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ARTICLE

Rituximab exposure-response in triweekly R-CHOP treatment in DLBCL: A loading dose is recommended to improve clinical outcomes

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Abstract

Previous exposure-response analyses for rituximab suggest that higher rituximab concentrations were associated with an improvement in efficacy, however, clinical studies investigating a higher rituximab dose had mixed results. To further explore the exposure-response relationship of rituximab, a prospective observational analysis was performed involving 121 newly diagnosed patients with diffuse large B-cell lymphoma treated with triweekly rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). The trough concentration in the first cycle ($C_{1\text{-trough}}$) was significantly higher in patients achieving complete response (CR) compared with patients that did not achieve CR (22.00 μg/ml vs. 16.62 μg/ml, $p = 0.0016$), however, this difference between the two groups disappeared in later cycles. The relationship between rituximab $C_{1\text{-trough}}$ and achieving a CR was confirmed by matched-pair logistic regression analysis (odds ratio, 0.79; $p = 0.0020$). In addition, a higher $C_{1\text{-trough}}$ (≥18.40 μg/ml) was associated with longer progression-free survival ($p < 0.0001$) and overall survival ($p = 0.0038$). The percentages of patients that did not achieve a CR and had recurrence after CR within 24 months were 35% and 22.50%, respectively, for patients with a $C_{1\text{-trough}}$ less than or equal to 18.40 μg/ml, compared with 12.35% and 6.17% for

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patients with $C_{1\text{-trough}}$ greater than 18.40 μg/ml. Disease stage was found to be the most significant influencing factor of $C_{1\text{-trough}}$, with 51.02% of patients at stage IV with an observed $C_{1\text{-trough}}$ less than 18.40 μg/ml. For these advanced patients, population pharmacokinetic simulations using an established model suggest that a loading dose of 800 mg/m^2 may help to improve clinical outcomes.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Several studies reported a good clinical response was correlated with a high rituximab concentration, however, not all trials that increased the dosage of rituximab exhibited clinical benefits.

WHAT QUESTION DID THIS STUDY ADDRESS?

Systemic investigation is warranted to explore the pharmacokinetic mechanism underlying this confusing dose/concentration-effect relationship.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Lower rituximab concentration in the first cycle rather than other cycles was significantly associated with lower complete response rate and early disease recurrence. The recommendatory minimum optimal trough concentration in the first cycle ($C_{1\text{-trough}}$) was 18.40 μg/ml, and a loading dose was recommended for advanced patients to obtain optimal exposure. Moreover, correction of hypoproteinemia and liver dysfunction before treatment was recommended to improve clinical benefits.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The ideal administration of rituximab may involve a high initial dose and then maintenance at modest levels for a sufficient time, and increasing the initial dose of rituximab may be a new direction for future studies.

INTRODUCTION

The CD20-specific monoclonal antibody rituximab (MabThera and Rituxan) is used as the backbone of treatment for patients with diffuse large B-cell lymphoma (DLBCL), which is the most common subtype of non-Hodgkin lymphoma (NHL).[1] Although DLBCL is curable in a large proportion of patients, approximately 30–40% of patients eventually relapse or are primarily refractory and do not achieve complete response (CR),[2,3] and patients who fail to obtain CR from the first-line rituximab-based regimen commonly have a dismal outcome.[4] The currently available treatment strategies still aim to achieve and maintain complete disease remission and to prolong and increase the rate of disease-free survival and overall survival (OS).[5]

The significant variability in the therapeutic response was thought to be partially due to the variability in pharmacokinetics (PKs), and a good clinical response has been found to be correlated with high rituximab concentrations.[6–8] However, the conclusions drawn from different clinical trials that have tried to adjust the dosing schedule of rituximab are inconsistent. In a multicenter phase II study[9] of rituximab monotherapy in relapsed or refractory patients with aggressive B-cell lymphoma, rituximab was given in two dosing schedules: eight consecutive weekly infusions at 375 mg/m^2 ($n = 28$) or one infusion at 375 mg/m^2 followed by seven consecutive weekly infusions at 500 mg/m^2 ($n = 26$), and the clinical response showed little difference between the two arms. In contrast, in Pfreundschuh's study,[10] elderly men on an initial dosage of 500 mg/m^2 rituximab were associated with a 32.5% improvement in progression-free survival (PFS; $p = 0.0390$), with a trend toward a better OS (30%) compared with those at an initial dosage of 375 mg/m^2 dose. In other studies,[11,12] patients with DLBCL with poor prognosis receiving initial dense-dose rituximab had a more promising response than those receiving the standard regimen. According to these studies, increasing the dose is not destined to improve efficacy and it seems that an increase in the initial phase leads to a better clinical outcome, whereas an increase in the later phase does not. Systemic investigation is warranted to explore the PK mechanism underlying this confusing dose-effect relationship. In addition, a clear threshold of effective

concentration and a simpler dosage calculation scheme for rituximab are urgently needed for clinical application.

Noticeably, rituximab PKs show wide interindividual variability. When a dosage of 375 mg/m^2 once weekly is administered to patients with relapsed or refractory follicular lymphoma, the interindividual variability in serum concentration could be more than 100-fold.[6] In addition, large variability (54%) in elimination half-life was found in Tran's study.[13] Moreover, Blasco[14] observed that some patients had a very different concentration time course from other patients (clearance value, 19.50 vs. 4.87 ml/h). Therefore, in this study, we aimed to gain insight into the factors that influence the PKs of rituximab, including the stage (indicating the tumor burden - the target), the baseline circulating CD20-positive B cells, liver and renal functions, and so on, which may further our understanding of the variability of treatment outcomes.

The objectives of the present study were to re-explore the exposure-response relationship in patients with DLBCL who received triweekly rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) therapy, quantify the impacts of physiological and pathological characteristics on the PKs of rituximab and propose an individual rituximab dose-adjustment regimen.

METHODS

Patients and therapy

Eligible patients were over 16 years old with previously untreated and histologically proven CD20 DLBCL according to the national guidelines for the treatment of NHL. Rituximab was administered at a dose of 375 mg/m^2 repeated every 3 weeks in combination with CHOP chemotherapy. Clinical response was evaluated according to the revised response criteria for malignant lymphoma.[15]

The study was conducted in accordance with Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. The study was approved by the ethics committee of the Sun Yat-Sen University Cancer Center and registered in the Chinese Clinical Trial Registry as ChiCTR1800017001 (http://www.chictr.org.cn/index.aspx).

