CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology Review

NDA or BLA Number	BLA761064
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Submission Date	August 26, 2016
Submission Type	Standard Review
Brand Name	Rituxan Hycela
Generic Name	Rituximab and hyaluronidase
Dosage Form and Strength	120 mg/mL Rituximab and 2000 U/mL hyaluronidase
Route of Administration	Subcutaneous injection
Proposed Indication	Indicated for the treatment of patients with: -Follicular Lymphoma (FL) -Diffuse Large B-cell Lymphoma (DLBCL) -Chronic Lymphocytic Leukemia (CLL)
Applicant	Genentech Inc
Associated IND	IND 126650
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1. EXECUTIVE SUMMARY

Rituximab SC, a co-formulation of rituximab and hyaluronidase, is a subcutaneously administered formulation that offers a different route of administration from intravenous rituximab (rituximab IV). Rituximab IV is a CD20-directed cytolytic antibody approved for the treatment of patients with Non-Hodgkin lymphoma (NHL) and chronic lymphocytic lymphoma (CLL). Hyaluronidase is approved for facilitating the absorption and dispersion of subcutaneously injected drugs.

The clinical development of rituximab SC was based on a comparative pharmacokinetic (PK) non-inferiority paradigm in which the PK of rituximab SC was compared to the PK of rituximab IV in patients with follicular lymphoma (FL) and CLL. The objective of the comparative PK program was to select rituximab SC doses that achieve similar or higher rituximab plasma C_{trough} compared to rituximab IV doses. The PK data showed that the proposed fixed rituximab SC doses of 1400 mg and 1600 mg achieved equal or higher rituximab C_{trough} relative to the approved 375 and 500 mg/m^2 rituximab IV doses for NHL and CLL, respectively. Exposure-safety analysis did not show a significant relationship between rituximab C_{trough} and Grade 3+ adverse events, which is consistent with published data that showed rituximab IV tolerated up to a dose of 2250 mg/m^2 (PMID 11226005). This exposure-safety evaluation provided sufficient evidence to conclude that the higher average rituximab plasma concentrations with the SC formulation would not result in higher rates of systemic adverse events compared to what would be expected with IV administration. No significant difference in immunogenicity rates was observed between the two formulations.

The PK comparability assessment employed in this development program served as the pivotal evidence of effectiveness for rituximab SC, and is consistent with OCP and CDER policy as described in the *Guidance to Industry: Providing Clinical Evidence of Effectiveness for Human Drug and Biologic Products*.

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the information contained in BLA 761,064 and recommends approval. Additionally, OCP supports the use of PK comparability assessment as the preferred paradigm for the development of similar combinations (i.e., previously approved antibodies with well understood efficacy and safety profiles being co-formulated with hyaluronidase or other modalities approved to enhance absorption). Key review issues are summarized below.

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	The primary evidence of effectiveness comes from two Phase 3 studies in patients with FL and DLBCL (Study BO22334 and MO28107) and one phase 1b study in patients with CLL (Study BO25341). Consistent with the primary endpoint, the rituximab SC doses achieved exposures equal or higher than that achieved by the rituximab IV doses.
General dosing instructions	OCP agrees with the proposed dosing regimens (after initial IV dose): FL/DLBCL - 1400 mg rituximab/23,400 U hyaluronidase SC CLL - 1600 mg rituximab/26,800U hyaluronidase SC

Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose individualization is recommended based on intrinsic and extrinsic factors. Body-size based dosing is not required (see **Section 2.2.2**)
Labeling	Generally acceptable. The review team has specific content and formatting change recommendations

1.2 Post-Marketing Requirements and Commitments

None

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Rituximab SC is a co-formulation of rituximab and hyaluronidase. Rituximab is a monoclonal antibody that binds CD20 and induces B-cell lysis. Rituximab IV has been approved and marketed for oncology indications such as NHL and CLL since 1997. Hyaluronidase is a purified preparation of the enzyme recombinant human hyaluronidase. Hyaluronidase facilitates absorption and dispersion of subcutaneously injected drugs by cleaving glycosidic bonds of hyaluronic acid and other acid mucopolysaccharides of the connective tissue. Hyaluronidase has been approved as an adjuvant to facilitate absorption and dispersion in a variety of settings.

In the current submission, the Applicant has co-formulated these two previously approved drugs, for subcutaneous administration. The Applicant applied a PK bridging strategy for the development of rituximab SC. Such a development approach is predicated on rituximab SC as "a different dose, regimen, or dosage form" of rituximab IV and that PK data can be used to bridge the two different formulations of the same molecular entity. The main differences between the two formulations are shown in **Table 1**.

Table 1: **Comparison between rituximab IV and rituximab SC**

Characteristics	Rituximab IV	Rituximab SC
Administration	IV infusion over 1.5 to 2.5 hours	SC injection over 5 minutes
Rituximab Concentration	10 milligrams (mg)/milliliters (mL)	120 mg/mL
Dosing regimen	Body surface area - based	Fixed
Co-formulation	none	Hyaluronidase
Doses	375 mg/m^2 and 500 mg/m^2	1400 mg and 1600 mg

The PK of rituximab in patients with NHL and CLL was described by a using a two-compartment model with time-independent clearance and additional target-mediated (time-dependent) elimination with non-renewable target. The subcutaneous absorption was characterized using a first-order absorption process. A summary of the clinical pharmacokinetics of rituximab SC is provided in **Table 2**.

Table 2: **Pharmacokinetic Parameters of Rituximab SC**[a]

	FL	**CLL**
Absorption		
Estimated Absolute Bioavailability[b]	0.646 (NA)	0.633 (21)
Distribution		
Volume of Central compartment[c] (L)	4.06 (26)[d]	4.80 (18)
Apparent Volume of Distribution at steady state[c] (L)	8.09 (19)[d]	8.52 (13)
Elimination		
Terminal Half-life (hours)	34.1 (27)	32 (24)
Effective Clearance (L/day)	0.18 (34)	0.204 (31)

[a] Parameters represented as geometric mean (%CV) unless otherwise specified;
[b] Compared to rituximab IV
[c] Volume of central compartment and peripheral compartment
[d] NA=Not available

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The applicant has proposed rituximab SC doses of 1400 and 1600 mg for NHL and CLL indications, respectively. These doses contained 23,400 U and 26,800 U of hyaluronidase, respectively. The doses were selected to achieve similar or higher rituximab C_{trough} compared to the approved rituximab IV doses. These doses are appropriate for their intended indications and were evaluated in the pivotal trials.

Patients with FL enrolled in study BO22334 were randomized to receive rituximab IV 375 mg/m^2 or rituximab SC 1400 mg in Cycles 2 – 8 after an initial IV dose in Cycle 1. In Stage 2 of Study BO23451, conducted in patients with CLL, patients were randomized to receive rituximab SC 1600 mg or rituximab IV 500 mg/m^2 in Cycles 2 to 6 after an initial IV dose in Cycle 1. In both trials, rituximab SC achieved comparable if not slightly higher average C_{trough} compared to rituximab IV. Comparable objective response rates (ORR) were seen between arms within these trials.

2.2.2 Therapeutic individualization

Rituximab IV is dosed using body surface area (BSA) and BSA was a significant covariate in the population PK analysis of rituximab. The proposed doses of 1400 mg for patients with NHL and 1600 mg for patients with CLL provided consistently higher exposure than the 375 mg/m^2 and 500 mg/m^2 IV doses, respectively. Because rituximab has a wide therapeutic window, the higher C_{trough} following the fixed rituximab SC doses is not expected to influence the safety profile of rituximab. No dose individualization is required for adult patients. For further details, see **Section 3.3.3**.

2.3 Outstanding Issues

None

2.4 Summary of Labeling Recommendations

The applicant's proposed labeling is generally acceptable. The Office of Clinical Pharmacology recommends that certain information be provided in a tabular format for ease of communication.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Rituximab SC is provided as single-dose vials containing either 1400 mg rituximab and 23400 USP units of hyaluronidase per 11.7 mL or 1600 mg rituximab and 26800 USP units of hyaluronidase per 13.4 mL.

The proposed indications are for the treatment of adult patients with FL, DLBCL, or CLL. These are the same oncology indications for which rituximab IV has been approved for. The applicant is not seeking any of the non-oncologic indications at this time. The applicant used a PK bridging strategy for the development of rituximab SC by leveraging the known and established efficacy of rituximab IV.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	Rituximab is a monoclonal antibody that targets the CD20 antigen expressed on the surface of pre-B and mature B-lymphocytes. Upon binding to CD20, rituximab mediates B-cell lysis. Possible mechanisms of cell lysis include complement dependent cytotoxicity (CDC) and antibody dependent cell mediated cytotoxicity (ADCC). The antibody induced apoptosis in the DHL 4 human B cell lymphoma cell line.
	Hyaluronidase is a dispersion agent, which modifies the permeability of connective tissue through the hydrolysis of hyaluronic acid, a polysaccharide found in the intercellular ground substance of connective tissue. Hyaluronidase hydrolyzes hyaluronic acid by splitting the glucosaminidic bond between C1 of an N-acetylglucosamine moiety and C4 of a glucuronic acid moiety. This temporarily decreases the viscosity of the cellular cement and promotes dispersion of injected fluids or of localized transudates or exudates, thus facilitating their absorption.
General Information	
Bioanalysis	Serum rituximab concentrations were measured in serum by a validated Enzyme-Linked Immunosorbent Assay (ELISA). The assay used polyclonal goat anti-rituximab antibodies (rituximab affinity purified, anti-efalizumab depleted), followed by washing. Bound samples were detected by incubation with goat antimouse IgG F(ab')2 conjugated to horseradish peroxidase. Following a further wash to remove any unbound conjugate, a substrate solution (tetramethylbenzidine/ hydrogen peroxide) was added to the wells, resulting in a color development in proportion to the amount of rituximab in the samples. The reporting range in human serum was 5 - 150 ng/mL. Analytical reports were submitted and QC reports were summarized for the use of the method for each study. Analytical methods for detection of anti-drug antibodies (ADAs) in serum were validated and demonstrated adequate sensitivity and drug tolerance for detection of ADA responses in human serum. Samples that were positive for the presence of ADA were then further analyzed for anti-drug NAbs.

Healthy Volunteers vs Patients	No studies were conducted in healthy subjects.
Drug exposure at steady state following the therapeutic dosing regimen	The overall geometric mean (%CV) rituximab exposure at the steady state (Cycle 7 in NHL treatment) after SC administration was 3677 µg.day/mL (40%).
ADME	
Absorption	The mean (CV%) relative bioavailability of rituximab SC was estimated to be 0.65 in patients with follicular lymphoma and 0.633 (21) in patients with CLL
Distribution	The estimated man (%CV) volume of distribution for the central compartment was 4.54 (25.7) L. Peripheral volume and inter-compartment clearance were estimated at 4.27 L and 0.573 L/day
Metabolism	Not studied. In general antibodies are expected to be metabolized into peptides and amino acids via catabolic pathway.
Elimination	The mean (%CV) initial time-dependent clearance was 0.398 (142) L/day which declined with a half-life of 9.3 days. The time-independent clearance was 0.2 (27.9) L/day.
IMMUNOGENICITY	
Immunogenicity	The post-baseline incidence of anti-rituximab antibodies (treatment-induced and treatment-enhanced HACA responses) was 1 - 4% in the IV group versus 1 – 2 % in the SC arm in patients with FL. In patient with CLL, the incidence of anti-rituximab antibodies was ~ 7% (6/89 patients) in the IV group compared with 2% (2/85 patients) in the SC group.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

The PK comparability assessment between rituximab SC and IV is the pivotal evidence of effectiveness in this development program. Exposure-safety analyses and immunogenicity assessment suggest that the overall benefit/risk profile is unlikely to differ between rituximab SC and IV.

The development program utilized a PK comparability approach to bridge efficacy to rituximab SC. The applicant has co-formulated two previously approved drugs, for which effectiveness and safety have been established for the individual components. Such a development approach is consistent with the *Guidance to Industry: Providing Clinical Evidence of Effectiveness for Human Drug and Biologic Products*, which states the following:

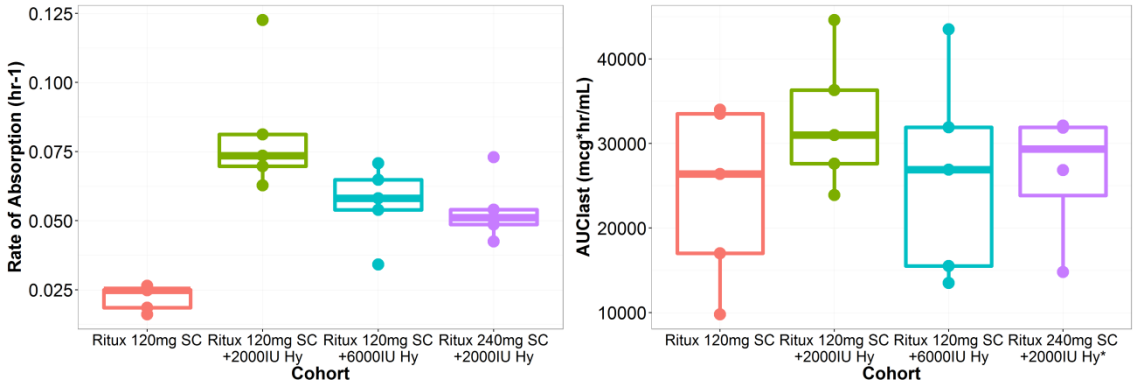
> "In certain cases, effectiveness of an approved drug product for a new indication, or effectiveness of a new product, may be adequately demonstrated without additional adequate and well-controlled clinical efficacy trials. Ordinarily, this will be because other types of data provide a

way to apply the known effectiveness to a new population or a different dose, regimen or dosage form."

