# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

# 761103Orig1s000

# NON-CLINICAL REVIEW(S)

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

# PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

| | |
|---|---|
| Application number: | 761103 |
| Supporting document/s: | 1 |
| Applicant's letter date: | July 25, 2018 |
| CDER stamp date: | July 25, 2018 |
| Product: | Ruxience, a proposed biosimilar to Rituxan |
| Indication: | See reference product |
| Applicant: | Pfizer |
| Review Division: | Division of Hematology Oncology Toxicology (DHOT) |
| Reviewer: | Pedro L Del Valle, PhD |
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# 1 Executive Summary

## 1.1 Introduction

Pfizer is requesting marketing approval for Ruxience (also known as PF-05280586 or rituximab), a proposed biosimilar product to the reference product US-licensed Rituxan. Rituxan (BLA 103765 by Genentech, Inc) was approved in the US in 1997 and the current label includes several indications and usage information related to hematological malignancies [Non-Hodgkin's Lymphoma (NHL) and Chronic Lymphocytic Leukemia (CLL)], rheumatoid arthritis (RA) in combination with methotrexate, and Wegener's Granulomatosis and microscopic polyangiitis (MPA). Pfizer is not seeking approval for the indication of pemphigus vulgaris as this indication was approved recently and granted an orphan drug designation with exclusivity rights.

## 1.2 Brief Discussion of Nonclinical Findings

PF-05280586 (Rituximab-Pfizer) is a genetically engineered chimeric mouse/human immunoglobin G1kappa (IgG1k) monoclonal antibodies directed against the CD20 antigen with the intended effect of depleting B cells. CD20 is a 32-kDa, non-glycosylated transmembrane phosphoprotein, located on the surface of normal precursor-B cells, mature B lymphocytes and malignant B cells but it is not located on hematopoietic stem cells, pro-B-cells, normal plasma cells or other normal cells. CD20 does not internalize upon antibody binding and is not shed from the cell surface. CD20 does not circulate in the plasma as a free antigen and, consequently, does not compete for antibody binding. Rituximab binds to a discontinuous conformational epitope on CD20, comprising amino acid residues 170-173 (ANPS) and 182-185 (YCYSI). Upon binding, rituximab initiates multiple immune effector functions including antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC) leading to target cell lysis.

Pfizer conducted a GLP-compliant comparative nonclinical single-dose toxicokinetic and tolerability study and a GLP-compliant 4-week toxicity and toxicokinetic study in cynomolgus monkeys that included a comparison of the potential toxicity, local tolerance, hematology and immunophenotyping assessment of absolute lymphocyte counts and B cells as PD biomarker, PK/TK, and immunogenicity of PF-05280586 and rituximab-EU. The studies compared the effects of PF-05280586 to rituximab-EU (MabThera), but not rituximab-US because, according to Pfizer, the physicochemical and functional data showed that PF-05280586, rituximab-US, and rituximab-EU were all similar to each other in the in vitro assays that evaluated biological activity and Fc-based functionality. PF-05280586 and rituximab-EU were administered by the IV route because it is consistent with the clinical route of administration. PF-05280586 and rituximab-EU produced similar PD effects and safety data in both toxicity and toxicokinetic studies in monkeys. No biologically-relevant or systemic exposure differences occurred between test articles. In addition, the in vitro pharmacology of PF-05280586, as compared to rituximab-US and rituximab-EU, was assessed with respect to its Fab and Fc-based functionality in several functional and binding assays (see CMC review). In vivo primary and secondary PD studies were not conducted. From the

perspective of nonclinical pharmacology and toxicology, there are no residual uncertainties regarding the similarity of PF-05280586 and rituximab-EU.

In the single-dose toxicology study in cynomolgus monkeys, comparative doses were 2, 10 or 20 mg/kg. Observations of red or swollen at the injection site occurred across groups including controls (primarily in males) for both test articles on Day 1 that resolved by Day 3. A minimal to mild decrease (29 -54% decrease from Week -1) in group mean absolute total lymphocyte counts occurred on Day 4 with no clear dose-response that corresponded with decreased absolute B cell values determined by immunophenotyping. The decrease in peripheral blood absolute total lymphocyte counts on Day 4 were consistent with the expected pharmacologic effect of the test articles. No clear test article-related lymphocyte effects occurred at other time points due to inter-animal variability and procedure-related changes. By Day 92, the increase in individual animal CD3-CD20+CD40+ B cells (compared to Day 4 values) ranged from 46.6-174.3% (rituximab-Pfizer) and 18.3-220% (rituximab-EU). Rituximab-Pfizer and rituximab-EU appeared similar in all immunophenotyping parameters tested (both the non-GLP and GLP markers). No consistent sex-related differences in systemic exposure occurred after a single dose of either test article. The group mean ratios (males/females) of serum test article concentrations between dose-matched males and females at the 2, 10, and 20 mg/kg dose levels were 0.9, 1.4, and 1.4 for rituximab-Pfizer $AUC_{0\text{-}672h}$ values and 1.0, 1.2, and 1.9 for rituximab-EU $AUC_{0\text{-}672h}$ values. Exposure ratios (rituximab-Pfizer - rituximab-EU) calculated based on group mean values ranged from 0.92 to 1.24. All monkeys that received either test article were positive for anti-drug antibodies (ADA) by Day 29 and remained positive for ADA through the last collection time point (Day 85). Circulating levels of ADA may have interfered with the detection of test article after Day 29 (672 h).