Rituximab concentration

Two samples per cycle were obtained to determine rituximab peak and trough plasma levels from the first to fifth cycles. Peak-level plasma samples were collected ~ 0 to 2 h after rituximab infusion, and trough samples were collected immediately before rituximab infusion in the subsequent cycle. Almost 40 samples at other timepoints were obtained after obtaining patient consent when they returned to the hospital for other requirements. Plasma levels of rituximab were determined by the commercial Matriks Biotek kit (SHIKARI Q-RITUX) through solid phase enzyme-linked immunosorbent assay.[16]

Population pharmacokinetic model

Population PK analysis was performed by using Phoenix NLME (version 1.3; Certara L.P., St. Louis, MO) Phoenix WinNonlin 6.4. The individual PK parameters were estimated using a Bayesian approach.[16–20] The basic PK parameters used were volume of distribution for the central compartment, clearance of the central compartment (CL, ml/h), volume of distribution for the peripheral compartment (V_2, L) and intercompartmental clearance (CL_2, ml/h). Two complementary methods were used to evaluate the developed model[21,22]: a nonparametric bootstrap and a visual predictive check (VPC). The VPC used Monte Carlo simulation to generate concentration-time profiles of 1000 patients. The observed (dependent variable) concentration data should be approximately distributed within the 5th to 95th prediction interval. The recommended dosage to obtain optimal exposure of rituximab was determined through an off-the-shelf simulation platform in Phoenix WinNonlin via 1000 Monte Carlo simulations.

Association between concentration and outcome

All data in this study were analyzed using the SPSS Statistics version 24.0 software package (SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed via *t*-tests and χ^2 tests. The associations of drug concentrations with metabolic response, PFS and OS were assessed using logistic regression and Cox models. Cutoff values for patient outcome were determined using receiver operating characteristic (ROC) curve analysis. Multiple linear regression models were constructed with a stepwise variable selection method, and only those variables with a *p* value of less than 0.05 in the univariate analysis were included in the multivariate analysis.

RESULTS

Patient characteristics

A total of 121 patients with DLBCL were included, and the patient characteristics at baseline are shown in Table 1.

TABLE 1 Summary of patients' characteristics at baseline and clinical outcome

	Total	Stage Ⅰ/II	Stage III	Stage IV
Characteristics	121 (100%)	54 (44.63%)	18 (14.88%)	49 (40.49%)
Age, years	54 (18–78)	52 (18–77)	60 (31–73)	56 (29–78)
Age, years >60	45 (37.19%)	16 (30.19%)	9 (34.62%)	18 (42.86%)
Sex, male	53 (43.80%)	25 (47.17%)	9 (34.62%)	23 (54.76%)
BMI	22.79 (16.56–32.46)	22.21 (16.56–32.46)	23.12 (16.83–27.97)	23.04 (16.89–27.24)
BSA, m^2	1.64 (1.25–2.06)	1.60 (1.37–2.06)	1.70 (1.36–1.87)	1.64 (1.25–2.03)
LDH (U/L)	201 (101–3590)	181 (101–336)	252 (126–741)	330 (127–3590)
Hemoglobin, g/L	128 (49–171)	135 (91–171)	127 (96–156)	117 (49–160)
β2-MG, mg/L	2.085 (1.18–16.26)	1.81 (1.19–3.38)	2.09 (1.42–4.09)	2.55 (1.18–16.26)
ESR, mm/h	22 (1–148)	13 (1–78)	22 (4–70)	38 (2–148)
PLT, $*10^9$/L	263 (67–564)	261 (121–556)	25 (167–350)	275 (67–564)
Non-GCB type	70 (57.85%)	30 (55.56%)	8 (44.44%)	32 (65.31%)
IPI 0–1	56 (46.28%)	50 (92.59%)	4 (22.22%)	2 (4.08%)
IPI 2–3	52 (42.96%)	4 (7.41%)	14 (77.78%)	34 (69.39%)
IPI 4–5	13 (10.74%)	0	0	13 (26.53%)
Treatment response (CR rate)				
After cycle 2	62 (51.24%)	36 (66.67%)	11 (61.11%)	15 (30.61%)
After cycle 4	92 (76.03%)	50 (92.59%)	15 (83.33%)	27 (55.10%)
After cycle 6	97 (80.17%)	51 (94.44%)	15 (83.33%)	31 (63.27%)

Note: All continuous values are reported with mean (minimum - maximum), while categories are reported in numbers (percentages).

Abbreviations: BMI, body mass index; BSA, body surface area; CR, complete response; ESR, erythrocyte sedimentation rate; GCB, germinal center beta-cell; IPI, International Prognostic Index score; LDH, lactate dehydrogenase; PLT, platelet count; β2-MG, β2-microglobulin.

The association of rituximab concentration with response

Median plasma concentrations of rituximab quantified before (trough concentration, C_{trough}) and after (peak concentration, C_{peak}) infusions in all cycles are reported in Table 2. The numbers of patients who achieved CR after cycles 2, 4 and 6 were 62, 92 and 97, respectively (Table 1). The first cycle C_{trough} ($C_{1\text{-trough}}$) was significantly higher in the CR patients than in the non-CR patients (response after cycle 2: 24.41 vs. 18.87, $p = 0.0065$; cycle 4: 22.09 vs. 17.99, $p = 0.0041$; cycle 6: 22.00 vs. 16.62, $p = 0.0016$). However, the differences in the trough concentrations between the two groups gradually disappeared in later cycles, with the trough concentration eventually becoming equal between the groups (Figure 1, Table 2). There was no difference in C_{peak} between the CR and non-CR groups in any cycle.