When applied to different doses, regimens, or dosage forms, the above FDA Guidance states that "it may be possible to conclude that a new dose, regimen, or dosage form is effective on the basis of PK data without an additional clinical efficacy trial". In the current application, PK data, together with a well-defined PK-efficacy relationship, are used to bridge the established safety and efficacy results of rituximab IV to rituximab SC.

One major difference between rituximab SC and rituximab IV is the inclusion of hyaluronidase in the rituximab SC formulation. In order to justify the inclusion of this adjuvant, the applicant conducted a proof-of-concept study in minipigs. As shown in **Figure 1**, co-administration of rituximab SC with hyaluronidase increased the absorption rate of rituximab 3-fold, but did not affect the extent of absorption. The study further showed that hyaluronidase doses of greater than 2000 U/mL did not result in a faster rate of absorption.

Figure 1: **Absorption rate and exposure for rituximab SC in minipigs with and without hyaluronidase**



The applicant provided evidence of efficacy by conducting studies to show that rituximab SC doses achieved equal to or higher rituximab C_{trough} than that achieved with rituximab IV. PK data were collected from patients with FL and CLL who took part in three clinical studies (**Table 3**).

Table 3: **Studies conducted to support development of rituximab SC**

Study (Population)	Objective per Study Stage	
	Dose Selection Stage	Dose Confirmation Stage
BP22333 (FL)	Identify a SC dose that yielded comparable C_{trough} to the IV dose	Demonstrate C_{trough} non-inferiority of SC dose when given every 2 or 3 months
BO22334 (FL)	Not Applicable	Demonstrate C_{trough} non-inferiority of 1400 mg SC compared to 375 mg/m^2 IV
BO25341 (CLL)	Determine a SC dose that yielded comparable C_{trough} to IV dose	Demonstrate C_{trough} non-inferiority of 1600 mg SC compared to 500 mg/m^2 IV

Two of the three studies had dose selection and dose confirmation components. These studies led to identification of rituximab SC doses of 1400 mg and 1600 mg for the treatment of patients with NHL and CLL, respectively.

Dose Exploration for Non-Hodgkin's Lymphoma

The initial selection of an appropriate dose for NHL was evaluated in the maintenance phase of treatment of patients with previously untreated and relapsed/refractory FL, who had received rituximab-containing induction therapy, achieved at least a partial response PR, and received at least one cycle of rituximab IV in maintenance (Stage 1 of Study BP22333). In this study, patients were administered rituximab IV 375 mg/m^2 IV, or rituximab SC 375 mg/m^2, 650 mg/m^2, or 800 mg/m^2 to determine which SC dose would yield comparable C_{trough} to that observed following rituximab IV 375 mg/m^2. The geometric means of the C_{trough} and the SC/IV geometric mean ratios (GMR) at Cycle 2 of the maintenance phase are shown in **Table 4**. As shown in **Table 4**, for the Q2M and Q3M regimens, the C_{trough} in rituximab SC 800 mg/m^2 arm was consistently comparable to that in the rituximab IV 375 mg/m^2 arm.

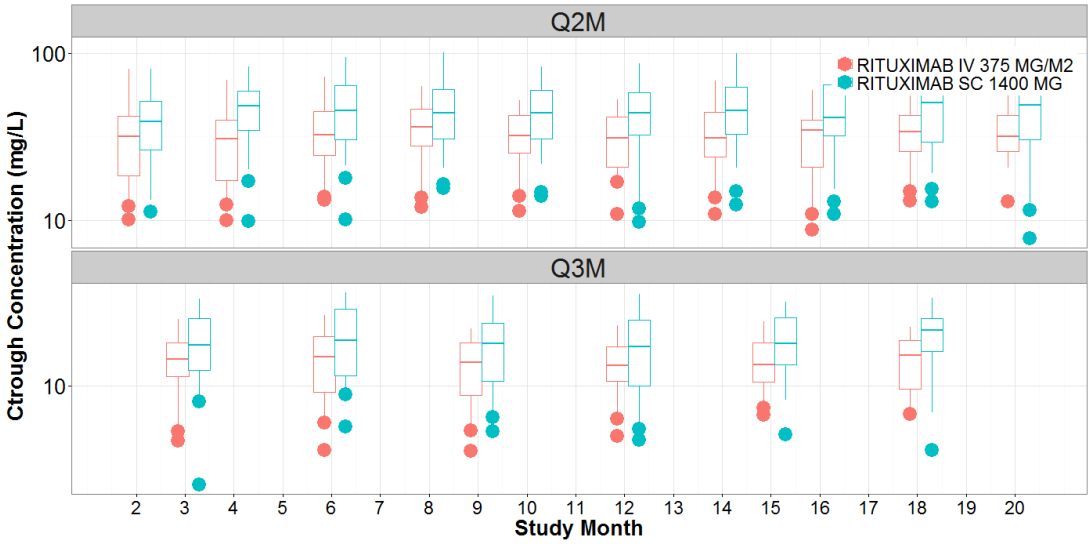
Table 4: **Geometric mean (%CV) of the C_{trough} by regimen and dosing schedule and SC to IV geometric mean ratio (90% CI) for Study BP22333 (Stage 1) at Cycle 2 of the Maintenance Phase**

Dosing Schedule	Regimen	N	Geometric mean (CV%)	GMR for SC/IV (90% CI)
Every 2 months	375 mg/m^2 IV	9	38.1 (69)	
	375 mg/m^2 SC	17	18.1 (85)	0.475 (0.279 – 0.81)
	625 mg/m^2 SC	17	35.6 (51)	0.933 (0.588 – 1.48)
	800 mg/m^2 SC	21	39.3 (80)	1.03 (0.625 – 1.70)
Every 3 months	375 mg/m^2 IV	6	14.2 (44)	
	375 mg/m^2 SC	15	11.7 (56)	0.823 (0.546 – 1.24)
	625 mg/m^2 SC	13	12.4 (65)	0.871 (0.558 – 1.36)
	800 mg/m^2 SC	17	16.8 (86)	1.18 (0.735 – 1.90)

Based on the findings from stage 1, the 800 mg/m^2 SC dose, for both the Q2M and Q3M dosing schedules, was shown to achieve equal or greater rituximab C_{trough} relative to the 375 mg/m^2 IV dose. Using modeling and simulation approaches, the BSA-based dose of 800 mg/m^2 SC was converted to a fixed 1400 mg SC dose. The 1400 mg dose of rituximab SC was then evaluated in the maintenance phase of the treatment in patients with FL (Stage 2 of BO22333).

In Stage 2 of BO22333, patients were randomized to receive either rituximab SC 1400 mg or rituximab IV 375 mg/m^2 every 2 or 3 months. A comparison of the C_{trough} over time for the Rituximab SC 1400 mg and the rituximab IV 375 mg/m^2 are shown in **Figure 2**. The geometric means of the C_{trough} and the C_{trough} geometric mean ratios (GMR) of the SC to IV dosing regimen are shown in **Table 5**. As shown in **Figure 2** and **Table 5**, the C_{trough} in rituximab SC 1400 mg arm was consistently higher than that in the rituximab IV 375 mg/m^2 arm for both Q2M and Q3M regimens. Based on these findings, the 1400 mg dose of rituximab SC was selected for further evaluation in the dose confirmation component of study BP22333.

Figure 2: **Comparisons of the C_{trough} after the administration of rituximab SC 1400 mg and rituximab IV 375 mg/m^2 over time by dosing schedule**



Note for above figure: The horizontal line represents the median, the top and bottom of the box represent the 25 and 75 percentile, the top and bottom line represent the 5 and 95 percentile and the circles represent the values lower than the 5 percentile.

Table 5: **Geometric mean (%CV) of the observed C_{trough} by regimen and dosing schedule and the SC to IV geometric mean ratio (90% CI) for Study BO22333 (Stage 2) at Maintenance Cycle 2**

Dosing Schedule	Regimen	N	Geometric mean (%CV)	GMR for SC/IV (90% CI)
Q2Months	375 mg/m^2 IV	37	30.2 (57)	--
	1400 mg SC	41	36.6 (54)	1.21 (0.98 – 1.50)
Q3Months	375 mg/m^2 IV	34	14.5 (56)	--
	1400 mg SC	34	19.0 (65)	1.32 (1.03 – 1.68)

Dose Exploration for Chronic Lymphocytic Leukemia (CLL)

Dose exploration evaluation for the CLL indication was evaluated in the Stage 1 of Study BO25341, a two-part study. In Stage 1, all patients received rituximab IV 375 mg/m^2 in Cycle 1. In Cycles 2-5, patients were assigned to receive either rituximab IV 500 mg/m^2, rituximab SC 1400 mg, 1600 mg, or 1870 mg. Pre-dose Cycle 5 and Cycle 6 C_{trough} were then evaluated. The geometric means and GMRs of rituximab C_{trough} are shown in **Table 6**. Based on these PK data, the rituximab SC 1600 mg dose was selected for the dose confirmation part of Study BO23451

Table 6: Rituximab C_{trough} Geometric Mean (%CV) and the geometric mean ratios (90% CI) for Study BO25341 (Stage 1)

Dose	28 days after 500 mg/m^2 IV	28 days after SC dose	GMR for SC/IV (90% CI)
	Geometric mean (%CV)	Geometric mean (%CV)	
1400 mg (n=16)	59.2 (45)	77.5 (38)	1.31 (1.17 – 1.46)
1600 mg (n=17)	49.6 (117)	83.8 (96)	1.69 (1.30 – 2.19)
1870 mg (n=22)	61.7 (46)	93.6 (44)	1.52 (1.41 – 1.64)

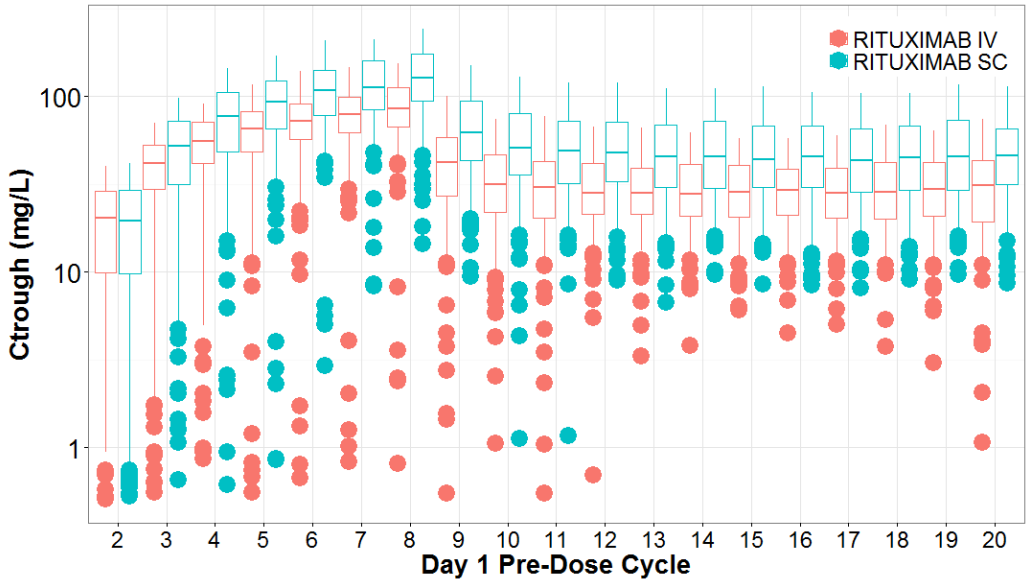
3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes. The selected dose and dosing regimen for the proposed indications in NHL and CLL are appropriate. The doses selected from the dose exploration arms of the relevant studies (see above) were confirmed to result in adequate concentrations of rituximab when tested in dose confirmation trials.