In the repeat-dose toxicology study in Cynomolgus monkeys, the comparative dose was 20 mg/kg given weekly for 5 doses. Clinical signs included non-adverse, transient, higher incidence of vomitus (containing food, foamy, yellow, or clear) or emesis on Day 22 after administration of the fourth dose of either test article and low incidence of red or swollen observations at the injection site across groups including controls (primarily in males) for both test articles on different days. A trend for lower group mean absolute lymphocyte counts occurred in rituximab-Pfizer and rituximab-EU compared to concurrent vehicle controls. A decrease in absolute lymphocyte counts was also observed during the recovery phase. Immunophenotyping assessments showed decreases in mean absolute B cell values compared with predose values or concurrent controls that suggested possible test article-related effects, but due to the large variability in values, these changes could not clearly be attributed to administration of rituximab-Pfizer or rituximab-EU. Rituximab-Pfizer and rituximab-EU appeared similar in all immunophenotyping parameters tested (both the non-GLP and GLP markers). No sex differences in systemic exposures after single or repeat dosing of either test article occurred although there was high inter-animal variability. After weekly IV dosing, mean exposure ratios ($AUC_{168h}$) in males and females on Day 22 relative to Day 1 (Day 22/Day 1) were 0.9 and 1.0, respectively for rituximab-Pfizer, and 1.2 and 1.8, respectively, for rituximab-EU. Exposure ratios (rituximab-Pfizer-rituximab-EU)

calculated based on group mean values ranged from 0.94 to 1.04 after a single dose (Day 1) and from 0.67 to 0.79 after multiple doses on Day 22. The overall induction of an immune response (dosing and recovery phases) was 79% (11 of 14 animals) in the rituximab-Pfizer group and 43% (6 of 14 animals) in the rituximab-EU group. ADA-positive animals declined from 10/14 animals on Day 22 to 1/14 animals on Day 30 (recovery Day 1) in the rituximab-Pfizer group and from 5/14 animals to 2/14 animals in the rituximab-EU group. At the end of the dosing phase, decreased mean absolute and relative spleen weights ranged from 13 to 17% lower in males and 30 to 42% lower in females for rituximab-Pfizer, and 39 to 44% lower in males and 15 to 25% lower in females for rituximab-EU, compared with respective controls. At the end of the recovery phase, decreases in spleen weight were still present in rituximab-Pfizer males and rituximab-EU females. The lower spleen weights corresponded with microscopic findings of decreased cellularity and decreased CD20 cells found in the spleen and mesenteric and axillary lymph nodes of rituximab-Pfizer- and rituximab-EU-dosed monkeys. At the end of the recovery phase, those microscopic findings were still present albeit with less severity and incidence compared to terminal sacrifice.

The nonclinical data submitted to the BLA demonstrate that PF-05280586 is similar to rituximab-EU.

### 1.3 Recommendations

#### 1.3.1 Approvability

Approvable. From the Pharmacology/Toxicology perspective there are no residual uncertainties regarding the similarity of PF-05280586 to rituximab-EU.

#### 1.3.2 Additional Non Clinical Recommendations

None

#### 1.3.3 Labeling

The nonclinical sections of the label will be comparable to the label of the reference product US-licensed Rituxan.

## 2 Drug Information

### 2.1 Drug

CAS Registry Number
174722-31-7

Code Name
PF-05280586 or rituximab-Pfizer or PF7

International Nonproprietary Name (INN) for licensed product
Rituximab

INN name for Pfizer Biosimilar
To be determined

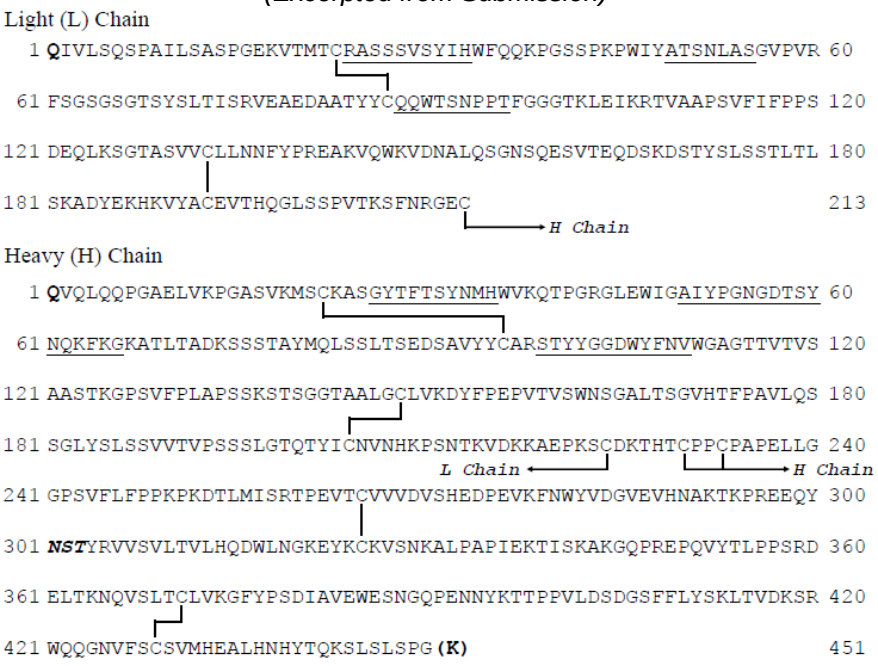
Chemical Name (IUPAC)
Not Applicable

Molecular Formula/Molecular Weight
The experimental molecular masses of the three predominant N-linked glycoforms are 147080.2, 147240.3 and 147403.1 Da.

Structure or Biochemical Description
PF-05280586 is an IgG1 kappa monoclonal antibody composed of complementarity-determining regions derived from mouse anti-human B-lymphocyte antigen CD20 monoclonal antibody, and the framework and constant regions derived from human IgG1 produced in Chinese Hamster Ovary (CHO) cell line.