Because tumor stage, age, and germinal center beta-cell (GCB) types are known factors influencing treatment response, conditional logistic regression (case-control) was used to further determine the relative risk of rituximab $C_{1\text{-trough}}$ for response. There were 29 matched pairs, each consisting of one non-CR patient and any number of CR patients who matched the conditions of same stage, same GCB type and age within 3 years. The matched-pair logistic regression analysis showed that $C_{1\text{-trough}}$ was significantly associated with achieving CR (odds ratio, 0.79; 95% confidence interval [CI], 0.68–0.92; $p = 0.0020$; Figure 2).

The optimal predictive $C_{1\text{-trough}}$ cutoff and its correlation with survival

Before subsequent analyses, the analyses of the relationship of exposure-quartile with outcome were conducted to confirm that a higher $C_{1\text{-trough}}$ was related to higher efficacy (Figure S1). The optimal predictive $C_{1\text{-trough}}$ cutoff using the Youden index was 18.40 μg/ml for both PFS and OS, with ROC AUCs of 0.81 (95% CI, 0.72–0.90; $p < 0.0001$) and 0.82 (95% CI, 0.68–0.96; $p = 0.0260$), respectively. The sensitivity and specificity were 0.90 and 0.71, respectively, for PFS and 0.69 and 1.00, respectively, for OS. The median cutoff values obtained by bootstrap analysis were nearly identical to the 18.40 μg/ml.

With a median follow-up of 28 months (range: 24–49), PFS was 68.60%, and OS was 96.70%. Patients with a $C_{1\text{-trough}}$ greater than or equal to 18.40 μg/ml ($n = 81$; 66.94%) had a significantly better PFS (81.48% vs. 42.50%; log-rank $p < 0.0001$) and OS (100% vs. 90%; log-rank $p = 0.0038$) than those with a $C_{1\text{-trough}}$ under the cutoff value (Figure 3).

For patients with $C_{1\text{-trough}}$ less than or equal to 18.40 μg/ml, the percentages of patients who did not achieve CR,

TABLE 2 Rituximab trough concentration (μg/ml) for patients with DLBCL in each cycle

Cycle	All patients	Response after cycle 2		Response after cycle 4		Response after cycle 6	
		CR	Non-CR	CR	Non-CR	CR	Non-CR
Cycle 1	21.04 (0.82–47.94)	24.41 (6.61–47.94)**	18.87 (0.82–34.46)**	22.09 (6.61–47.94)***	17.99 (0.82–31.73)***	22.00 (6.61–47.94)***	16.62 (0.82–31.73)***
Cycle 2	37.92 (0.50–75.77)	41.12 (12.80–75.77)	36.75 (0.50–57.68)	38.55 (12.80–75.77)*	34.70 (0.50–57.68)*	39.03 (12.80–75.77)*	34.14 (0.50–52.98)*
Cycle 3	42.61 (2.23–85.11)			42.99 (23.08–85.11)	42.25 (2.23–69.09)	43.19 (26.36–85.11)	42.06 (2.23–69.09)
Cycle 4	45.55 (10.93–88.45)			46.00 (10.93–79.92)	45.12 (21.99–88.45)	47.17 (10.93–88.45)	45.89 (21.99–83.40)
Cycle 5	51.63 (11.81–108.07)					48.03 (11.81–108.07)	46.49 (19.59–89.99)

Note: All concentrations values are reported with mean (minimum - maximum).

Abbreviations: CR, patients achieved complete response; DLBCL, diffuse large B-cell lymphoma; non-CR, patients without complete response.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$.

achieved CR but experienced recurrence within 24 months, and had a PFS greater than 24 months were 35%, 22.50%, and 42.50%, respectively, whereas the percentages for patients with $C_{1\text{-trough}}$ greater than or equal to 18.40 μg/ml were 12.35%, 6.17%, and 81.48%, respectively.

Because stage is a recognized prognostic factor for clinical outcome, subgroup analyses based on stage were conducted. The $C_{1\text{-trough}}$ greater than or equal to 18.40 μg/ml was significantly associated with better PFS and OS in both patients with stages I/II/Ⅲ disease ($n = 72$, PFS, $p < 0.0001$, OS, $p = 0.0210$) and patients with stage IV disease ($n = 49$, PFS, $p < 0.0001$, OS, $p = 0.0210$; Figure 3).

No correlation between the incidence of adverse reactions and rituximab concentration was observed during treatment.

Influencing factors of rituximab $C_{1\text{-trough}}$

The correlations among $C_{1\ trough}$ and sex, age, body surface area (BSA), body mass index (BMI), tumor stage (indicating the tumor burden - the target), initial bone marrow infiltration, baseline circulating CD20-positive B cells, GCB type, lactate dehydrogenase (LDH), beta 2-microglobulin (β2-MG), erythrocyte sedimentation rate, albumin, aspartate transaminase (AST), alanine transaminase (ALT), creatinine, globulin, total protein, albumin-globulin ratio, and uric acid (UA) were analyzed by linear regression.

In the univariate linear regression analyses, weight ($p = 0.0490$, adjusted R^2, 0.03), BMI ($p = 0.0440$, adjusted R^2, 0.03), total protein ($p = 0.0280$, adjusted R^2, 0.04), ALT ($p = 0.0180$, adjusted R^2, 0.05), AST ($p = 0.0370$, adjusted R^2, 0.03), LDH ($p = 0.0020$, adjusted R^2, 0.04), albumin ($p < 0.0010$, adjusted R^2, 0.11), and stage ($p < 0.0010$, adjusted R^2, 0.21) were significantly associated with $C_{1\text{-trough}}$.

Only tumor stage, albumin, and ALT were significantly associated with $C_{1\text{-trough}}$ in the multiple linear regressions: $C_{1\text{-trough}} = 22.76 - 3.08 *$ stage III $-6.14 *$ stage IV $+4.88 *$ albumin $- 4.02 *$ ALT (albumin <35 g/L = 0, albumin ≥35 g/L = 1; ALT <40 U/L = 0, ALT ≥40 U/L = 1), and the adjusted R square was 26.80% (Figure 4).

The percentages of patients with $C_{1\text{-trough}}$ below 18.40 μg/ml in stages I/II, III, and IV were 18.52%, 27.78%, and 51.02%, respectively.