Dose Confirmation for Non-Hodgkin's Lymphoma

The rituximab SC 1400 mg dose was confirmed in Study BO22334, a two-stage, phase III study designed to investigate the PK, efficacy, and safety of rituximab SC in combination with CHOP or CVP chemotherapy in induction followed by maintenance with rituximab as monotherapy in patients with previously untreated FL. In this study, patients were randomized to receive either rituximab IV 375 mg/m^2 or rituximab SC 1400 mg starting from Cycle 2. In the induction phase, patients received 8 cycles of chemotherapy including rituximab every 3 weeks. A comparison of the C_{trough} over time for the Rituximab SC 1400 mg and the rituximab IV 375 mg/m^2 are shown in
Figure 3.

Figure 3: **Comparisons of the C_{trough} after the administration of rituximab SC 1400 mg and rituximab IV 375 mg/m^2 over time for Study BO22334**



The GMR of the SC to IV C_{trough} over time is shown in **Figure 4** and the geometric means of the C_{trough} and the GMR of the SC to IV dosing regimen at Cycle 7 (induction Phase) and Cycle 18 (Maintenance Phase) is shown in **Table 7**.

Figure 4: **Rituximab SC/IV C_{trough} GMR (90% CI) over time for Study BO22334**

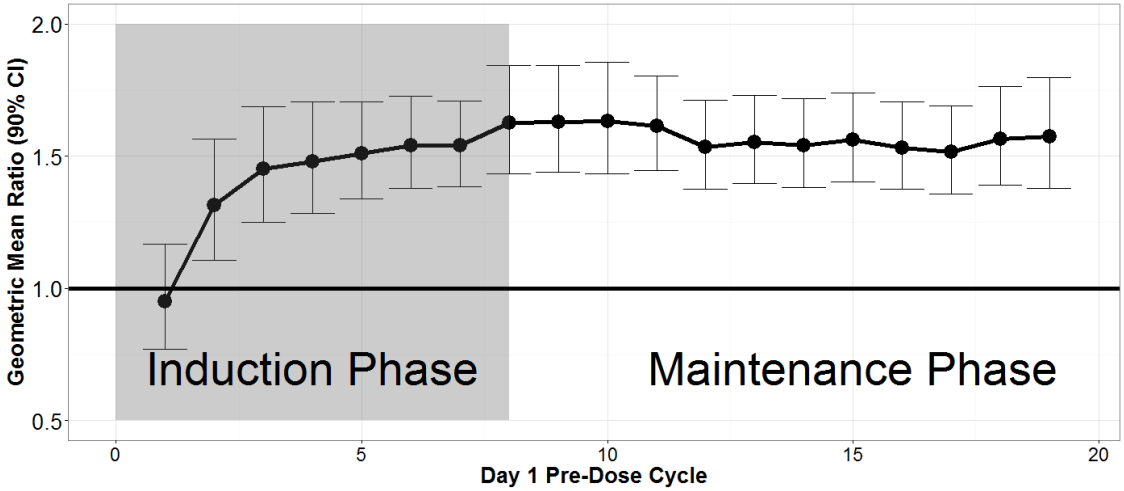


Table 7: **Rituximab C_{trough} geometric mean (%CV) and the SC to IV geometric mean ratio (90% CI) for Study BO22334 for Cycle 7 (Induction Phase) and Cycle 18 (Maintenance Phase)**

Phase	Cycle	Rituximab IV		Rituximab SC		GMR for SC/IV (90% CI)
		N	Geometric mean (%CV)	N	Geometric mean (%CV)	
Induction	7	185	78.7 (68.6)	175	121 (52)	1.53 (1.39 – 1.71)
Maintenance	18	143	28.6 (57.1)	132	44.8 (62)	1.58 (1.39 – 1.76)

As shown in **Figure 4** and **Table 7**, the SC/IV C_{trough} geometric mean ratios (90% CI) were higher than 1 over the duration of the study indicating that rituximab SC leads to consistently higher C_{trough} relative to rituximab IV, supporting the rituximab SC 1400 mg dose for the treatment of NHL. It is important to note that no meaningful difference in efficacy was observed between the two products (see Clinical and Statistical Review by Dr. Schwarsin and Dr. Ye).

Dose Confirmation for Chronic Lymphocytic Leukemia (CLL)

The rituximab SC 1600 mg dose was confirmed in Stage 2 of Study BO25431. Patients enrolled in this Stage were randomized to receive either rituximab SC 1600 mg or rituximab IV 500 mg/m^2 in Cycles 2 to 6, after an initial dose of rituximab IV 375 mg/m^2 in Cycle 1. As shown in **Table 8**, the SC/IV C_{trough} geometric mean ratios (90% CI) were consistently higher than 1 over the duration of the study indicating that the C_{trough} after the SC administration was consistently higher than after IV dosing. No meaningful difference in efficacy was observed between the two products (see Clinical and Statistical Review by Dr. Schwarsin and Dr. Ye.)

Table 8: **Geometric mean (%CV) of the C_{trough} for the SC and IV arm and the SC to IV geometric mean ratio (90% CI) for Study BO25341 after Cycle 2**

Cycle	Sample Day	Rituximab IV		Rituximab SC		SC/IV GMR (90% CI)
		N	Geometric mean (%CV)	N	Geometric mean(%CV)	
2	Pre dose	72	2.40 (102)	75	3.15 (105)	1.31 (0.99 – 1.74)
3	Pre dose	76	15.7 (124)	80	28.1 (108)	1.79 (1.32 – 2.44)
4	Pre dose	74	33.6 (105)	78	55.1 (80)	1.64 (1.28 – 2.10)
5	Pre dose	72	52.5 (88)	78	72.8 (73)	1.39 (1.11 – 1.73)

6	Pre dose	72	60.2 (76)	74	86.5 (66)	1.44 (1.18 – 1.44)
	28 day	71	75.9 (71)	72	96.6 (71)	1.27 (1.04 – 1.55)
	56 day	72	34.1 (100)	72	47.1 (87)	1.38 (1.06 – 1.79)

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

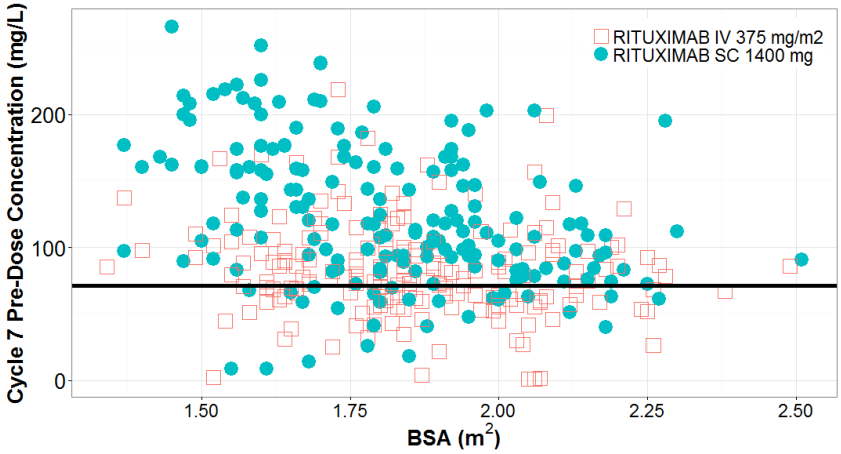
No. There is no need for an alternative dose or dosing regimen for subpopulation based on the intrinsic factors as described below. While rituximab IV is dosed using the patient's BSA, no BSA-based regimen is necessary for rituximab SC for the indications being sought.

Body Size: Body size (BSA and BMI) was identified as a significant covariate in the population PK analysis for rituximab (**See Section 4.1**). Given that BSA-based dosing allows individualization of doses based on BSA, a transition to fixed dosing could lead to under- or over-dosing of patients in the extremes of the BSA spectrum. This possibility was explored in our review and our findings are summarized below.

1400 mg Fixed Dose

As shown in **Figure 5**, data from Study BO22334 indicate that the distribution of rituximab C_{trough} are fairly consistent across BSA sizes following rituximab IV 375 mg/m^2 and rituximab SC 1400mg. The solid black line represents the median C_{trough} after the IV dose. However, as expected, the C_{trough} following the rituximab SC 1400 mg dose was generally equal or higher than that observed after the rituximab IV dose. We note that the Rituximab SC C_{trough}s gradually converged towards the median rituximab IV C_{trough} with increasing BSA.

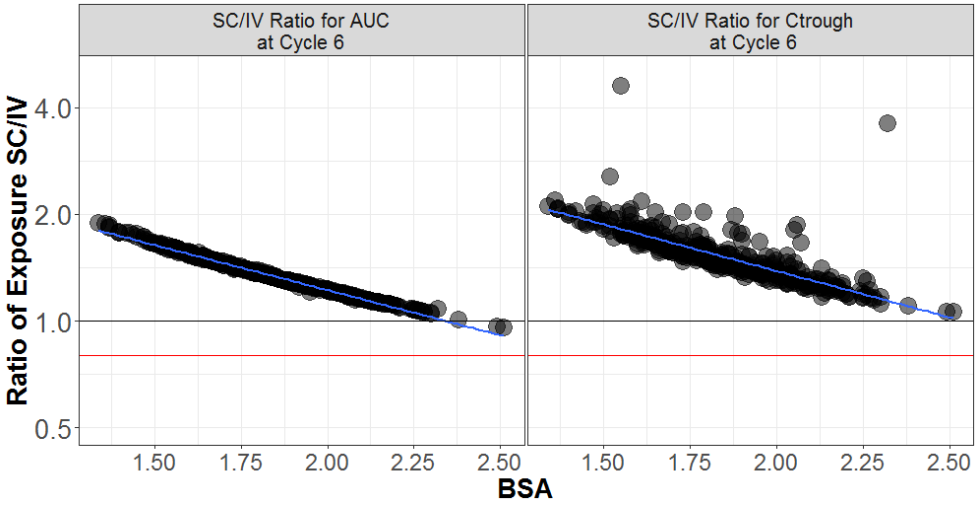
Figure 5: **Relationship between observed C_{trough} and body surface area for rituximab IV 375 mg/m^2 and rituximab SC 1400 mg**



In addition, the applicant's final population PK model was used to simulate and compare the predicted AUC, Cmax, and C_{trough} at the end of Cycle 6 (Pre-dose Cycle 7) and 17 (Pre-dose Cycle 18) after the administration of rituximab IV and rituximab SC for all patients in Study BO22334 to determine if the SC dose will consistently result in higher C_{trough} compared to the IV dose regardless of BSA. The relationship between BSA and the SC/IV ratio for AUC, Cmax, and C_{trough} at the end of Cycle 6 for patients simulated

to receive rituximab IV and SC are shown in **Figure 6**. Similar such relationship was observed at the end of Cycle 17.

Figure 6: The relationship between BSA and the SC/IV AUC, Cmax, and C_{trough} Geometric Mean Ratio (90% CI) at the end of Cycle 6 for patients enrolled in Study BO22334 simulated to receive rituximab IV and SC

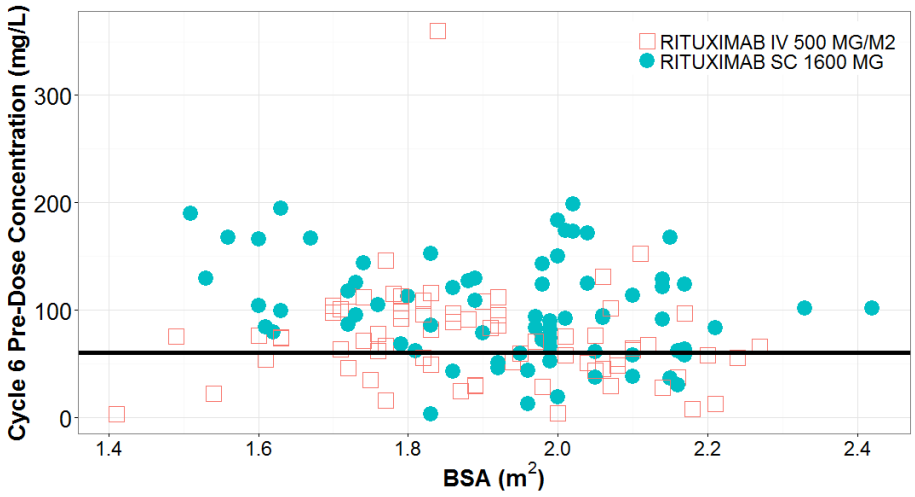


All patients were expected to have higher C_{trough} (ratio of SC/IV > 1) when administered rituximab SC 1400 mg compared to rituximab 375 mg/m^2 IV, regardless of the BSA. While fixed rituximab doses could lead to 2-fold higher C_{trough} relative to the BSA-based rituximab IV doses in some patients, we note that rituximab has been well tolerated at doses up to 2250 mg/m^2 or approximately 4000 mg on a fixed dose scale, indicating that rituximab has wide therapeutic window. In addition, higher rates of adverse events were not observed in patients with low BSA (see Clinical review by Dr. Schwarsin).

1600 mg Fixed Dose

Because the rituximab 1600 mg SC dose proposed for the CLL indication was derived using the same exposure matching principles as the 1400 mg SC dose proposed for the NHL indication, we observed a similar relationship between the observed C_{trough} and BSA as shown in **Figure 7**.