**Figure 1 PF-05280586 Primary Structure (Amino Acid Sequence)**

*(Excerpted from Submission)*




The predicted intra- and inter-chain disulfide bonds are illustrated with connecting lines. Q is bolded to represent pyroglutamic acid, the putative complementarity-determining regions are underlined, the N-linked glycosylation consensus sequence appears in bold italics, and the C-terminal K is shown in parentheses.

Pharmacologic Class
CD20-directed cytolytic antibody

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 110426

## 2.3 Drug Formulation

The drug product contains no preservative and is for single use only.

**Table 1 Composition of PF-05280586 Drug Product, 100 mg/10 mL and 500 mg/50 mL**

*(Excerpted from Submission)*

| Ingredient | Reference to Standard | Function | Unit Formula (mg/mL) |
|---|---|---|---|
| PF-05280586 | In-house specification | Active ingredient | 10 |
| L-histidine | Ph. Eur., USP, JP | (b) (4) | 1.20 |
| L-histidine hydrochloride monohydrate | Ph. Eur., JP | | 2.57 |
| Edetate disodium dihydrate | Ph. Eur., USP, JP | | 0.056 |
| Polysorbate 80 | Ph. Eur., NF, JP | | 0.2 |
| Sucrose | Ph. Eur., NF, JP | | 85 |
| Water for Injection | Ph. Eur., USP, JP | | q.s. to 1 mL |

## 2.4 Comments on Novel Excipients

None; the excipients used are all compendial.

## 2.5 Comments on Impurities/Degradants of Concern

None

## 2.6 Proposed Clinical Population and Dosing Regimen

Pfizer proposes clinical populations and dosing regimens that are consistent with current US-licensed Rituxan labeling except for the indication of moderate to severe pemphigus vulgaris in adult patients. See approved US-licensed Rituxan label for more detailed information.

## 2.7 Regulatory Background

BLA 761103 was submitted on July 25, 2018 for the biologic product PF-05280586 under Section 351(k) of the Public Health Service Act (42 U.S.C. 262(k)). A Type B pre-IND meeting was held on March 23, 2011 with the Division of Pulmonary, Allergy and Rheumatology Products and the Division of Hematology Products to discuss the regulatory development of PF-05280586 as a biosimilar. IND 110426 was submitted for PF-05280586 (Rituximab-Pfizer) on December 27, 2011. A Biosimilar Product Development (BPD) Type 2 meeting was held on May 16, 2017 and a BPD Type 4 was held on April 6, 2018 where the overall content and format of the planned 351(k) BLA for PF-06881893 was discussed.

Pfizer is seeking approval for PF-05280586 as a proposed biosimilar product to the single reference biologic product US-licensed Rituxan. To fulfill the nonclinical requirements for a biosimilar BLA, Pfizer submitted a single-dose toxicokinetic and

tolerability study in Cynomolgus monkeys with a 13-week observation period and a 4-week repeat-dose toxicity study in Cynomolgus monkeys with a 13-week recovery phase comparing rituximab-Pfizer to EU-licensed MabThera (rituximab-EU). The repeat-dose toxicity study included an assessment of the potential toxicity, toxicokinetic, pharmacodynamics, and potential associated immunogenicity of rituximab-Pfizer compared to rituximab-EU.

# 3 Studies Submitted

## 3.1 Studies Reviewed

| Study# | Title | Module |
|---|---|---|
| | **Toxicology** | |
| 8246556 (11GR111) | Single-Dose Intravenous Toxicokinetic and Tolerability Study with Rituximab-Pfizer and Rituximab-EU in Cynomolgus Monkeys with a 13-Week Observation Period | 4.2.3.1 |
| 8246557 (11GR112) | 4-Week Intravenous Injection Toxicity and Toxicokinetic Study with Rituximab-Pfizer and Rituximab-EU in Cynomolgus Monkeys with a 13-Week Recovery Phase | 4.2.3.2 |

## 3.2 Studies Not Reviewed

| Study# | Title | Module |
|---|---|---|
| | **Pharmacokinetics** | |
| 15-1096 | Determination of Rituximab in Cynomolgus Monkey Serum By ELISA | 4.2.2.1 |
| 15-1097 | The Validation of an ECL Assay for the Detection of Anti-Drug Antibodies (ADA) Directed Against Mabthera (Rituximab-EU) in Monkey Serum | 4.2.2.1 |
| 15-1098 | The Validation of an ECL Assay for the Detection of Anti-Drug Antibodies (ADA) Directed Against PF-05280586 (Rituximab-Pfizer) in Monkey Serum | 4.2.2.1 |
| | **Toxicology** | |
| 17GR338 | Investigative Report: Single Nucleotide Polymorphisms in Cynomolgus monkeys receiving Rituximab-Pfizer and Rituximab-EU in Studies 11GR111 and 11GR112 | 4.2.3.7.7 |

## 3.3 Previous Reviews Referenced

None

# 4 Pharmacology

## 4.1 Primary Pharmacology

The in vitro pharmacology of PF-05280586, as compared to rituximab-US and rituximab-EU, was assessed with respect to its Fab- and Fc-based functionality in a number of functional and binding assays. In each assay, the results for PF-05280586 were compared for similarity to rituximab-US and rituximab-EU. These studies were submitted to Module 3, Section 3.2.R.3.2.2 Biological Activity and reviewed by the

Product Quality team. The evaluation of a well-established pharmacodynamic marker (depletion of peripheral blood B cells) for rituximab was incorporated into the comparative 4-week toxicology study. No other studies were submitted for review.