Recommended rituximab dosage calculated by Monte Carlo simulation

These concentrations were described using a two-compartment population pharmacokinetic (PopPK) model. Of all covariate relationships tested (listed in the

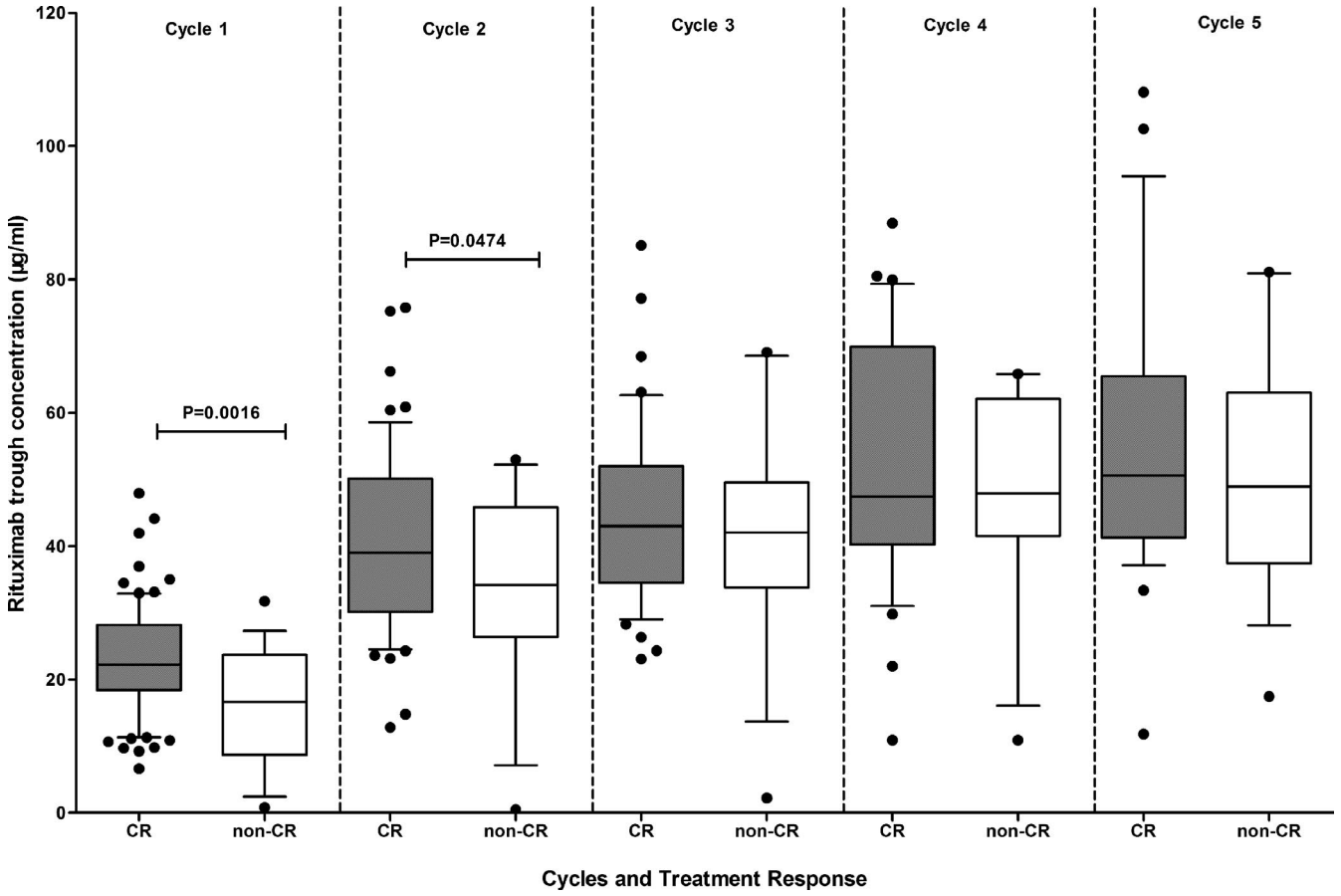

FIGURE 1 Rituximab trough concentrations (μg/mL) in the CR and non-CR groups in each cycle. CR, complete response. Whiskers: 10–90 percentile

above section), we observed significant associations of BSA with CL and a significant association of tumor stage with CL_2. The estimated covariates and 1000 bootstrap replicates for rituximab indicated qualified stability for the final model (Table 3). The PopPK model diagnostic plots are shown in Figure S2.

To determine the initial dose of rituximab suitable for achieving the effective concentration of 18.40 μg/ml, different initial dosages were simulated by the established PopPK model through 1000 Monte Carlo simulations to predict the concentration profiles. For patients with stages Ⅰ/Ⅱ and III disease, 850 mg and 900 mg, respectively, were predicted to yield a sufficient initial level in ~ 95% of patients. For patients with stage IV disease, the dosage should be increased to 1200 mg or 800 mg/m^2.

DISCUSSION

To our knowledge, this is the first prospective observational study to describe the influence of each cycle's C_{peak} and C_{trough} of rituximab on clinical outcome in patients with DLBCL who received triweekly R-CHOP treatment. A lower initial C_{trough} of rituximab was significantly associated with lower CR rate and early disease recurrence. The recommended minimum optimal $C_{1\text{-trough}}$ was 18.40 μg/ml, and a loading dose was recommended for advanced patients to obtain optimal exposure; therefore, the optimal outcome was predicted. Moreover, correction of hypoproteinemia and liver dysfunction before treatment was recommended to improve clinical benefits.