Figure 7: **Relationship between observed C_{trough} and body surface area for rituximab IV 500 mg/m² and rituximab SC 1600 mg**



We observed a similar relationship between BSA and the SC/IV ratio for AUC and C_{trough} based on simulations using the applicant's final population PK model as that observed with NHL.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

No. Since rituximab is not given orally, food-drug interactions are not anticipated or applicable. Drug-drug interactions are not expected based on the CYPs, other metabolizing enzymes, or transporters. As such, no *in vivo* or *in vitro* drug-drug interaction studies were conducted.

4. APPENDICES

4.1 Population PK and/or PD Analyses

The applicant's population PK models are adequate to describe the C_{trough} and AUC of rituximab after IV and SC administration.

4.1.1 Population PK Analysis for NHL

The applicant conducted a population PK analysis to characterize rituximab PK following IV and SC administration, identify covariate factors that could influence rituximab disposition, and compute individual exposure estimates for subsequent exposure-response analyses. Data from Study BO22334 was used in the population PK study.

Serum samples were obtained predose for every cycle from patients in Stage 1. Additional serum samples were obtained at the end of infusion for Cycle 1, Days 1, 2, 6, and 14 for Cycles 2 and 7and Day 28 for Cycle 8. Samples were also obtained at follow-up every 12 weeks after the last rituximab administration until 96 weeks after the last rituximab administration or until undetectable rituximab levels. Additionally, to capture peak values, serum samples were collected at the end of infusion in Cycles 2, 5, and 7 for the IV arm, and 6 days post-dose in Cycle 5 for the SC arm.

Serum samples were collected in Stage 2 pre-dose at all cycles in the induction and maintenance periods. Also, samples were collected during follow-up every 12 weeks after the last rituximab administration until 96 weeks after the last rituximab administration.

The population PK analysis was conducted via nonlinear mixed-effects modeling with the NONMEM software, version 7.3.0 using first-order conditional estimation with INTERACTION option (FOCEI). Visual inspection was used to identify potential outlying errors in the dataset. Concentration values before the first dose were excluded from the PK analysis as well as post-dose observations that were below the limit of quantification (BLQ).

A linear two-compartment population PK model with a combined time-dependent and time-independent elimination clearances, additional target-mediated elimination with non-renewable target, and first order SC absorption described rituximab serum concentrations following IV and SC administration.

Rituximab PK parameters depended on body size measures. Clearance (both, CLinf and CLT terms), Vc, Vp, and Q increased with BSA. CLT was higher in patients with higher B-cell count and higher tumor size at baseline. The rate constant of decay of CLT with time (kdes) was lower in patients with higher baseline tumor size. In addition to BSA, central volume also increased with age, but the dependency was minor. Gender had no clinically relevant effects on rituximab PK and exposure. A summary of the parameter estimates, relevant covariates, and goodness of fit plots are provided in **Table 9, Table 10**, and **Figure 8**.

Table 9: **Summary of the Population PK Parameter Estimates for Rituximab based on data from Study BO223334**

Parameter		Estimate	%RSE	95%CI	Variability	Shrinkage (%)
k_{des} (1/day)	θ_1	0.0745	8.57	0.062 - 0.087		
CL_T (mL/day)	θ_2	398	11.1	311 - 485		
CL_{inf} (mL/day)	θ_3	200	1.78	193 - 207		
V_C (mL)	θ_4	4540	2.20	4350 - 4740		
V_P (mL)	θ_5	4270	1.61	4140 - 4400		
Q (mL/day)	θ_6	573	3.59	533 - 613		
k_a (1/day)	θ_7	0.344	7.17	0.295 - 0.392		
F_{SC}	θ_8	0.646	0.992	0.634 - 0.659		
Kvmax (10^{-5} ng/mL/day)	θ_9	9.73	3.94	8.98 - 10.5		
V_{max} (1/day)	θ_{10}	0.0187	4.5	0.0171 - 0.0204		
$V_{C,AGE}$	θ_{11}	0.211	23.9	0.112 - 0.309		
$k_{a,AGE}$	θ_{12}	-2.40	22.1	-3.45 - -1.36		
$CL_{BSA} = CL_{T,BSA} = Q_{BSA}$	θ_{13}	1.54	8.25	1.29 - 1.79		
$V_{C,BSA} = V_{P,BSA}$	θ_{14}	1.25	4.69	1.14 - 1.37		
σ_L	θ_{15}	0.602	6.94	0.52 - 0.684		
σ_H	θ_{16}	0.113	3.00	0.106 - 0.119		
σ_{50}	θ_{17}	4.41	12.6	3.32 - 5.49		
$k_{des, BSIZ}$	θ_{18}	-0.424	17.4	-0.568 - -0.279		
$CL_{T, BSIZ}$	θ_{19}	0.355	20.2	0.215 - 0.496		
$CL_{T, B\text{-}cell}$	θ_{20}	0.683	12.1	0.522 - 0.845		
ω^2	$\Omega(1,1)$	1.07	14.5	0.765 - 1.37	CV=103%	23.1%
ω^2	$\Omega(2,2)$	2.01	11.8	1.55 - 2.48	CV=142%	15.4%
ω^2	$\Omega(3,3)$	0.0776	8.07	0.0653 - 0.0898	CV=27.9%	3.8%
ω^2	$\Omega(4,4)$	0.0662	12.9	0.0495 - 0.0829	CV=25.7%	20.4%
ω^2	$\Omega(5,5)$	0.288	17.7	0.188 - 0.388	CV=53.7%	34.4%
ω^2	$\Omega(6,6)$	0.136	8.32	0.114 - 0.158	CV=36.9%	2.5%
σ^2	$\Sigma(1,1)$	1	fixed			1.4%

Source: Applicant's analysis

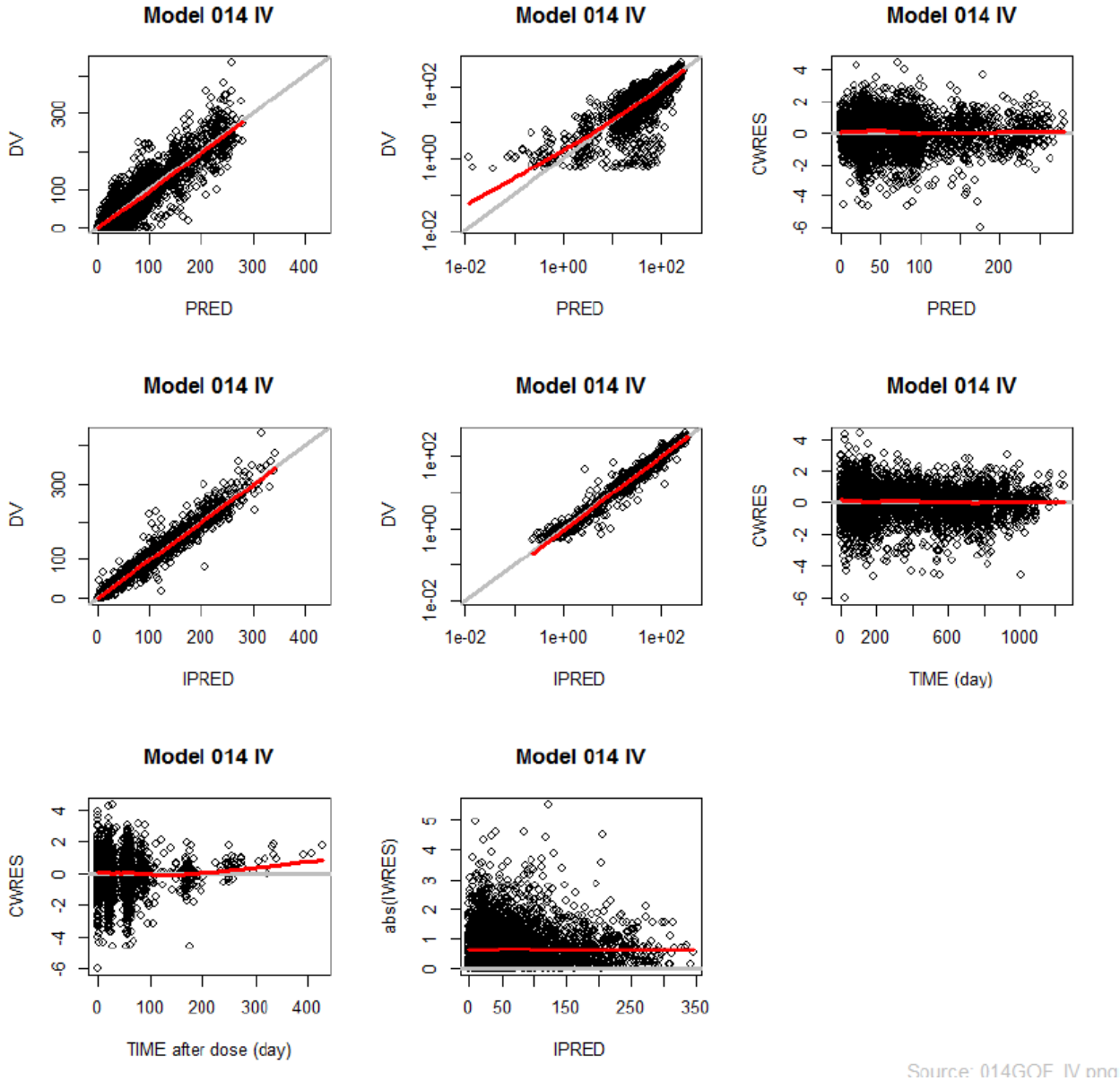
Table 10: **Covariate Effects in the Population PK Parameter Estimates for Rituximab based on data from Study BO223334**

Parameter	Covariate	Reference Value	Covariate Value	Covariate Effect Value [95%CI] (%)
CL_{inf}, CL_T, Q	BSA (m^2)	1.9	1.44[a]	-34.7 [-39; -30]
			2.26[b]	30.6 [25; 36.3]
Vc, Vp	BSA (m^2)	1.9	1.44[a]	-29.4 [-31.6; -27.1]
			2.26[b]	24.3 [21.9; 26.8]
Vc	Age (years)	60 years	33[a]	-11.8 [-16.9; -6.47]
			80[b]	6.25 [3.27; 9.31]
CL_T	BSIZ (mm^2)	4420[c]	477[a]	-54.7 [-66.9; -38]
			29500[b]	96.3 [50.3; 156]
	BBCE (10^6/L)	74[c]	≤ 74	0 [0; 0]
			9610[b]	2680 [1170; 6010]
k_a	Age (years)	60	≤ 60	0 [0; 0]
			80[b]	-49.9 [-62.9; -32.4]

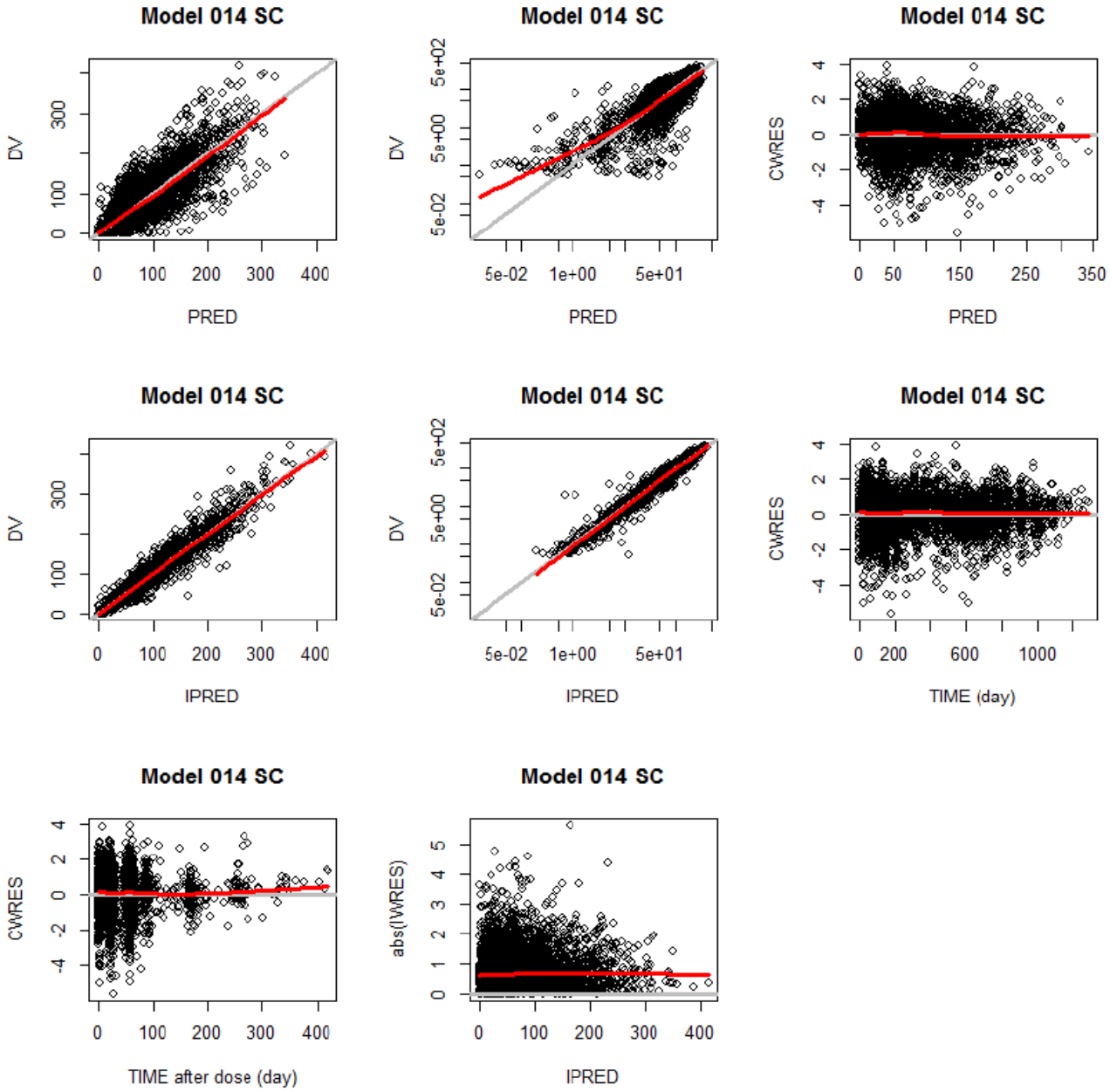
k_{des}	BSIZ (mm^2)	4420 [c]	477[a]	157 [86.1; 254]
			29500[b]	-55.2 [-66; -41.1]