### 4.2 Secondary Pharmacology

No studies were submitted for review.

### 4.3 Safety Pharmacology

Comparative safety pharmacology studies with PF-05280586 and Rituxan were not performed.

# 5 Pharmacokinetics/ADME/Toxicokinetics

PK/TK assessment of PF-05280586 was included in the single-dose and the repeat-dose toxicology studies in Cynomolgus monkeys.

# 6 General Toxicology

### 6.1 Single-Dose Toxicity

**Study title: Single-Dose Intravenous Toxicokinetic and Tolerability Study with Rituximab-Pfizer and Rituximab-EU in Cynomolgus Monkeys with a 13-Week Observation Period**

| | |
|---|---|
| Study no.: | 8246556 (11GR111) |
| Study report location: | eCTD 4.2.3.1 |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | May 11, 2011 |
| GLP compliance: | Yes. Report amended on March 11, 2016 |
| QA statement: | Yes |
| Drug, lot #, and % purity: | PF-05280586; 87201; 110.2 mg/mL protein concentration<br>MabThera; H0104B01; 10 mg/mL protein concentration |

**Key Study Findings**

Rituximab-Pfizer and rituximab-EU produced decreases in total lymphocyte counts that corresponded with decreases in B cells. No biologically-relevant or systemic exposure differences occurred between test articles.

Methods

| | |
|---|---|
| Doses: | 0, 2, 10, or 20 mg/kg each test article |
| Frequency of dosing: | Single dose |
| Route of administration: | Intravenous bolus injection |
| Dose volume: | 2 mL/kg |
| Formulation/Vehicle: | PF-05280586 diluted in 20 mM histidine, 8.5% w/v sucrose, 0.15 mM EDTA, and 0.02% w/v polysorbate 80, pH 5.8, in water<br>MabThera diluted in 25 mM sodium citrate, 0.9% w/v sodium chloride, and 0.07% w/v polysorbate 80, pH 6.5, in water |
| Species/Strain: | Cynomolgus monkeys (*Macaca fascicularis*) |
| Number/Sex/Group: | 3/sex/group |
| Age: | 4-5 years old |
| Weight: | M: 5.2 to 9.0 kg; F: 3.1 to 5.2 kg |
| Unique study design: | None |
| Deviation from study protocol: | Protocol deviations neither affected the overall interpretation of study findings nor compromised the integrity of the study |

## Results

| **Parameters** | **Major findings** |
|---|---|
| **Mortality** | All animals survived through Day 92. |
| **Clinical Signs** | No test-article related clinical signs.<br>Red or swollen injection sites occurred across groups including controls (primarily in males) for both test articles on Day 1 that resolved by Day 3. |
| **Body Weights** | No differences occurred among groups or between the two test articles. |
| **Hematology** | A minimal to mild decrease (29 -54% decrease from Week -1) in group mean absolute total lymphocyte count occurred on Day 4 in monkeys administered 2, 10, or 20 mg/kg rituximab-Pfizer or rituximab-EU. This finding corresponded with decreased peripheral B cells in immunophenotyping data (See Table 2). A clear dose-dependent response was not identified on Day 4. No clear test article-related lymphocyte effects occurred at other time points due to inter-animal variability and procedure-related changes.<br><br>Decreased peripheral blood absolute total lymphocyte counts on Day 4 were consistent with the expected pharmacologic effect of the test articles. No biologically-relevant differences were observed between rituximab-Pfizer and rituximab-EU.<br><br>Decreases of group mean absolute total lymphocyte count > Day 4 compared to Week -1:<br>Rituximab-Pfizer 20 mg/kg: Males 16-30% Day 92; Females 23-36% Day 57.<br>Rituximab-EU 20 mg/kg: Males 22-38% Day 57; all dose-groups Females 18-36% Day 92 |

**Immunophenotyping**

**Table 2 Percent Decrease of Absolute B Cell Values compared to Week -1 over all three dose levels both sex combined**

*(Excerpt from Submission)*

| | Rituximab-Pfizer | Rituximab-EU |
|---|---|---|
| CD3-CD20+CD40+ (range of individual animal values) | | |
| Day 4 | 100% | 100% |
| Day 15 | 80-100% | 81-100% |
| Day 92 | 0-53% | 0-82% |
| CD3-CD20+ (range of individual animal values) | | |
| Day 4 | 97-100% | 93-100% |
| Day 15 | 80-100% | 79-100% |
| Day 92 | 0-72% | 0-65% |
| CD3-CD19+ (range of group means) | | |
| Day 4 | 79-94% | 86-90% |
| Day 15 | 78-95% | 74-94% |
| Day 92 | 0-40% | 0-23% |
| CD3-CD40+ (range of group means) | | |
| Day 4 | 81-94% | 86-91% |
| Day 15 | 77-96% | 80-94% |
| Day 92 | 0-32% | 0-61% |

Note: Where there was no decrease from baseline (i.e. an increase) values are represented as zero.

Time-Course for repopulation of CD3-CD20+CD40+ B cell subset of either test article
Dose-related:
2 mg/kg on Day 15
10 mg/kg on Day 29
20 mg/kg on Day 57

By Day 92, the increase in individual animal CD3-CD20+CD40+ B cells (compared to Day 4 values) ranged from 46.6-174.3% (rituximab-Pfizer) and 18.3-220% (rituximab-EU).
In all immunophenotyping parameters tested (both the non-GLP and GLP markers) rituximab-Pfizer and rituximab-EU appeared similar.

**Coagulation, Clinical Chemistry, and Urinalysis**

No test articles-related changes occurred.