Several studies have been performed to investigate the PK-response relationships of rituximab in recurrent or refractory patients. In a phase III clinical trial[6] with patients with recurrent or refractory low-grade NHL, the authors reported that during the single-agent rituximab induction treatment of four cycles, once weekly, 3 months after the last infusion, median rituximab levels of 5.9 μg/ml were found for nonresponders and 25.4 μg/ml for responders. In a Japanese multicenter phase II PK study[23] of rituximab in relapsed or refractory patients with aggressive B-cell lymphoma, serum rituximab levels were assayed in 12 patients, and the mean ± SD values of trough levels and areas under the curve (AUCs) of the responders were significantly higher than those of nonresponders ($p = 0.021$; $p = 0.037$). In 2017, Tout[8] reported that a high AUC (≥9400 mg*h per liter) was associated with better response and longer PFS and OS in patients with DLBCL treated

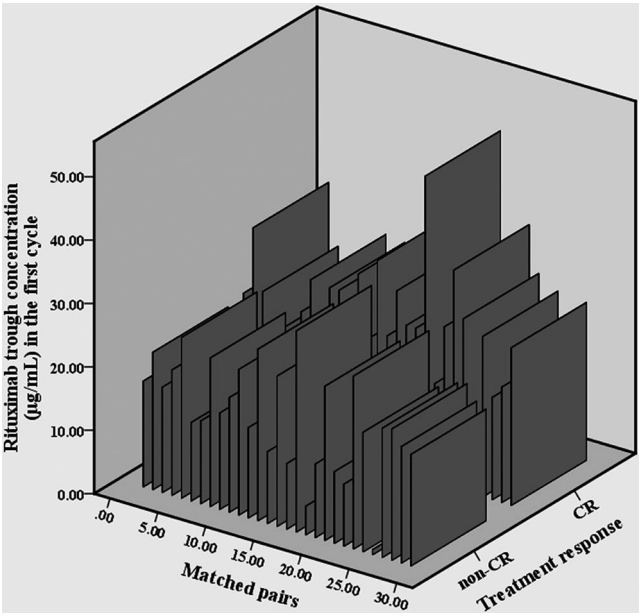

FIGURE 2 The distributions of trough concentration in the first cycle ($C_{1\text{-trough}}$) in the matched-pair groups of complete response (CR) vs. non-CR patients. In the matched-pair logistic analysis, the matching variables included stage, age and germinal center beta-cell (GCB) type. There were 29 matched pairs, each consisting of one non-CR patient and any number of CR patients who matched the conditions of same stage, same GCB type and age within 3 years. The matched-pair logistic regression analysis showed that the $C_{1\text{-trough}}$ was significantly associated with CR (odds ratio, 0.79; 95% confidence interval, 0.68–0.92; $p = 0.0020$)

with rituximab-based chemotherapy every 2 weeks. The PK characteristics of rituximab have also been described in some other studies[13,14] with a sample size of no more than 10 patients.

However, at present, rituximab is mainly used as the first-line treatment for newly diagnosed patients in combination with chemotherapy once every 3 weeks. Based on the above research, the influence of the drug exposure level in different cycles on the response rate to the treatment, and even the long-term survival is still pending. Therefore, we conducted this prospective clinical trial in more than 100 newly diagnosed patients to assess the relationships among rituximab PK and CR rate, PFS, and OS, and the C_{peak} and C_{trough} were assayed in each cycle. The present study suggested a more important role of rituximab C_{trough} in the first cycle in DLBCL treatment.

In our study, the $C_{1\text{-trough}}$ was found to be significantly correlated with achieving CR. Once the $C_{1\text{-trough}}$ reached one unit, the risk of not achieving CR was reduced by 21% ($p = 0.0020$), as determined from matched-pair logistic analysis, which controlled for the effects of tumor stage, GCB type, and age to confirm the independent effect of the $C_{1\text{-trough}}$ on the clinical response. Patients with a $C_{1\text{-trough}}$ greater than or equal to 18.40 μg/ml had a significantly better PFS (81.48% vs. 42.50%; log-rank $p < 0.0001$) and OS (100% vs. 90%; log-rank $p = 0.0038$) than those with a $C_{1\text{-trough}}$ under the cutoff value. The C_{trough} and C_{peak} in cycles two to six were not found to significantly influence clinical outcomes.

A C_{trough} of 18.40 μg/ml, was recommended as the minimum optimal $C_{1\text{-trough}}$ value. The percentages of patients with rituximab $C_{1\text{-trough}}$ below 18.40 μg/ml in stages I/II, III, and IV were 18.52%, 27.78%, and 51.02%, respectively. For patients with stage IV, the $C_{1\text{-trough}}$ (ranging from ~ 0.82–47.94 μg/ml) was significantly lower than that of patients in other stages, according to the PopPK simulation results, the simulated initial dose of 1200 mg or 800 mg/m^2 could induce ~ 95% of patients to overcome the "sink effect" of the baseline tumor burden. The "sink effect"[7,24] refers to the target (CD20)-mediated disposition phenomenon for rituximab, wherein the tumor cells act as a sink to adsorb rituximab and influence the rate and extent of rituximab distribution and elimination. In a dose-escalation trial[25] of rituximab from 500 to 2250 mg/m^2 conducted in patients with chronic lymphocytic leukemia, toxicity was minimal until a dose of 2250 mg/m^2 was achieved. Therefore, an adjusted rituximab initial dose could be considered for patients with advanced tumor stage.

The associations among rituximab exposure and sex, age, tumor burden, and initial bone marrow infiltration have been explored in several studies.[7,26] In this study, we had a larger sample size than those in previous studies, and stage, ALT, and albumin were the ultimate factors identified by stepwise multiple linear regression analysis as associated with rituximab $C_{1\text{-trough}}$. Rituximab $C_{1\text{-trough}}$ decreased with increased tumor burden (stage), ALT outside the upper limit of normal, and albumin outside the lower limit of normal. ALT was also independently associated with plasma nivolumab C_{trough} in the multivariate analysis in Puszkiel's study,[27] indicating the important role of liver function in the metabolism of monoclonal antibodies (mAbs). The mechanism might involve the hepatic neonatal crystallizable fragment receptor (FcRn), which regulates homeostasis of immunoglobulin G,[28] and liver dysfunction may increase the loss of rituximab, an unconjugated IgG1 antibody. The impact of albumin on the PKs of mAbs has also been previously reported for infliximab,[29] bevacizumab,[30] ustekinumab,[31] pertuzumab,[32] and durvalumab,[33] which may be explained by the fact that albumin and IgG share the same FcRn-salvaging pathway; moreover, hypoalbuminemia could reflect a higher protein catabolic rate in patients with cancer, which also affects the CL of rituximab and other IgG mAbs. Therefore, correction of hypoproteinemia and liver dysfunction before treatment, or increasing the dose of rituximab for patients with lower albumin or higher ALT can result in clinical benefits.