a. 2.5th percentile of the parameter distribution;
b. 97.5th percentile of the parameter distribution;
c. median of the parameter distribution
Source: Applicant analysis

Figure 8: **Goodness of fit for the applicant's final population PK model (Model 14) in patients with follicular lymphoma by Study arm (IV – top panel, SC-bottom panel).**



Source: 014GOF_IV.png



DV: Observed concentrations; PRED: population predictions of the model; IPRED: individual predictions of the model; CWRES: conditional weighted residuals; IWRES: individual weighted residuals; TIME: time after the first dose. The gray solid y=x or y=0 lines are included for reference. The bold red lines are the lowess (local regression smoother).

(Source Applicant's Population PK Report 1071510, Figures 27-28)

Using the final population PK model, the AUC and total clearance after each cycle in Study BO22334, by arm was calculated and is provided in **Table 11**.

Table 11: **Mean (CV) of AUC and Total Clearance after each Cycle for the Rituximab IV and Rituximab SC Arm based on data from Study BO223334**								
Cycle	Rituximab IV Arm				Rituximab SC Arm			
	AUC (µg/mL•day)		Total Clearance (mL/day)		AUC (µg/mL•day)		Total Clearance (mL/day)	
	Mean (CV)	Geo. Mean (CV)	Mean (CV)	Geo. Mean (CV)	Mean (CV)	Geo. Mean (CV)	Mean (CV)	Geo. Mean (CV)
1	818 (0.53)	621 (0.94)	36764 (3.19)	8370 (1.5)	755 (0.54)	562 (0.97)	42384 (2.59)	9587 (1.57)
2	1396 (0.41)	1194 (0.69)	9484 (2.93)	1581 (1.7)	1625 (0.41)	1415 (0.63)	10118 (2.77)	2097 (1.68)
3	1838 (0.37)	1645 (0.56)	4122 (2.85)	696 (1.61)	2319 (0.36)	2096 (0.53)	4425 (3.45)	887 (1.59)
4	2197 (0.32)	2030 (0.46)	2113 (3.55)	446 (1.34)	2871 (0.35)	2633 (0.48)	2471 (3.84)	563 (1.37)
5	2470 (0.31)	2309 (0.42)	1448 (4.05)	353 (1.17)	3319 (0.35)	3068 (0.44)	1541 (3.99)	415 (1.2)
6	2698 (0.31)	2534 (0.4)	1096 (4.4)	304 (1.03)	3686 (0.36)	3463 (0.36)	725 (3.22)	322 (0.96)
7	2903 (0.32)	2726 (0.39)	868 (4.6)	275 (0.93)	3970 (0.34)	3744 (0.35)	556 (3.42)	283 (0.85)
8	5545 (0.37)	5149 (0.41)	504 (3.46)	247 (0.75)	8118 (0.39)	7482 (0.42)	465 (3.61)	260 (0.75)
9	4060 (0.37)	3808 (0.36)	323 (2.71)	219 (0.56)	6030 (0.43)	5514 (0.43)	337 (3.6)	222 (0.57)
10	3856 (0.4)	3632 (0.33)	237 (1.39)	204 (0.42)	5481 (0.39)	5082 (0.39)	286 (3.21)	203 (0.5)
11	3717 (0.32)	3553 (0.3)	221 (0.84)	201 (0.37)	5320 (0.37)	4981 (0.36)	204 (0.42)	191 (0.36)
12	3726 (0.29)	3580 (0.28)	211 (0.52)	197 (0.34)	5334 (0.37)	4995 (0.36)	198 (0.38)	186 (0.35)
13	3727 (0.3)	3582 (0.28)	206 (0.39)	195 (0.33)	5286 (0.36)	4971 (0.35)	196 (0.36)	184 (0.35)
14	3699 (0.28)	3563 (0.27)	204 (0.33)	194 (0.32)	5322 (0.36)	5000 (0.35)	194 (0.36)	183 (0.35)
15	3782 (0.3)	3631 (0.28)	202 (0.32)	193 (0.31)	5300 (0.35)	4986 (0.35)	194 (0.35)	183 (0.35)
16	3756 (0.27)	3627 (0.26)	201 (0.32)	192 (0.31)	5337 (0.37)	4999 (0.36)	193 (0.35)	182 (0.35)
17	3782 (0.28)	3640 (0.28)	201 (0.32)	192 (0.32)	5326 (0.36)	4996 (0.36)	194 (0.35)	182 (0.35)
18	3765 (0.29)	3618 (0.28)	201 (0.32)	191 (0.32)	5269 (0.35)	4964 (0.35)	194 (0.35)	183 (0.35)
19	3826 (0.28)	3687 (0.27)	199 (0.31)	190 (0.31)	5271 (0.36)	4962 (0.35)	194 (0.35)	183 (0.35)

As shown in **Table 11**, the variability of the AUC and total clearance were high due in part to the variability around the time-dependent clearance (%CV ~ 142%). As expected, there was a decline in the time-dependent clearance over time. Given that the time dependent clearance was variable, the total clearance and AUC were highly variable in the early cycles of treatment. As shown in **Table 12**, the variability around the AUC and total clearance gradually decreased, given the decrease in the percentage of total clearance that could be attributed to the time-dependent clearance.

Table 12: **Summary of the Percentage of Total Clearance Attributed to Time-dependent Clearance for Rituximab IV and Rituximab SC in NHL based on data from Study BO223334**				
Pre-dose Cycle	Rituximab IV Arm		Rituximab SC Arm	
	% of time-dependent clearance[1]		% of time-dependent clearance	
	Mean (CV)	Min - max	Mean (CV)	Min - max
1	95 (0.05)	100 - 100	96 (0.04)	100 - 100
2	71.7 (0.4)	0 - 92.58	77.2 (0.34)	0 - 90.11
3	46.6 (0.83)	0 - 85.61	55.2 (0.7)	0 - 80.34
4	33.26 (1.1)	0 - 79.22	43.95 (0.9)	0 - 71.99
5	25.2 (1.4)	0 - 73.31	35.13 (1.1)	0 - 63.18
6	19.68 (1.6)	0 - 67.82	26.91 (1.3)	0 - 56.6
7	15.92 (1.8)	0 - 62.74	21.94 (1.5)	0 - 49.96

8	11.92 (2.1)	0 - 56.76	18.75 (1.6)	0 - 44.78
9	6.89 (2.8)	0 - 45.98	11.47 (2)	0 - 32.74
10	3.95 (3.5)	0 - 37.51	7.31 (2.3)	0 - 24.44
11	2.65 (4.2)	0 - 26.79	4.01 (2.7)	0 - 13.02
12	1.89 (5)	0 - 21.77	2.5 (3)	0 - 9.04
13	1.4 (5.9)	0 - 17.69	1.35 (3.5)	0 - 6.28
14	1.07 (6.5)	0 - 12.87	0.81 (4)	0 - 4.36
15	0.81 (7.1)	0 - 10.46	0.49 (4.6)	0 - 3.03
16	0.62 (7.7)	0 - 8.32	0.3 (5.1)	0 - 2.11
17	0.48 (8.5)	0 - 6.73	0.19 (5.7)	0 - 1.45
18	0.37 (9.3)	0 - 5.37	0.12 (6.2)	0 - 1.01
19	0.3 (9.9)	0 - 4.26	0.08 (6.7)	0 - 0.7
1 Percent of total clearance attributed to time-dependent clearance = time-dependent clearance*100/total clearance				

4.1.2 Population PK Analysis for CLL

Data from Study BO17072 and BO23541 were used in this population PK study. Patients enrolled in study BO17072 were treated for 6 cycles. Subjects received rituximab IV 375 mg/m^2 in Cycle 1 and 500 mg/m^2 on Day 1 of Cycle 2 to 6. Blood samples for determination of rituximab concentrations were collected in Cycles 1, 3, and 6, prior to dosing, at 8, 11, and 24 hours post dosing, and at 3, 5, 7, 14, 21, and 28 days post dose. In addition, blood samples were collected at Months 7, 8, 9, and 12.

Study BO25341 was a two-part study. Stage 1 was used to select the rituximab SC fixed dose and Stage 2 was used to ensure that the selected SC dose resulted in rituximab serum C_{trough} levels that are comparable to that following rituximab IV administration. In Stage 2, approximately 170 patients were randomized to receive either rituximab IV or SC. Rituximab IV was administered in the first cycle for all subjects. In the rituximab IV arm, subjects received 375 mg/m^2 rituximab IV in Cycle 1, and 500 mg/m2 rituximab IV in Cycles 2-6. In the SC arm, subjects received 375 mg/m^2 rituximab IV in Cycle 1, and rituximab SC at the dose of 1600 mg in Cycles 2-6. For Stage 1, blood samples for determination of rituximab concentrations were collected during Cycles 5 and 6. In Cycle 5, samples were collected pre-dose, end of infusion, 1, 4, 10, and 14 days post-dose. In Cycle 6, samples were collected pre-dose, 1, 2, 4, 10, 14, 28, and 56 days post-dose.

In Part 2, the pharmacokinetic samples were collected for both IV and SC arms pre-dose for Cycles 1 to 6; at the end of infusion, 1, 2, 7, and 14 days post-dose in Cycle 1 and 6; 14 days post-dose in Cycle 2; and 56 days post-dose in Cycle 6. For the IV arm, additional samples were collected following the end of infusion in Cycles 4 and 6. For the SC arm, additional samples were collected 1, 2, and 7 days post dose in Cycle 2.

The population PK analysis was conducted using nonlinear mixed-effects modeling with the NONMEM software, version 7.3.0 using first-order conditional estimation with INTERACTION option (FOCEI). Visual inspection was used to identify potential outliers in the dataset. Concentration values before the first dose were excluded from the PK analysis as well as post-dose observations that were below the limit of quantification (BLQ).