**Toxicokinetics**

No consistent sex-related differences in systemic exposure occurred for either test article. Exposure ratios were calculated based on group mean values presented in the following table.

**Table 3 Mean Toxicokinetic Parameters of Rituximab in Cynomolgus Monkeys**

| Dose (mg/kg) | $C_{max}$ (ng/mL) | $t_{1/2}$ (h) | $AUC_{672h}$ (ng/mL*h) | $AUC_{1344h}$ (ng/mL*h) |
|---|---|---|---|---|
| | Rituximab-Pfizer | | | |
| 2 | 74,000 | 46.6 | 5,930,000 | 5,930,000 |
| 10 | 481,000 | 53.6 | 48,800,000 | 48,900,000 |
| 20 | 912,000 | 82.8 | 101,000,000 | 102,000,000 |
| | Rituximab-EU | | | |
| 2 | 80,300 | 43.2 | 6,230,000 | 6,230,000 |
| 10 | 497,000 | 50.0 | 48,800,000 | 48,800,000 |
| 20 | 726,000 | 69.8 | 81,700,000 | 82,200,000 |

| | |
|---|---|
| | <table><tr><td></td><td colspan="4">Ratio Rituximab-Pfizer / Rituximab-EU</td></tr><tr><td>2</td><td>0.92</td><td></td><td>0.95</td><td>0.95</td></tr><tr><td>10</td><td>0.97</td><td></td><td>1.00</td><td>1.00</td></tr><tr><td>20</td><td>1.26</td><td></td><td>1.24</td><td>1.24</td></tr></table> |
| **Anti-Drug Antibody (ADA)** | All animals given either test article were positive by Day 29 (672 hours postdose) and remained positive for ADA through the last collection time point (Day 85). Circulating levels of ADA may have interfered with the detection of test article after Day 29. |
| **Dose Formulation Analysis** | Dose formulations for both test articles were within ±10% of the target concentrations (mean results ranging from 95.6 to 99.7% of target). The test articles were not detected in any vehicle control article formulations. All formulations met the acceptance criteria for study use. |
| **Anatomic Pathology** | Not provided since all animal survived through Day 92. |

## 6.2 Repeat-Dose Toxicity

**Study title: 4-Week Intravenous Injection Toxicity and Toxicokinetic Study with Rituximab-Pfizer and Rituximab-EU in Cynomolgus Monkeys with a 13-Week Recovery Phase**

| | |
|---|---|
| Study no.: | 8246557 (11GR112) |
| Study report location: | eCTD 4.2.3.2 |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | May 17, 2011 |
| GLP compliance: | Yes. Report amended on March 11, 2016 |
| QA statement: | Yes |
| Drug, lot #, and % purity: | PF-05280586; 87201; 110.2 mg/mL protein concentration<br>MabThera; H0104B01; 10 mg/mL protein concentration |

### Key Study Findings

- Test article-related changes were consistent with the expected pharmacologic activity of an anti-CD20 monoclonal antibody and the ADA incidence was similar between the test articles.
- Toxicological and pharmacodynamic responses to rituximab-Pfizer and rituximab-EU appeared similar

Methods

| | |
|---|---|
| Doses: | 0 or 20 mg/kg each test article |
| Frequency of dosing: | Weekly for 5 doses Days 1, 8, 15, 22 and 29 |
| Route of administration: | Intravenous bolus injection |
| Dose volume: | 2 mL/kg |
| Formulation/Vehicle: | PF-05280586 diluted on 20 mM histidine, 8.5% w/v sucrose, 0.15 mM EDTA, and 0.02% w/v polysorbate 80, pH 5.8, in water<br>MabThera diluted on 25 mM sodium citrate, 0.9% w/v sodium chloride, and 0.07% w/v polysorbate 80, pH 6.5, in water |
| Species/Strain: | Cynomolgus monkeys (*Macaca fascicularis*) |
| Number/Sex/Group: | 7 sex/group |
| Age: | 4-5 years old |
| Weight: | M: 4.6 to 7.8 kg; F: 3.0 to 6.4 kg |
| Unique study design: | None |
| Deviation from study protocol: | Protocol deviations neither affected the overall interpretation of study findings nor compromised the integrity of the study |

## Results

| Parameters | Major findings |
|---|---|
| **Mortality** | All animals survived through the scheduled dosing or recovery sacrifice. |
| **Clinical Signs** | Non-adverse, transient, higher incidence of vomitus (containing food, foamy, yellow, or clear) or emesis occurred on Day 22 after administration of either test article.<br><br>**Table 4 Incidence of Clinical Signs**<br><br>**Dose (mg/kg)** / **Rituximab-Pfizer** / **Rituximab-EU**<br>Control: 1 / 14 / 6 / 14<br>20: 2 / 14 / 7 / 14<br><br>Low incidence of red or swollen injection sites occurred across groups including controls (primarily in males) for both test articles on different days. |
| **Body Weights** | No differences occurred among groups or between the two test articles. One 20 mg/kg/dose male given rituximab-Pfizer lost 1.1 kg during the recovery phase. A specific cause for the weight loss was not determined. |
| **Ophthalmic Examinations** | No test articles-related findings occurred during the dosing or recovery phase. |
| **Electrocardiographic Measurements** | No test article-related abnormalities in rhythm or effect on the PR, QRS, QT or QTc intervals were found at any dose level compared to predose and postdose group mean values and control values. |
| **Hematology** | -Group mean absolute lymphocyte counts of rituximab-Pfizer and rituximab-EU- tended to be lower than those of the concurrent vehicle control groups. |

-Percent decreases from their own group baseline (Week -1) value appeared slightly greater in rituximab-Pfizer and rituximab-EU-dosed groups relative to the respective controls on Days 4, 15, and 30 (see Table 5).
-Decreased absolute lymphocyte counts was also observed during the recovery phase.
-These differences could not be clearly attributed to either of the test articles based on the high variability in the peripheral blood lymphocyte count of individual animals.