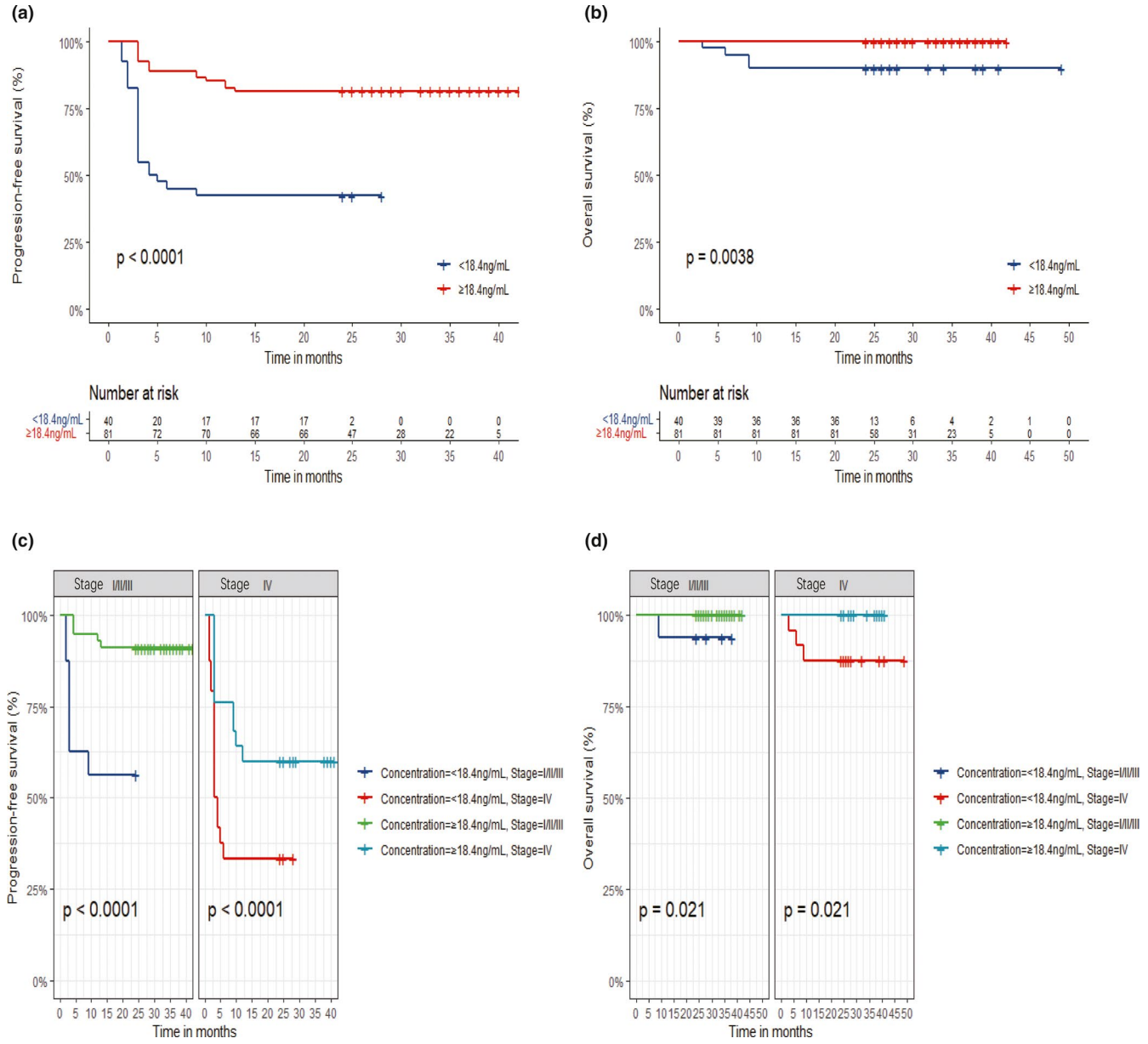

FIGURE 3 Kaplan-Meier estimates of PFS and OS by rituximab trough concentration in cycle 1 ($C_{1\text{-trough}}$). (a, b) Patients with a $C_{1\text{-trough}}$ greater than or equal to 18.40 μg/ml ($n = 81$; 66.94%) had a significantly better PFS (81.48% vs. 42.50%; log-rank $p < 0.0001$) and OS (100% vs. 90%; log-rank $p = 0.0038$) than those with a $C_{1\text{-trough}}$ under this cutoff value. (c, d) Subgroup analyses based on stage, $C_{1\text{-trough}}$ greater than or equal to 18.40 μg/ml was significantly associated with better PFS and OS in both patients with stages I/II/III disease ($n = 72$, PFS, $p < 0.0001$, OS, $p = 0.0210$) and patients with stages IV disease ($n = 49$, PFS, $p < 0.0001$, OS, $p = 0.0210$), respectively. PFS, progression free survival, OS, overall survival

This study has several limitations. Although this was a prospective observational study, some of the conclusions presented were based on retrospective analysis. Moreover, the tumor stage is not an accurate indicator of the number of rituximab target CD20-positive cells expressed on the tumors, which may limit the accuracy of the recommended dosage. This research only recommended a quick and simple scheme to confirm the initial dose of rituximab for clinical practice. The sample size in this study was moderate; therefore, the recommended optimal $C_{1\text{-trough}}$ and administration strategy need to be confirmed in prospective interventional clinical trials.