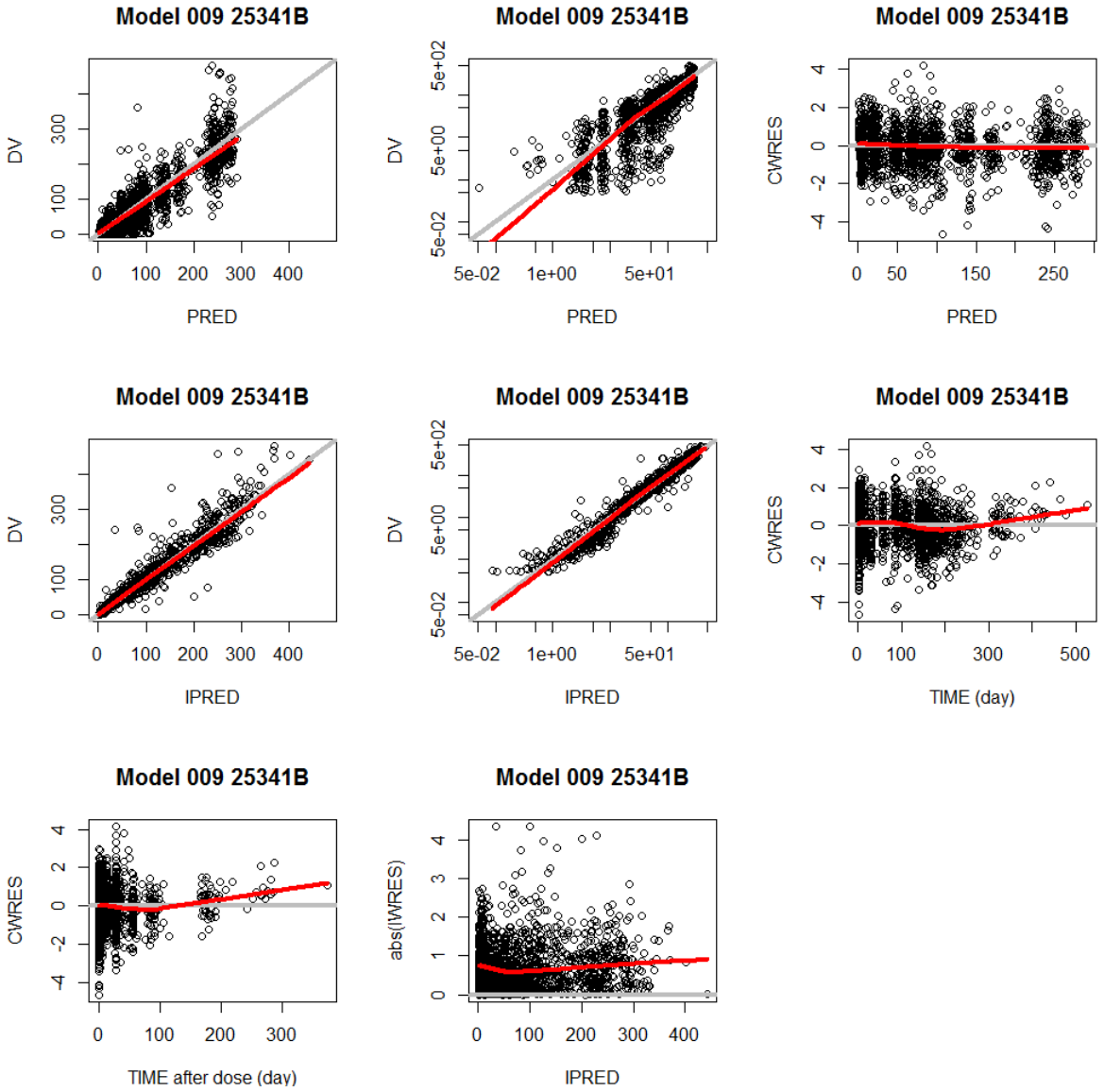
The data was described using a linear two-compartment population PK model with a combined time-dependent (CLT) and time-independent elimination clearances (CLinf), and first order SC absorption. All clearance (CLT, CLinf, Q) and volume (Vc and Vp) parameters were related to body surface area. The time-dependent clearance was also related to the baseline white blood cell count and B-cell count. The rate of absorption (ka) and fraction absorbed was also related to body mass index (BMI) and gender was related to the volume of the central compartment. A summary of the parameter estimates, relevant covariates, and goodness-of-fit plots are provided in **Table 13**, **Table 14,** and **Figure 9**.

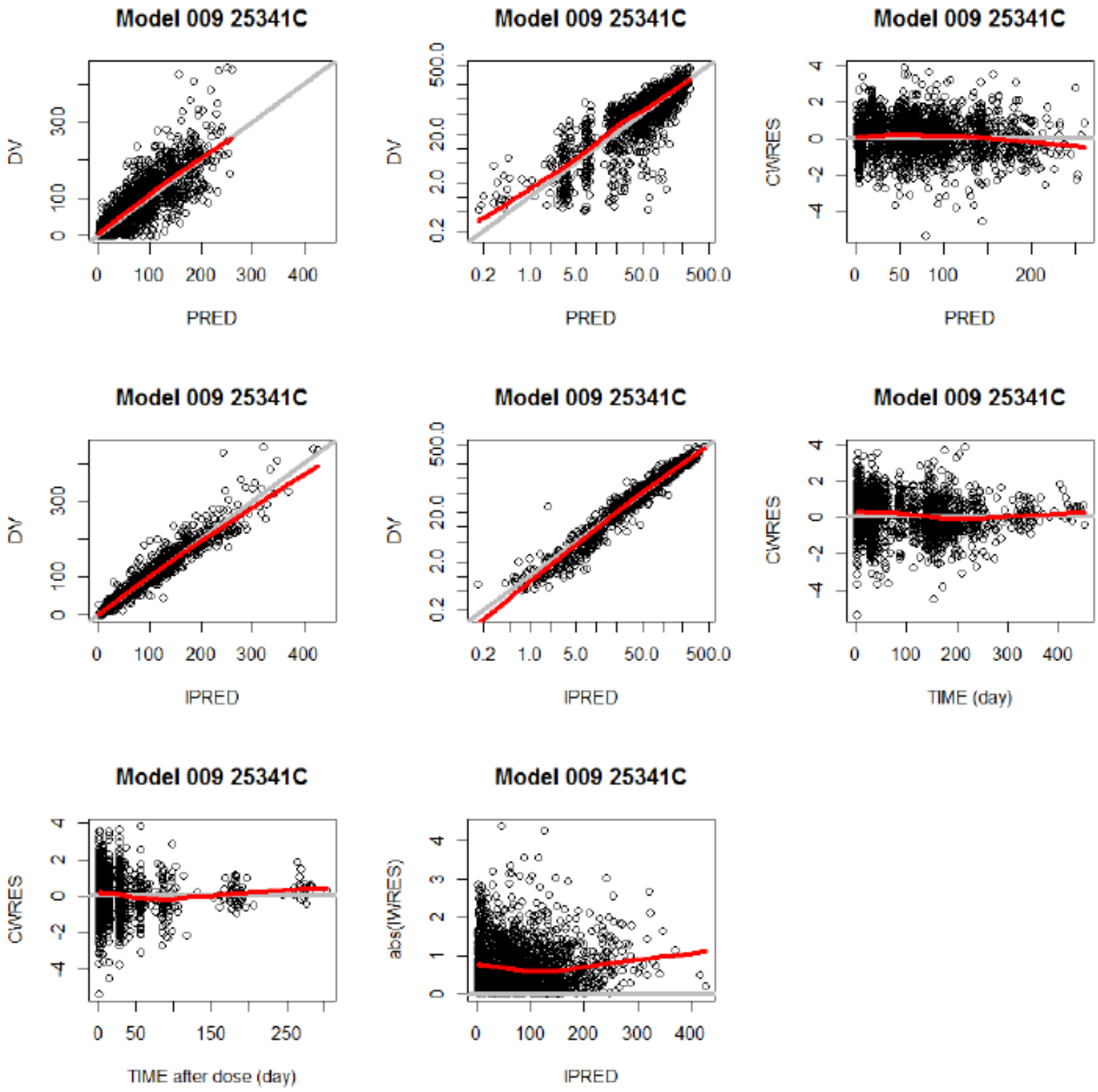
Table 13: **Summary of the Population PK Parameter Estimates for Rituximab in CLL based on data from Study BO17072 and BO25341**

Parameter		**Estimate**	**%RSE**	**95%CI**	**Variability**	**Shrinkage (%)**
k_{des} (1/day)	θ_1	0.0399	5.19	0.0359 - 0.044		
CL_T (mL/day)	θ_2	1550	8.14	1300 - 1800		
CL_{inf} (mL/day)	θ_3	207	2.62	196 - 217		
V_C (mL)	θ_4	4990	1.82	4820 - 5170		
V_P (mL)	θ_5	3700	1.97	3560 - 3840		
Q (mL/day)	θ_6	420	3.23	393 - 446		
k_a (1/day)	θ_7	0.372	3.86	0.344 - 0.4		
F_{SC}	θ_8	0.633	2.52	0.601 - 0.664		
$CL_{BSA} = Q_{BSA}$	θ_{14}	1.37	12.3	1.04 - 1.7		
$V_{C,BSA} = V_{P,BSA}$	θ_{15}	0.8	11.4	0.622 - 0.978		
$CL_{T,WBC}$	θ_{16}	0.223	34.2	0.0737 - 0.373		
$V_{C,SEXF}$	θ_{17}	0.909	2.96	0.856 - 0.961		
$k_{a,BMI}$	θ_{18}	-1.01	23.8	-1.48 - -0.537		
$F_{SC,BMI}$	θ_{19}	-0.465	35.6	-0.789 - -0.141		
$CL_{T,BSIZ}$	θ_{20}	0.261	21.1	0.153 - 0.369		
σ_L	θ_9	0.81	8.69	0.672 - 0.948		
σ_H	θ_{10}	0.134	4.32	0.123 - 0.146		
σ_{50}	θ_{11}	6.35	17.5	4.17 - 8.54		
σ_{17072}	θ_{12}	1.42	7.6	1.21 - 1.63		
σ_{CohA}	θ_{13}	0.568	5.7	0.505 - 0.632		
ω^2	$\Omega(1,1)$	0.357	10.3	0.285 - 0.428	CV=59.7%	13.2%
ω^2	$\Omega(2,2)$	0.691	9.4	0.563 - 0.818	CV=83.1%	10.5%
ω^2	$\Omega(3,3)$	0.106	10.5	0.0839 - 0.127	CV=32.5%	7.6%
$R\omega_{CLinf}\omega_{Vc}$	$\Omega(3,4)$	0.0277	15.7	0.0191 - 0.0362	R=0.47	
ω^2	$\Omega(4,4)$	0.0323	11.3	0.0251 - 0.0394	CV=18.0%	9.9%
ω^2_{ka}	$\Omega(5,5)$	0.115	17.7	0.0753 - 0.156	CV=34.0%	9.1%
$R\omega_{ka}\,\omega_{Fsc}$	$\Omega(5,6)$	0.0265	36.4	0.00759 -0.0454	R= 0.37	
ω^2_{Fsc}	$\Omega(6,6)$	0.0453	19.0	0.0285 - 0.0622	CV=21.3%	7.9%
ω^2	$\Omega(7,7)$	0.0929	10.2	0.0743 - 0.111	CV=30.5%	0%
σ^2	$\Sigma(1,1)$	1	fixed			2.8%
Source: Applicant analysis						

Table 14: Covariate Effects in the Population PK Parameter Estimates for Rituximab in NHL based on data from Study BO17072 and BO25341				
Parameter	**Covariate**	**Reference Value**	**Covariate Value**	**Covariate Effect Value [95%CI] (%)**
CLinf, CLT, Q	BSA (m^2)	1.9	1.53	-25.6 [-30.7; -20.1]
			2.23	24.5 [18.1; 31.2]
Vc, Vp	BSA (m^2)	1.9	1.53	-15.9 [-19.1; -12.6]
			2.23	13.7 [10.5; 17]
Vc	SEX	Male	Female	-9.1 [-14.4; -3.9]
CLT	WBC (10^9/L)	100	11.6	-38.2 [-55.2; -14.7]
			281	26 [7.9; 47]
	BSIZ (mm^2)	7000	400	-52.7 [-65.2; -35.5]
			27000	42.3 [23; 64.6]
ka	BMI (kg/m^2)	27	20.7	30.7 [15.3; 48.1]
			36.5	-26.2 [-36; -14.9]
Fsc	BMI (kg/m^2)	27	20.7	13.1 [3.8; 23.3]
			36.5	-13.1 [-21.2; -4.1]
Source: Applicant analysis				

Figure 9: Goodness of fit for the applicant's final population PK model (Model 09) in patients with CLL by Study arm (IV – top panel, SC-bottom panel)





DV: Observed concentrations; PRED: population predictions of the model; IPRED: individual predictions of the model; CWRES: conditional weighted residuals; IWRES: individual weighted residuals; TIME: time after the first dose. The gray solid y=x or y=0 lines are included for reference. The bold red lines are the lowess (local regression smoother). *(Source Applicant's Population PK Report 1063749, Figures 15-16)*

4.2 Exposure-Response Analyses

Efficacy

The impact of exposure on efficacy was explored by evaluating the relationship between C_{trough} at Cycle 6 (Pre-dose Cycle 7) and Best Observed Response (CR/CRu) in patients with FL using data from Study BO22334 and in patients with CLL using data from Study BO23541 and a logistic regression model (all data pooled). As shown in the **Figure 10** and **Figure 11**, a weak but significant relationship between C_{trough} and CR/CRu was observed for both FL and CLL.