**Table 5 Percent Change in Absolute Lymphocyte Counts compared to Week -1**

**MALES**

| Day | Control | Rituximab-Pfizer | Control | Rituximab-EU |
|---|---|---|---|---|
| **Dosing N=** | **7** | **7** | **7** | **7** |
| 4 | ↓21 | ↓32 | ↓12 | ↓36 |
| 15 | ↓19 | ↓34 | ↓31 | ↓50 |
| 30 | ↓28 | ↓38 | ↓19 | ↓42 |
| **Recovery N=** | **3** | **3** | **3** | **3** |
| 29 | ↓49 | ↓24 | ↓19 | ↓31 |
| 57 | ↓51 | ↓37 | ↓22 | ↓29 |
| 92 | ↓49 | ↓37 | ↓15 | ↓45 |

**FEMALES**

| Day | Control | Rituximab-Pfizer | Control | Rituximab-EU |
|---|---|---|---|---|
| **Dosing N=** | **7** | **7** | **7** | **7** |
| 4 | ↓11 | ↓37 | ↓18 | ↓30 |
| 15 | ↓3 | ↓9 | | ↓12 |
| 30 | ↓13 | ↓46 | ↓22 | ↓30 |
| **Recovery N=** | **3** | **3** | **3** | **3** |
| 29 | ↓35 | | ↓24 | ↓35 |
| 57 | ↓20 | | ↓27 | ↓39 |
| 92 | ↓27 | | ↓15 | ↓37 |

Empty spaces denote no change compare to concurrent values on Week -1

**Immunophenotyping**

GLP Markers
Decreases in mean absolute lymphocyte counts compared with predose values or concurrent controls suggested possible test article-related effects, but due to the large variability in values, these changes could not clearly be attributed to administration of rituximab-Pfizer or rituximab-EU.

**Table 6 Individual Animal Ranges for CD3-CD20+ Absolute and Relative B Cells, expressed as a Percent of or Decrease from Week-1 Predose Values**

*(Excerpt from Submission)*

| Group (Combined Sex) | CD3-CD20+ Absolute Range % of Predose Week -1 | CD3-CD20+ Absolute Range Decrease from Predose Week -1 | CD3-CD20+ Relative Range % of Predose Week -1 | CD3-CD20+ Relative Range Decrease from Predose Week -1 |
|---|---|---|---|---|
| Rituximab-Pfizer | | | | |
| Day 4 | 0 to 0% | 100 to 100% | 0 to 0% | 100 to 100% |
| Day 15 | 0 to 2% | 98 to 100% | 0 to 2% | 98 to 100% |
| Day 30 | 0 to 6% | 94 to 100% | 0 to 12% | 88 to 100% |
| Recovery Day 29 | 0 to 34% | 66 to 100% | 0 to 31% | 69 to 100% |
| Recovery Day 57 | 0 to 55% | 45 to 100% | 0 to 58% | 42 to 100% |
| Recovery Day 92 | 0 to 59% | 41 to 100% | 0 to 76% | 24 to 100% |
| Rituximab-EU | | | | |
| Day 4 | 0 to 0% | 100 to 100% | 0 to 1% | 99 to 100% |
| Day 15 | 0 to 2% | 98 to 100% | 0 to 2% | 98 to 100% |
| Day 30 | 0 to 14% | 86 to 100% | 0 to 28% | 72 to 100% |
| Recovery Day 29 | 0 to 72% | 28 to 100% | 0 to 82% | 18 to 100% |
| Recovery Day 57 | 0 to 74% | 26 to 100% | 0 to 101% | 0 to 100% |
| Recovery Day 92 | 0 to 72% | 28 to 100% | 0 to 114% | 0 to 100% |

No test article-related changes were noted in peripheral blood for absolute T cell values (total T, helper T, and cytotoxic T cells) or natural killer cell values.

Non-GLP Markers

An additional panel of B cell markers (CD3-CD19+, CD3-CD40+ and CD3-CD20+CD40+) was included to verify the observations for the CD3-CD20+ subset. Results from this panel, conducted non-GLP, confirmed depletion of B cell populations identified using CD3-CD20+ as a marker.

**Table 7 Range of Absolute Decreases in B Cell Markers, expressed as a Percent of or Decrease from Week-1 Predose Values**

| Parameter | Rituximab-Pfizer | Rituximab-EU |
|---|---|---|
| CD3-CD19+ | 86 to 97 | 86 to 96 |
| CD3-CD40+ | 96 to 99 | 88 to 99 |
| CD3-CD20+CD40+ | 98 to 100 | 91 to 100 |
| **Recovery Day 92** | | |
| CD3-CD19+ | 59 to 89 | 86 to 89 |
| CD3-CD40+ | 52 to 82 | 77 to 90 |
| CD3-CD20+CD40+ | 62 to 81 | 77 to 94 |

Decreases in relative B cell values were of a similar magnitude to the absolute values for B cell markers across time points, and between test articles.

| | |
|---|---|
| **Coagulation, Clinical Chemistry, and Urinalysis** | No test articles-related changes occurred |
| **Toxicokinetics** | No sex differences in systemic exposure after single or repeat dosing of either test article based on high inter-animal variability and a ratio of less than 2.0 in serum test article concentrations between dose-matched males and females. After weekly IV dosing, mean exposure ratios ($AUC_{168h}$) in males and females on Day 22 relative to Day 1 (Day 22/Day 1) were 0.9 and 1.0, |

respectively for rituximab-Pfizer, and 1.2 and 1.8, respectively, for rituximab-EU. Exposure ratios (rituximab-Pfizer-rituximab-EU) after multiple doses were calculated based on group mean values and ranged from 0.67 to 1.04.