In summary, the C_{peak} and C_{trough} of each cycle in the induction phase and their effect on clinical outcomes were systemically explored in patients with DLBCL who received 375 mg/m^2 rituximab every 3 weeks in combination with chemotherapy. A key role of rituximab C_{trough} was observed, and a lower $C_{1\text{-trough}}$ was significantly associated with a lower CR rate and early disease recurrence. Based on our established PopPK model, a loading dose

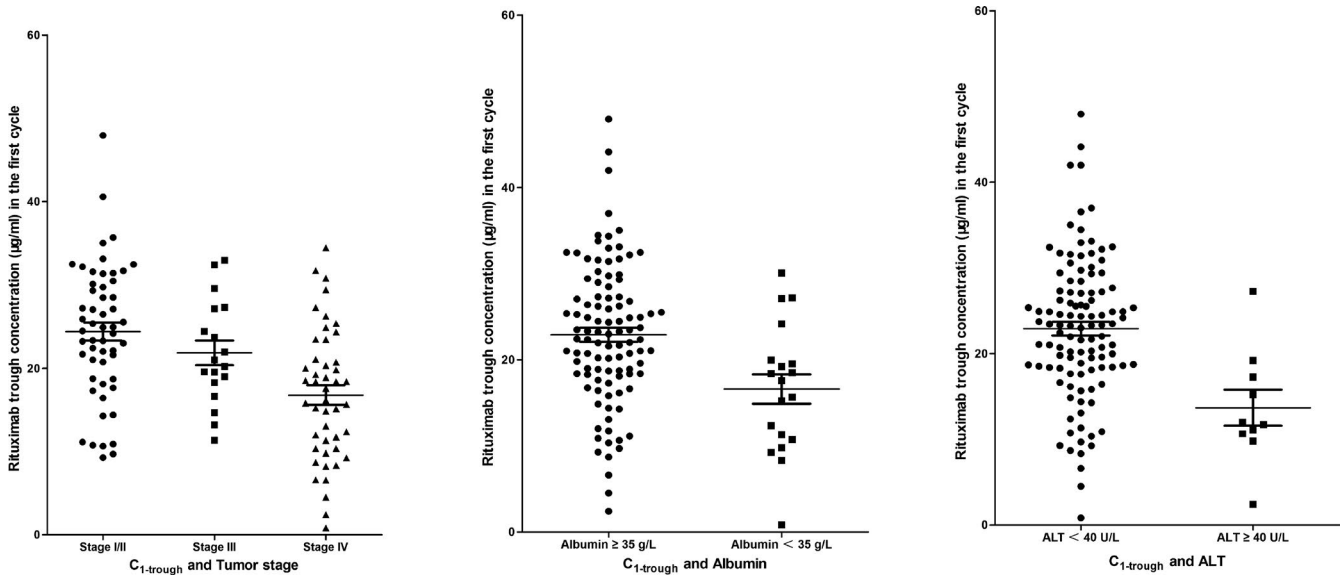

FIGURE 4 Associations of rituximab trough concentration in the first cycle ($C_{1\text{-trough}}$) with tumor stage, albumin and ALT. ALT, alanine transaminase

TABLE 3 Population pharmacokinetic parameters of rituximab in patients with DLBCL and the results of bootstrap validation (final models)

	Final model					Bootstrap replicates			
Model 1	**Estimate**	**%RES**	**2.5% CI**	**97.5% CI**	**Shrinkage (%)**	**Median**	**%RES**	**2.5% CI**	**97.5% CI**
tvV (L)	3.43	5.39	3.07	3.80	0.39	3.41	11.10	2.07	3.78
tvV_2 (L)	7.37	13.03	5.48	9.25	0.34	7.49	15.57	5.60	10.35
tvCl (L/d)	0.32	4.49	0.29	0.35	0.25	0.32	5.69	0.29	0.36
$tvCl_2$ (L/d)	0.65	26.61	0.31	0.99		0.68	34.14	0.36	1.43
tvCMultStdev	−0.17	−7.63	−0.19	−0.14		−0.17	−7.99	−0.20	−0.14
dCl-BSA	1.32	24.46	0.69	1.96		1.31	23.61	0.73	1.92
dCl_2-stage Ⅲ	0.77	58.89	−0.12	1.66		0.75	45.66	0.11	1.46
dCl_2-stage IV	−0.49	−52.89	−1.00	0.02		−0.49	−58.45	−0.95	0.21
Residual variability (CV%)									
$\sigma_{add+Mult}$	3.34	15.17	2.34	4.34		3.29	18.18	1.95	4.36
Interindividual variability									
ω^2V	0.08								
ω^2V2	0.29								
ω^2CL	0.06								

Abbreviations: CI, confidence interval; CV%, percent coefficient of variation; dCL2-BSA, fixed parameter coefficient of body surface area; dCL2-stage3, fixed parameter coefficient of tumor stage 3; dCL2-stage4, fixed parameter coefficient of tumor stage 4; DLBCL, diffuse large B-cell lymphoma; tvCL, typical value of clearance; tvCL2, typical value of CL2; tvCMultStdev, typical value of standard deviation (additive +multiplicative error model); tvV, typical value of V; tvV2, typical value of V2; ω2V, variance of the interindividual variability of V; ω2V2, variance of the inter-individual variability of V2; ω2CL, variance of the interindividual variability of clearance.

was recommended for advanced patients to overcome the initial "sink" effect for better clinical outcomes. Therefore, the ideal administration of rituximab may involve a high initial dose and then maintenance at modest levels for a sufficient time, and increasing the initial dose of rituximab may be a new direction for future studies.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

S.L., R.C., and X.W. wrote the manuscript. S.L., X.W., T.L., and M.H. designed the research. Z.W., He H., C.P., X.F.,

and S.G. performed the research. S.L., R.C., Hongbing H., and T.L. analyzed the data. Y.G. and S.G. contributed new reagents/analytical tools.