Figure 10: **Relationship between observed C_{trough} at Cycle 7 and Probability of CR/CRu for NHL using data from Study BO22334**

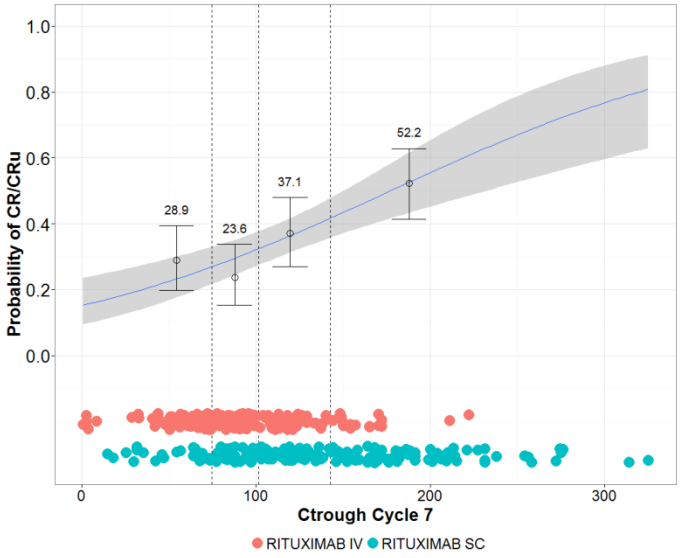
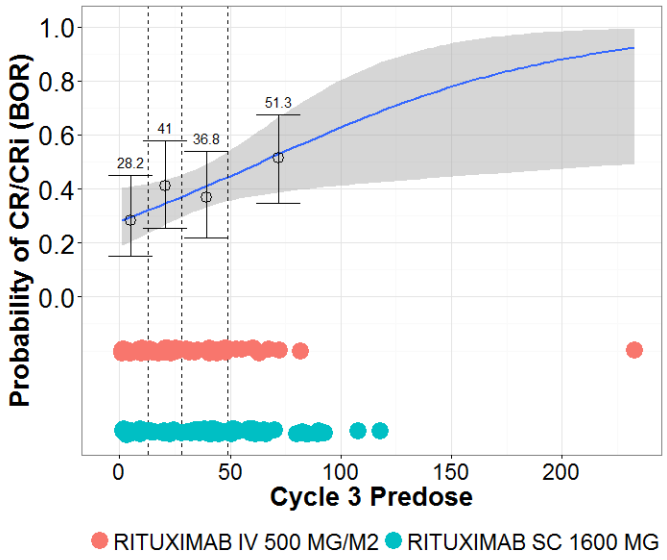


Figure 11: **Relationship between observed C_{trough} at Cycle 3 and Probability of CR/CRu for CLL using data from Study BO25341**

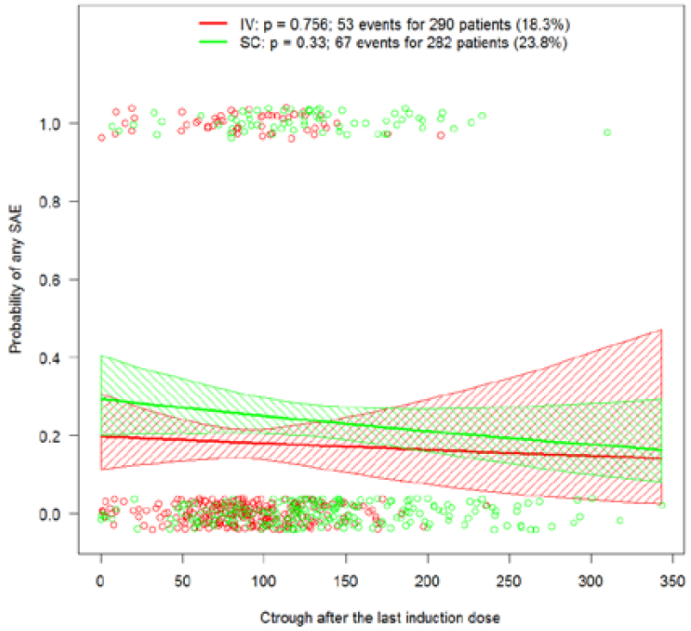


Safety

Neutropenia, serious adverse events (SAE), and Grade 3+ adverse events were identified as potentially clinically important safety endpoints to be considered with higher rituximab exposures. As such, the impact of exposure on these safety endpoints were explored by evaluating the relationship between C_{trough} and each of these safety endpoints in patients with FL using data from Study BO22334 and in patients with CLL using data from Study BO23541. These exposure-response relationships for safety were evaluated using a logistic regression model.

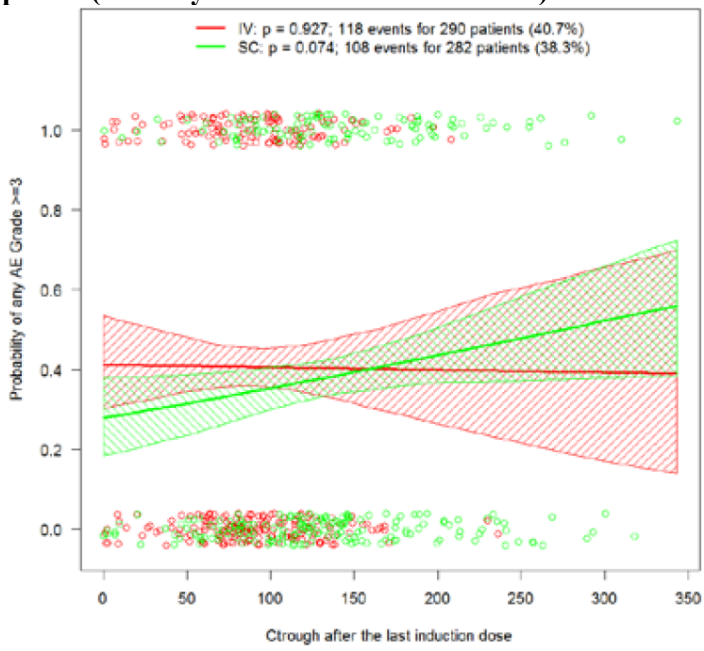
Based on the univariate evaluations relating exposure to each of the safety endpoints, (**Figure 12** and **Figure 13**) in patients with NHL and CLL, no relationships between C_{trough} and each of the safety endpoints were observed. As such, the FDA concluded that higher increases in exposure are not related to higher probability of higher safety events.

Figure 12: Relationship between probability of any SAE and C_{trough} during induction period (From cycle 1 until end of induction)



Source: Applicant's Response to FDA Information Request 04Jan2017

Figure 13: Relationship between probability of any Grade 3+ AE and C_{trough} during induction period (From cycle 1 until end of induction)



Source: Applicant's Response to FDA Information Request 04Jan2017

4.3 Immunogenicity

Patients were tested for ADAs in all clinical trials (BO22333, BO22334, and BO25341). Anti-drug antibodies in human serum samples were detected as described in **Section 4.4.1**. A summary of the prevalence of anti-rituximab antibodies are provided in **Table 15** for FL and **Table 16** for CLL.

Table 15: **Baseline Prevalence and Post-Baseline Incidence of Anti-Rituximab Antibodies (Safety Population) by Study for FL population**

	Study BP22333			**Study BO25341**		
	IV[N =77]	**SC [N =77]**	**All [N=154]**	**IV [N =210]**	**SC [N =197]**	**All [N=407]**
Baseline *(Prevalence of Anti-Rituximab Antibodies [HACAs] predose at first cycle on study)*						
Evaluable patients	76	77	153	210	197	399
No. of patients positive for HACAs	0 (0%)	1 (1%)	1 (<1%)	12 (6%)	5 (3%)	17 (4%)
No. of patients negative for HACAs	76 (100%)	76 (99%)	152 (99%)	196 (94%)	186 (97%)	382 (96%)
Post-Baseline *(Incidence of Anti-Rituximab Antibodies [HACAs] following study treatment)*						
Evaluable patients	77	77		206	197	
No. of patients positive for HACAs	3 (4%)	1 (1%)		3 (1%)	4 (2%)	
Treatment-induced[a] HACAs	3	1		3	3	
Treatment-enhanced[b] HACAs	0	0		0	1	
No. of patients negative for HACAs	74	76		203[d]	193[d]	
Treatment-unaffected[c] HACAs	0	1		12[e]	4[e]	

a Post-baseline positive patients are those who have seroconverted (treatment-induced) or enhanced their baseline response (treatment-enhanced) during the study period following study drug administration.
b Treatment-induced HACAs: Patients who had baseline-negative HACA result who developed anti-rituximab antibodies at any time after initial drug administration.
c Treatment-enhanced HACAs: Patients who had baseline-positive HACA result in whom the assay signal was enhanced (greater than baseline titer by ≥ 0.60 titer units) at any time after initial drug administration.
d Post-baseline negative patients are those who are negative at all timepoints after baseline or for whom a baseline positive signal was not enhanced after baseline (i.e., treatment unaffected).
e Treatment-unaffected HACAs: Patients who had baseline-positive HACA result in whom the assay signal was not enhanced (not greater than baseline titer by ≥ 0.60 titer units) at any time after initial drug administration. These patients are considered post-baseline negative for HACAs.
Source: Applicant Analysis

Table 16: **Baseline Prevalence and Post-Baseline Incidence of Anti-Rituximab Antibodies (Safety Population) by Study for CLL population**

	IV N =77	**SC N =77**	**All Patients N=154**
Baseline *(Prevalence of Anti-Rituximab Antibodies [HACAs])*			
Evaluable patients	87	85	172
No. of patients positive for HACAs	0 (0%)	2 (2%)	2 (1%)
No. of patients negative for HACAs	87 (100%)	83 (98%)	170 (99%)
Post-Baseline *(Incidence of Anti-Rituximab Antibodies [HACAs] following study treatment)*			
Evaluable patients	89	85	
No. of patients positive for HACAs	6 (7%)	2 (2%)	
Treatment-induced[a] HACAs	6	2	
Treatment-enhanced[b] HACAs	0	0	
No. of patients negative for HACAs	83	83	
Treatment-unaffected[c] HACAs	0	2	

a Treatment-induced HACAs: Patients who had baseline-negative HACA result who developed anti-rituximab antibodies at any time after initial drug administration.
b Treatment-enhanced HACAs: Patients who had baseline-positive HACA result in whom the assay signal was enhanced (greater than baseline titer by ≥ 0.60 titer units) at any time after initial drug administration.
c Treatment-unaffected HACAs: Patients who had baseline-positive HACA result in whom the assay signal was not enhanced (not greater than baseline titer by ≥ 0.60 titer units) at any time after initial drug administration. These patients are considered post-baseline negative for HACAs.
Source: Applicant Analysis

Baseline prevalence rates of anti-rHuPH20 antibodies were in the range of 6% to 11% across the three studies. For the majority of patients who were HAHA-positive at baseline in the three rituximab SC studies, the baseline assay signal was not enhanced after the start of treatment and these patients were thus considered to be HAHA-negative post-baseline (treatment-unaffected).

The post-baseline incidence of anti-rHuPH20 antibodies (treatment-induced and treatment-enhanced responses) ranged between 5% and 13% across the three studies as shown in **Table 17**.

Table 17: Baseline Prevalence and Post-Baseline Incidence of Anti-Rituximab Antibodies (Safety Population) by Study for CLL population

	Rituximab IV n/N (%)	Rituximab SC n/N (%)	Median follow-up time (median treatment duration)
BP22333, Stage 2			
Baseline	-	5/77 (6%)	26 months follow-up
Post-baseline[a]	-	4/77 (5%)	(17 months treatment)
BO22334, Stage 1 and 2			
Baseline	7/68 (10%)[b]	21/188 (11%)	37 months follow-up
Post-baseline[a]	5/66 (8%)[b]	26/197 (13%)	(27 months treatment)
BO25341, Part 2			
Baseline	-	9/84 (11%)	14 months follow-up
Post-baseline[a]	-	9/85 (11%)	(5 months treatment)

a Treatment-induced/-enhanced HAHA response.
b Anti-rHuPH20 antibody testing conducted in rituximab IV patients (negative control) in Stage 1 only
Source: Applicant analysis

4.4 Summary of Bioanalytical Method Validation and Performance

4.4.1 How are the active moieties identified and measured in the clinical pharmacology and biopharmaceutics studies?

Rituximab

Serum rituximab concentrations were measured in plasma by a validated Enzyme-Linked Immunosorbent Assay (ELISA). Test samples, quality controls, and standards (rituximab in buffer) were incubated on plates pre-coated with polyclonal goat anti-rituximab antibodies (rituximab affinity purified, anti-efalizumab depleted), followed by washing. Bound samples were detected by incubation with goat anti-mouse IgG F(ab')2 conjugated to horseradish peroxidase. Following a further wash to remove any unbound conjugate, a substrate solution (tetramethylbenzidine/hydrogen peroxide) was added to the wells, resulting in a color development in proportion to the amount of rituximab in the samples. The reaction was stopped, and absorbance was measured photometrically. The lower limit of quantitation (LLOQ) in human serum was 2.5 ng/mL. Analytical reports were submitted and QC reports were summarized for the use of the method for each study.