**Table 8 Mean Toxicokinetic Parameters of Rituximab after Multiple Doses in Cynomolgus Monkeys**

| Day | Sex | $C_{max}$ (ng/mL) | $T_{max}$ (h) | $AUC_{168h}$ (ng/mL*h) |
|---|---|---|---|---|
| | | Rituximab-Pfizer | | |
| 1 | M | 910,000 | 1.059 | 64,000,000 |
| | F | 785,000 | 0.345 | 49,700,000 |
| | Overall | 848,000 | 0.702 | 56,800,000 |
| 22 | M | 1,020000 | 0.738 | 57,200,000 |
| | F | 915,000 | 0.214 | 49,200,000 |
| | Overall | 966,000 | 0.476 | 53,200,000 |
| | | Rituximab-EU | | |
| 1 | M | 1,010,000 | 0.476 | 59.600,000 |
| | F | 794,000 | 0.214 | 49,700,000 |
| | Overall | 903,000 | 0.345 | 54,600,000 |
| 22 | M | 1,210,000 | 0.476 | 70,900,000 |
| | F | 1,250,000 | 0.869 | 88,100,000 |
| | Overall | 1,230,000 | 0.673 | 79,500,000 |
| | | Ratio Rituximab-Pfizer / Rituximab-EU | | |
| 1 | | 0.94 | | 1.04 |
| 22 | | 0.79 | | 0.67 |

**Anti-Drug Antibody (ADA)**

- A small number of predose samples or samples from control animals tested positive (<4%, 2/56 animals). Given the presence of low titers ($log_{10}$) ranging from 1.52 to 1.60 compared to the titer cut point for a positive ADA response of 1.40, these positive results were not considered to impact data interpretation.
- No induction of an immune response to rituximab was considered in the animals from the control group or in the animals with detectable ADA at predose based on the difference between postdose and predose in $log_{10}$ titers.
- After repeat dosing, the overall induction of an immune response (dosing and recovery phases) was 79% (11 of 14 animals) in the rituximab-Pfizer group and 43% (6 of 14 animals) in the rituximab-EU group. Many of these positive animals had relatively low maximum signals based on the difference from assay cut points.
- ADA-positive animals declined from 10/14 animals on Day 22 to 1/14 animals on Day 30 (recovery Day 1) in the rituximab-Pfizer group and from 5/14 animals to 2/14 animals in the rituximab-EU group.
- Circulating levels of test article from the study Day 29 dose may have interfered with the detection of corresponding ADA on Day 30 (recovery Day 1).
- During the recovery phase, detectable ADA were observed in 3/6 animals in each of the two test article groups, suggesting no marked differences overall in the immune response of the two test articles.

**Dose Formulation Analysis**

Dose formulations for both test articles were within ±10% of the target concentrations (mean results ranging from 94.6 to 99.7% of target). The test articles were not detected in any vehicle control article formulations. All formulations met the acceptance criteria for study use.

**Anatomic Pathology**

Organ Weight

At the end of the dosing phase euthanasia, the mean absolute and relative spleen weights ranged from 13 to 17% lower in males and 30 to 42% lower in females for rituximab-Pfizer, and 39 to 44% lower in males and 15 to 25% lower in females for rituximab-EU, compared with respective controls.
At recovery phase euthanasia, lower mean absolute and relative spleen weights occurred in rituximab-Pfizer males and rituximab-EU females, compared with respective controls.

**Table 9 Rituximab-related Organ Weight Changes after Multiple Doses in Cynomolgus Monkeys**

| Spleen weight | Rituximab-Pfizer | | Rituximab-EU | |
|---|---|---|---|---|
| **Sex / N** | **M / 4** | **F / 4** | **M / 4** | **F / 4** |
| Absolute | ↓17 | ↓42 | ↓43 | ↓15 |
| Relative to body weight | ↓13 | ↓30 | ↓39 | ↓25 |
| Relative to brain weight | ↓17 | ↓42 | ↓44 | ↓16 |
| **Recovery N=** | **3** | **3** | **3** | **3** |
| Absolute | ↓20 | ↓7 | | ↓33 |
| Relative to body weight | ↓20 | ↓6 | | ↓16 |
| Relative to brain weight | ↓21 | ↓5 | | ↓30 |

Macroscopic findings

No test article-related macroscopic findings occurred at either sacrifice.

Microscopic findings

At the end of the dosing phase, test article-related findings of decreased cellularity and decreased CD20 cells occurred in the spleen and mesenteric and axillary lymph nodes of rituximab-Pfizer- and rituximab-EU-dosed monkeys, see Table 10.