REFERENCES

1. Pfreundschuh M. Current therapeutic strategies for diffuse large Bcell lymphoma. *Der Internist*. 2016;57:214-221.
2. Wasterlid T, Harrysson S, Andersson TM, et al. Outcome and determinants of failure to complete primary R-CHOP treatment for reasons other than non-response among patients with diffuse large B-cell lymphoma. *Am J Hematol*. 2020;95:740-748.
3. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol*. 2010;28:4184-4190.
4. Crump M, Neelapu SS, Farooq U, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. *Blood*. 2017;130(16):1800-1808. https://doi.org/10.1182/blood-2017-03-769620
5. Flodr P, Latalova P, Tichy M, et al. Diffuse large B-cell lymphoma: the history, current view and new perspectives. *Neoplasma*. 2014;61:491-504.
6. Berinstein NL, Grillo-López AJ, White CA, et al. Association of serum Rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. *Ann Oncol*. 1998;9:995-1001.
7. Golay J, Semenzato G, Rambaldi A, et al. Lessons for the clinic from rituximab pharmacokinetics and pharmacodynamics. *mAbs*. 2013;5:826-837.
8. Tout M, Casasnovas O, Meignan M, et al. Rituximab exposure is influenced by baseline metabolic tumor volume and predicts outcome of DLBCL patients: a Lymphoma Study Association report. *Blood*. 2017;129:2616-2623.
9. Coiffier B, Haioun C, Ketterer N, et al. Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. *Blood*. 1998;92:1927-1932.
10. Pfreundschuh M, Murawski N, Zeynalova S, et al. Optimization of rituximab for the treatment of DLBCL: increasing the dose for elderly male patients. *Br J Haematol*. 2017;179:410-420.
11. Strüßmann T, Fritsch K, Baumgarten A, et al. Favourable outcomes of poor prognosis diffuse large B-cell lymphoma patients treated with dose-dense Rituximab, high-dose Methotrexate and six cycles of CHOP-14 compared to first-line autologous transplantation. *Br J Haematol*. 2017;178:927-935.
12. Murawski N, Pfreundschuh M, Zeynalova S, et al. Optimization of rituximab for the treatment of DLBCL (I): dose-dense rituximab in the DENSE-R-CHOP-14 trial of the DSHNHL. *Ann Oncol*. 2014;25:1800-1806.
13. Tran L, Baars JW, Aarden L, Beijnen JH, Huitema AD. Pharmacokinetics of rituximab in patients with CD20 positive B-cell malignancies. *Hum Antibodies*. 2010;19:7-13.
14. Blasco H, Chatelut E, de Bretagne IB, Congy-Jolivet N, Le Guellec C. Pharmacokinetics of rituximab associated with CHOP chemotherapy in B-cell non-Hodgkin lymphoma. *Fundam Clin Pharmacol*. 2009;23:601-608.
15. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. *J Clin Oncol*. 2007;25:579-586.
16. Liu S, Huang H, Chen RX, et al. Low initial trough concentration of rituximab is associated with unsatisfactory response of first-line R-CHOP treatment in patients with follicular lymphoma with grade 1/2. *Acta Pharmacol Sin*. 2021;42:641-647.
17. Regazzi MB, Iacona I, Avanzini MA, et al. Pharmacokinetic behavior of rituximab: a study of different schedules of administration for heterogeneous clinical settings. *Ther Drug Monit*. 2005;27:785-792.
18. Li J, Zhi J, Wenger M, et al. Population pharmacokinetics of rituximab in patients with chronic lymphocytic leukemia. *J Clin Pharmacol*. 2012;52:1918-1926.
19. Song L, He CY, Yin NG, Liu F, Jia YT, Liu Y. A population pharmacokinetic model for individualised dosage regimens of vancomycin in Chinese neonates and young infants. *Oncotarget*. 2017;8:105211-105221.
20. Munekage M, Ichikawa K, Kitagawa H, et al. Population pharmacokinetic analysis of daikenchuto, a traditional Japanese medicine (Kampo) in Japanese and US health volunteers. *Drug Metab Dispos*. 2013;41:1256-1263.
21. Brendel K, Comets E, Laffont C, Laveille C, Mentré F. Metrics for external model evaluation with an application to the population pharmacokinetics of gliclazide. *Pharm Res*. 2006;23:2036-2049.
22. Candelaria M, Gonzalez D, Fernández Gómez FJ, et al. Comparative assessment of pharmacokinetics, and pharmacodynamics between RTXM83, a rituximab biosimilar, and rituximab in diffuse large B-cell lymphoma patients: a population PK model approach. *Cancer Chemother Pharmacol*. 2018;81:515-527.
23. Tobinai K, Igarashi T, Itoh K, et al. Japanese multicenter phase II and pharmacokinetic study of rituximab in relapsed or refractory patients with aggressive B-cell lymphoma. *Ann Oncol*. 2004;15:821-830.
24. Keating GM. Rituximab: a review of its use in chronic lymphocytic leukaemia, low-grade or follicular lymphoma and diffuse large B-cell lymphoma. *Drugs*. 2010;70:1445-1476.
25. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. *J Clin Oncol*. 2001;19:2165-2170.
26. Jager U, Fridrik M, Zeitlinger M, et al. Rituximab serum concentrations during immuno-chemotherapy of follicular lymphoma correlate with patient gender, bone marrow infiltration and clinical response. *Haematologica*. 2012;97:1431-1438.
27. Puszkiel A, Noé G, Boudou-Rouquette P, et al. Development and validation of an ELISA method for the quantification of nivolumab in plasma from non-small-cell lung cancer patients. *J Pharm Biomed Anal*. 2017;139:30-36.
28. Pyzik M, Rath T, Kuo TT, et al. Hepatic FcRn regulates albumin homeostasis and susceptibility to liver injury. *Proc Natl Acad Sci USA*. 2017;114:E2862-E2871.
29. Fasanmade AA, Adedokun OJ, Olson A, Strauss R, Davis HM. Serum albumin concentration: a predictive factor of infliximab pharmacokinetics and clinical response in patients with ulcerative colitis. *Int J Clin Pharmacol Ther*. 2010;48:297-308.
30. Lu JF, Bruno R, Eppler S, Novotny W, Lum B, Gaudreault J. Clinical pharmacokinetics of bevacizumab in

patients with solid tumors. *Cancer Chemother Pharmacol*. 2008;62:779-786.
31. Zhu Y, Hu C, Lu M, et al. Population pharmacokinetic modeling of ustekinumab, a human monoclonal antibody targeting IL-12/23p40, in patients with moderate to severe plaque psoriasis. *J Clin Pharmacol*. 2009;49:162-175.
32. Garg A, Quartino A, Li J, et al. Population pharmacokinetic and covariate analysis of pertuzumab, a HER2-targeted monoclonal antibody, and evaluation of a fixed, non-weight-based dose in patients with a variety of solid tumors. *Cancer Chemother Pharmacol*. 2014;74:819-829.
33. Baverel PG, Dubois VFS, Jin CY, et al. Population pharmacokinetics of durvalumab in cancer patients and association with longitudinal biomarkers of disease status. *Clin Pharmacol Ther*. 2018;103:631-642.

SUPPORTING INFORMATION

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