Hyaluronidase

Serum hyaluronidase concentrations were detected using a validated microplate-based colorimetric assay. Hyaluronidase activity was measured by incubating human plasma samples with biotinylated hyaluronic acid (bHA) bound to the plate for a fixed period of time. The remaining bound bHA was detected with streptavidin conjugated to horseradish peroxidase and subsequently a substrate solution (tetramethylbenzidine/ hydrogen peroxide). Color development is inversely proportional to the activity of

the hyaluronidase. The method has a hyaluronidase standard curve range of 0.00625 to 0.200 U/mL. All test samples are assayed at a minimum 50-fold dilution in assay diluent, and any additional dilution is made in standard diluent. Therefore, the effective hyaluronidase activity quantitation range is from 0.3125 to 10.0 U/mL for human K3EDTA plasma samples. Analytical reports were submitted and QC reports were summarized for the use of the method for each study.

Antibodies

Analytical methods for detection of anti-drug antibodies (ADAs) in serum were validated and demonstrated adequate sensitivity and drug tolerance for detection of ADA responses in human serum. The assay uses a two-tiered approach:

- a screening assay, which detects anti-drug antibodies (screen-positives);
- a confirmatory assay, which contains an immunodepletion (competitive binding) step to assess the specificity of initial positive results (confirmed-positives).

In the pivotal studies (BO22334, BP22333, and BO25341), samples that were positive for the presence of ADA were then further analyzed for anti-drug neutralizing anti-bodies (Nabs). All assays were validated and reports were submitted.

Rituximab

Serum anti-rituximab antibodies was evaluated using a validated ELISA assay. Briefly, test samples and quality controls (using rabbit polyclonal anti-rituximab antibodies as positive control) were pre-incubated overnight with biotinylated rituximab and digoxingenylated rituximab. Immune complexes were transferred to streptavidin-coated microtiter plates and incubated, followed by washing. Bound immune complexes were detected by incubation with polyclonal anti-digoxigenin antibodies conjugated to horseradish peroxidase. Following a further wash to remove any unbound conjugate, a substrate solution (tetramethylbenzidine/ hydrogen peroxide) was added to the wells, resulting in a color development in proportion to the amount of anti-rituximab antibodies in the initial step. The reaction was stopped, and absorbance was measured photometrically. Positivity for anti-rituximab antibodies was assessed by categorizing photometric absorbance in a particular sample against a screening cut-point defined during assay validation. Samples with absorbance signals equal to or above the screening cut-point were categorized as screen-positive. Those samples were further tested in a confirmatory assay.

The confirmatory assay was conducted identically to the screening assay, except that each putative positive sample was pre-incubated in the absence and in the presence of excess rituximab, which acts as an immunocompetitor, thereby reducing the absorbance signal only in samples containing specific antirituximab antibodies. Positivity for specific anti-rituximab antibodies in the confirmatory assay was assessed by categorizing the signal reduction of a particular immunodepleted sample relative to a matching non-immunodepleted sample against a confirmatory cut-point, which was defined during assay validation. For samples confirmed positive, titration was performed and an antibody titer value was reported. The titer was reported as the log10 of the highest dilution factor at which a confirmed-positive sample still produced an absorbance above the assay cut-point in the screening assay.

Hyaluronidase

Serum hyaluronidase was evaluated using a validated bridging ECLIA utilizing Meso Scale Discovery technology was used to detect and confirm the presence of anti-rHuPH20 antibodies in plasma. Briefly, ruthenylated and biotinylated rHuPH20 were used to form antibody rHuPH20-bridged complexes.

Samples with a screen result equal to or greater than the calculated cut-point value were categorized as screen-positive. The cut-point value was the sum of the mean negative control and a statistically derived normalization factor. All screen-positive samples were further tested by confirmatory assay in the absence and presence of excess rHuPH20.

For the confirmatory assay, titers (dilution factor at or above the cut-point) were determined for all confirmed-positive samples across the rituximab SC clinical studies. To estimate the titer of confirmed-positive samples, a minimum dilution (1:10) was performed followed by serial twofold dilutions. The endpoint titer is reported as the highest dilution factor (with the initial tenfold dilution reported as a titer of 1) at which the confirmed-positive sample still produced a signal above the assay cut-point in the screening assay. The sensitivity of the ECLIA used is ~ 1.51 ng/mL for the positive control (i.e., a Protein G purified rabbit polyclonal anti-rHuPH20 antibody preparation) evaluated during assay validation. The assay can tolerate an approximately 50-fold excess of rHuPH20 as shown by the anti-rHuPH20 low positive control (20 ng/mL), which tested positive in the presence of 1000 ng/mL of rHuPH20.

4.4.2 What bioanalytical methods are used to assess rituximab concentrations? Briefly describe the methods and summarize the assay performance.

Serum rituximab concentrations were measured in plasma by a validated ELISA. See **section 4.1.1**. The accuracy, precision, and other relevant parameters for the assay are described in **Table 9** below. This is sufficient to meet the requirements of the submitted studies.

Table 18: **Summary of Rituximab Assay Validation Report**	
Analyte	Rituximab
Matrix	Qualified in human serum from individuals with RA, MS, NHL, CLL, ANCA-AAV, UC, LN, SLE,
Reference or analytical standard	Rituxan, Lot C2B81298-2, 9.8 mg/mL
Minimum dilution	1/100
Limit of detection	1.25 ng/mL
LLOQ	5 ng/mL
ULOQ	160 ng/mL
Minimum Quantifiable concentration (MQC)	500 ng/mL in serum 250 ng/mL in CSF 500 ng/mL in urine
Accuracy (%Bias)	Low (93 – 134%)
Inter-assay Precision (%CV)	LLOQ: 6 – 9% Low: 13% Mid: 8% High: 6% ULOQ:15 – 25%
Intra-assay Precision	LLOQ: 5-7% Low: 13% Mid: 8% High: 9% ULOQ:12 – 19%
Hook effect	No hook effect was observed
Accuracy in Normal human serum sample (% recovery)	Low (800 ng/mL): 97 – 113% Mid (2500 ng/mL): 93 – 101% High (10000 ng/mL): 94 – 98 %
Linearity in normal human serum sample (%	-8% to 6%

difference from preceding dilution)	
Accuracy in human CLL serum sample (% recovery)	Low (797 ng/mL): 95 – 117% Mid (3000 ng/mL): 83 – 107% High (9800 ng/mL): 92 – 102 %
Linearity in human CLL serum sample (% difference from preceding dilution)	-15 to 25%
Accuracy in human NHL serum sample (% recovery)	Low (800 ng/mL): 108 – 134% Mid (2500 ng/mL): 97 – 124% High (9800 ng/mL): 95 – 124 %
Linearity in human NHL serum sample (% difference from preceding dilution)	-15 to 25%
Cross-reactivity	none
Interference	Anti-rituximab antibody
Analyte stability	Room temperature: 24 h a total of 8 freeze/thaw cycles in combination storage at 2°C−8°C for 5 days in between each freeze/thaw cycle
Long-term Stability	< -20º: up to 4 months < -60º: up to 3.5 yrs

Table 19: **Summary of rHuPH20 Assay Validation Report**	
Analyte	rHuPH20
Matrix	Human K3EDTA plasma
Reference or analytical standard	rHuPH20, Lot HUA0602MA, 115098 U/mL by Halozyme
Minimum dilution	1/50
Limit of detection	0.00625 U/mL
LLOQ	0.3125 U/mL
ULOQ	8 U/mL
Accuracy (%Bias)	89 – 92.5%
Inter-day Precision (%CV)	LLOQ (0.3125 U/mL): 7.229% Low (0.9375 U/mL): 7.36% Mid (4 U/mL): 8.21% High (8 U/mL): 8.5%
Inter-day Accuracy	LLOQ (0.3125 U/mL): -9.12% Low (0.9375 U/mL): -11% Mid (4 U/mL): -9.75% High (8 U/mL): -7.63%
Intra-day Precision	LLOQ (0.3125 U/mL): 1.89% Low (0.9375 U/mL): 3.86% Mid (4 U/mL): 4.11% High (8 U/mL): 9.10%
Intra-day Precision	LLOQ (0.3125 U/mL): -8.80% Low (0.9375 U/mL): -12.6% Mid (4 U/mL): -4.25% High (8 U/mL): -6.75%
Linearity in normal human serum sample (% difference from preceding dilution)	-14.9% to 9.6%
Analyte stability	Room temperature: 4 h Stable for a total of 4 freeze/thaw cycles in combination storage at room temperature and -70°C
Long-term Stability	< -20º: up to 12 months < -70º: up to 12 months

Serum anti-rituximab antibodies were measured using a validated ELISA assay (See Section 4.1.1). The immunoassay method for the determination of anti-Rituximab antibodies in human serum was validated using rabbit anti-Rituximab antibodies (polyclonal) as a Positive Control. In the confirmatory assay, samples were treated with excess Rituximab (12.5 µg/mL, concentration in assay).

Table 20: **Summary of Anti-Rituximab Antibody Assay Validation Report**	
Validation parameter	Validation result
Cut-point and normalization factor for healthy population	Cut point: 0.07858 Normalization factor: 1.36108
Confirmatory cut point	Cut point: 0.2606
Assay sensitivity	At least 1.42 ng/mL (concentration in serum)
Precision of titer determination	The titer was defined as log10(-dilution factor). The titer varied from 2.806 (7x) to 3.107 (2X), giving an overall CV of 4.6%.
LPC and HPC concentrations	4.06 ng/mL and 225 ng/mL
Assay precision	Intra-assay precision: 1.7% to 4.9% Inter-assay precision: 5.5% to 8.8%
Selectivity	No matrix effect was observed in different individual sera of 10 healthy volunteers.
Drug tolerance	Average drug tolerance factor was at least 587
Prozone effect	Absence of a prozone effect was demonstrated up to an anti-Rituximab antibody concentration in serum of 12.5 µg/mL
Bench top stability	At least 24 hours at room temperature
Freeze/ thaw stability at -20 °C and -70° C	At least five (5) cycles
Long-term frozen stability at -20 °C and -70 °C	At least 24 months

4.4.3 What bioanalytical methods are used to assess the formation of rituximab antidrug antibodies.

Serum anti-rituximab antibodies were evaluated using a validated ELISA assay. The assay uses a two-tiered approach:

- A screening assay, which detects anti-rituximab antibodies (screen-positives);
- A confirmatory assay, which contains an immunodepletion (competitive binding) step to assess the specificity of initial positive results (confirmed-positives).

Briefly, test samples and quality controls (using rabbit polyclonal anti-rituximab antibodies as positive control) were pre-incubated overnight with biotinylated rituximab and digoxingenylated rituximab. Immune complexes were transferred to streptavidin-coated microtiter plates and incubated, followed by washing. Bound immune complexes were detected by incubation with polyclonal anti-digoxigenin antibodies conjugated to horseradish peroxidase. Following a further wash to remove any unbound conjugate, a substrate solution (tetramethylbenzidine/ hydrogen peroxide) was added to the wells, resulting in a color development in proportion to the amount of anti-rituximab antibodies in the initial step. The reaction was stopped, and absorbance was measured photometrically. Positivity for anti-rituximab antibodies was assessed by categorizing photometric absorbance in a particular sample against a screening cut-point defined during assay validation. Samples with absorbance signals equal to or above

the screening cut-point were categorized as screen-positive. Those samples were further tested in a confirmatory assay.

The confirmatory assay was conducted identically to the screening assay, except that each putative positive sample was pre-incubated in the absence and in the presence of excess rituximab, which acts as an immunocompetitor, thereby reducing the absorbance signal only in samples containing specific antirituximab antibodies. Positivity for specific anti-rituximab antibodies in the confirmatory assay was assessed by categorizing the signal reduction of a particular immunodepleted sample relative to a matching non-immunodepleted sample against a confirmatory cut-point, which was defined during assay validation. For samples confirmed positive, titration was performed and an antibody titer value was reported. The titer was reported as the log10 of the highest dilution factor at which a confirmed-positive sample still produced an absorbance above the assay cut-point in the screening assay.

Neutralizing Assay for Anti-rituximab antibodies

A cell-based neutralization assay was developed to directly measure the neutralization potential of anti-rituximab antibodies. However, the resulting assay had a low sensitivity and poor drug tolerance and was considered unreliable.

Neutralizing Assay for Anti-rHuPH20 antibodies

For samples confirmed positive, the neutralizing capability was further assessed. A validated United States Pharmacopeia (USP) turbidimetric method was used to detect neutralizing anti-rHuPH20 antibodies in plasma. Hyaluronidase activity was measured by incubating rHuPH20 with hyaluronate. The resulting turbidity is inversely proportional to the concentration of hyaluronidase activity. The presence of neutralizing antibodies against hyaluronidase is expected to decrease the activity of rHuPH20, resulting in an increase of turbidity in the sample. The sensitivity of this neutralizing antibody assay is in the 200 – 400 ng/mL range.

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/s/

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NAM ATIQUR RAHMAN
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I concur.

ISSAM ZINEH
05/10/2017
I concur with the findings and conclusions of the review.