**Table 10 Rituximab-related Microscopic findings after Multiple Doses in Cynomolgus Monkeys**

*(Excerpted from Submission)*

| | Rituximab-Pfizer | | | | Rituximab-EU | | | |
|---|---|---|---|---|---|---|---|---|
| Group | 1 | | 2 | | 3 | | 4 | |
| Dose Level (mg/kg/dose) | 0 | | 20 | | 0 | | 20 | |
| Sex | M | F | M | F | M | F | M | F |
| Number Examined | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Spleen | | | | | | | | |
| Decreased cellularity: lymphoid follicles | | | | | | | | |
| Minimal | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Mild | 0 | 0 | 4 | 3 | 0 | 0 | 4 | 4 |
| Decreased CD20 cells (IHC)[b] | | | | | | | | |
| Moderate | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Marked | 0 | 0 | 4 | 3 | 0 | 0 | 3 | 3 |
| Severe | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Mesenteric lymph node | | | | | | | | |
| Decreased cellularity: germinal center | | | | | | | | |
| Minimal | 1 | 1 | 4 | 4 | 1 | 1 | 4 | 4 |
| Decreased CD20 cells (IHC)[b] | | | | | | | | |
| Moderate | 0 | 0 | 4 | 4 | 0 | 0 | 4 | 4 |
| Axillary lymph node | | | | | | | | |
| Decreased cellularity: germinal center | | | | | | | | |
| Minimal | 0 | 1 | 4 | 3 | 1 | 1 | 3 | 3 |
| Decreased CD20 cells (IHC)[b] | | | | | | | | |
| Moderate | 0 | 0 | 4 | 4 | 0 | 0 | 4 | 4 |

a Severity changes were graded as minimal, mild, moderate, marked, or severe.
b IHC = assessed by immunohistochemistry.

At the end of the 13-week recovery phase, test article-related findings were still present in the spleen and mesenteric and axillary lymph nodes of rituximab-Pfizer- and rituximab-EU-dosed monkeys with less severity and incidence compared to terminal sacrifice.

**Table 11 Rituximab-related Microscopic findings after 13-week Recovery in Cynomolgus Monkeys**

*(Excerpted from Submission)*

| | Rituximab-Pfizer | | | | Rituximab-EU | | | |
|---|---|---|---|---|---|---|---|---|
| Group | 1 | | 2 | | 3 | | 4 | |
| Dose (mg/kg/dose) | 0 | | 20 | | 0 | | 20 | |
| Sex | M | F | M | F | M | F | M | F |
| Number Examined | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Spleen | | | | | | | | |
| Decreased cellularity: lymphoid follicles | | | | | | | | |
| Minimal | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| Mild | 0 | 0 | 1 | 2 | 0 | 0 | 2 | 2 |
| Spleen | | | | | | | | |
| Decreased CD20 cells (IHC)[b] | | | | | | | | |
| Minimal | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Mild | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Moderate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Marked | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Severe | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1 |
| Mesenteric lymph node | | | | | | | | |
| Decreased cellularity: germinal center | | | | | | | | |
| Minimal | 1 | 1 | 2 | 2 | 0 | 1 | 2 | 3 |
| Mesenteric lymph node | | | | | | | | |
| Decreased CD20 cells (IHC)[b] | | | | | | | | |
| Mild | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 2 |
| Moderate | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1 |
| Axillary lymph node | | | | | | | | |
| Decreased cellularity: germinal center | | | | | | | | |
| Minimal | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| Axillary lymph node | | | | | | | | |
| Decreased CD20 cells (IHC)[b] | | | | | | | | |
| Minimal | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Mild | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 1 |
| Moderate | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |

a Severity changes were graded as minimal, mild, moderate, marked, or severe.
b IHC = assessed by immunohistochemistry.

# 7 Genetic Toxicology

Genetic toxicology studies are not required for biotechnology-derived pharmaceuticals per ICH S6 Guidance.

# 8 Carcinogenicity

Carcinogenicity studies are not required for biotechnology-derived pharmaceuticals per ICH S6 Guidance.

# 9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicity studies to assess the similarity of PF-05280586 to Rituxan were not conducted.

# 10 Special Toxicology Studies

**Study No. 17GR338: Investigative Report: Single Nucleotide Polymorphisms in Cynomolgus monkeys receiving Rituximab-Pfizer and Rituximab-EU in Studies 11GR111 and 11GR112**

*This study report was not reviewed. The abstract was excerpted from submission.*

In humans, genetic variation at specific loci have been shown to influence the clinical response to rituximab, a therapeutic anti-CD20 antibody designed to deplete B cells in inflammatory diseases and cancer. Variable B cell repletion rates were observed in cynomolgus monkeys receiving either rituximab-PF or rituximab-EU during the recovery phases of the studies [11GR111 (single-dose study) and 11GR112 (repeat-dose study)]. The objective of this assessment was to determine if the variable B cell counts and ADA observed at the end of these studies were associated with identified single nucleotide polymorphisms (SNPs) in cynomolgus monkeys. In the single dose study, two SNPs (17544G>A in the CD95 gene and 44058G>A in the IFNγR2 gene) were statistically associated ($p<0.05$) with absolute CD3-CD20+ B cell counts following a single 10 mg/kg dose of rituximab. Statistical associations with these 2 SNPs were not present with the 2 or 20 mg/kg doses, and no SNPs were associated with CD3-CD19+ B cell counts at any dose in this study. Because all animals were ADA positive at the end of the single-dose study, no significant association between SNPs and ADA titers were observed. In the repeat-dose study, 4 SNPs in the FcγR3A gene (1134A>C, 4942C>T, 5027A>G, and 5082A>G) were significantly associated with absolute CD3-CD20+ and CD3-CD19+ B cell counts at the end of the study ($p<0.05$). These same FcγR3A SNPs were also significantly associated with positive ADA at the end of the repeat dose study ($p<0.05$). These data demonstrate genetic associations between CD95, IFNγR2, and FcγR3A SNPs and CD3-CD20+ B cell counts, CD3-CD19+ B cell counts, and ADA titers in cynomolgus monkeys administered rituximab. However, the functional impact of these SNPs in cynomolgus monkeys and the mechanistic relationship to B cell counts and ADA titers is unknown.

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