monthly doses of 2000 mg/patient. The doses tested nonclinically (in the 4-week study, the 2-cycle study, the 7-month study, and in the embryofetal study) are the equivalent of 0.7-fold and 3.5-fold the clinical dose when scaled by body weight (assuming a patient weight of 70 kg).
 - As noted by the sponsor (m2.4): the highest dose of ofatumumab evaluated in the pivotal 7 month study was 100 mg/kg. This corresponds to a mean $AUC_{0-\infty}$ for the final dosing interval ranging from approximately 810 to 900 mg*h/mL and a mean C_{max} ranging from approximately 3100 to 3500 µg/mL. "This represents parity and 2-fold exposure, in terms of AUC and C_{max} , respectively, of the human therapeutic exposure observed at a dose of 2000 mg, the highest clinical dose." (m2.4).
 - The elimination half-life ($T_{1/2}$) of ofatumumab was approximately 16 days in cynomolgus monkeys. This may not be predictive for patients with CLL.
- The sponsor tested ofatumumab for an adequate duration in the cynomolgus monkey model to predict effects in humans.
 - The longest duration of testing was 7 months (5 weekly doses + 5 monthly doses).

Unresolved toxicology issues:

- Ofatumumab has been shown to cross the placenta, and deplete fetal B-cells. No data regarding postnatal recovery following fetal exposure are available.
 - For infants prenatally exposed to ofatumumab, information regarding the potential effects of B-cell depletion on infant-immunizations may be useful.
 - The sponsor noted (m1.9.1) that for patients with CLL, the median age at diagnosis is 72 years; this disease is extremely rare in patients younger

than 40 years of age. This information was considered in assessing the need for and relevance of nonclinical reproductive and developmental toxicology data to support the treatment of patients with CLL.

- Ofatumumab is the subject of (/ IND () (for the treatment of active RA; DAARP is the review division); DBOP and DARRP have communicated internally for consistency of review and advice, and DBOP consulted with Pediatric and Maternal Health Staff (PMHS) regarding the current draft labeling and in anticipation of future labeling. This reviewer recommended that DAARP consider recommending that the sponsor submit a nonclinical developmental toxicity study to assess the effects of prenatal exposure to ofatumumab on postnatal development prior to the treatment of large numbers of women who are or may be pregnant, or who may become pregnant shortly after taking ofatumumab. DBOP suggested that DAARP consider recommending that the assessment include evaluation of the effects of ofatumumab on immune function, specifically on the humoral response of infant animals from ofatumumab treated dams to immune challenge, to provide information useful relevant to infant vaccination scheduling.
- After weeks of treatment with ofatumumab, monkeys developed transient infusion-reaction-like signs (increased heart rate, increased heart force, tremors) and anemia. If these effects are secondary to the development of immune-responses to ofatumumab, and if these effects are relevant to humans, then chronically-treated patients who develop anti-ofatumumab antibodies may develop similar symptoms. Although the sponsor notes that the potential for patients developing ADA exists, the sponsor considers the risk to be low based on the primary sequence of ofatumumab, the mechanism of action, and the intended patient population (m2.5 Clinical Overview, subsection 3.5 Immunogenicity). The sponsor notes that two of 274 subjects tested positive for ADA using one assay and 0 of 45 subjects tested positive using another assay, but the assays had technical limitations (e.g. interference by circulating ofatumumab).

b(4)

Recommendations: Approval of Biologics Licensing Application (BLA) STN 125326 is recommended. No basis for objection was identified in the review of the nonclinical pharmacology and toxicology sections of the original BLA submission and amendments. Nonclinical toxicities of ofatumumab are extensions of the pharmacology of the product, were reflected in the clinical studies, and are monitorable in the clinical setting.

Suggested labeling: No additional labeling suggestions are documented in this review. The table below documents draft revisions (revised by FDA's BLA review team) to the original proposed labeling relevant to and supported by this nonclinical review and the draft revised label.

Section	Original	Revised
Highlights	[omitted]	USE IN SPECIFIC POPULATIONS

b(4)

Signatures (optional):

Reviewer Signature Andrew J McDougal 8/10/2009

Supervisor Signature [Signature] 8/10/09 Concurrence Yes Y No

APPENDIX/ATTACHMENTS

None.

b(4)

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

None.

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

NDA/BLA Number:

Applicant:

Stamp Date:

STN 125326

GSK

1/30/2009

Drug Name:

NDA/BLA Type:

Ofatumumab (HuMax-CD20)

BLA

On initial overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	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		Yes but see comment below.
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		
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		Claim made by sponsor in Module 2.6.1, and in the referenced m2.6.6s8.2.3 Comparability and Impurities. The actual batch analysis summary is at m3.2.S.4.4.
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		Intravenous 30-minute infusion. Claim made by sponsor in Module 2.6.1 p 4
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	Pivotal studies are GLP, but not explicitly 21CFR58 compliant. See comments below.

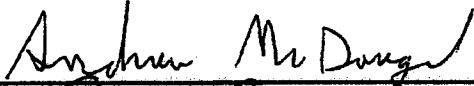
**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

3. In Module 4.3.3.2, study # "Pilot and pharmacokinetic study in Cynomolgus monkeys using HuMax-CD20" is not fully legible.
 - a. The pdf file does not appear to be loading correctly. Pages 274-313 have black boxes instead of graphs.
 - b. Pages 314-359, 361-369, and the signatures on pp 5 and 394 are not adequately readable (poor-quality image capture).
4. All but one (study #CD2007-01024) of the GLP nonclinical studies state performance in accordance with the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice, but do not explicitly state compliance with 21CFR58.

Provide a brief statement for each GLP report, indicating either that it is fully in compliance with US FDA GLP or detailing each deviations beyond those already documented in the study reports. Also provide statements of compliance for each subreport (e.g. histology, PK).

For each GLP study conducted outside the U.S., indicate whether the test facility has been inspected by US FDA and/or another US regulatory agency.

- a. Study #25052 "HuMax-CD20: multiple dose intravenous toxicity study in Cynomolgus monkeys followed by a 6 month recovery period"
- b. Study #23542, "HuMax-CD20: multiple dose toxicity study in Cynomolgus monkeys with a 6 month recovery period"
- c. Study #28366, "HuMax-CD20: cycled repeat dose toxicity study in Cynomolgus monkeys with a 4 month recovery period"
- d. Study #231379 "HuMax-CD20: an immunohistochemical investigation of cross-reactivity in human tissues"
- e. Study #2148-010, "Intravenous embryo-fetal development study with HuMax-CD320 in the Cynomolgus monkey"



Reviewing Pharmacologist

2/20/09

Date



Team Leader/Supervisor

2/23/2009
Date