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pharmacokinetic/pharmacodynamic associations of
ofatumumab, a human monoclonal CD20 antibody, in
patients with relapsed or refractory chronic lymphocytic
leukemia: a phase 1-2 study**

Bertrand Coiffier, Nedjad Losic, Birgitte Billmann Rönn, Stéphane Leprêtre,
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Pharmacokinetics and pharmacokinetic/pharmacodynamic associations of ofatumumab, a human monoclonal CD20 antibody, in patients with relapsed or refractory chronic lymphocytic leukemia: a phase 1-2 study

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5 **ofatumumab, a human monoclonal CD20 antibody, in patients with relapsed or**
6 **refractory chronic lymphocytic leukemia: a phase 1-2 study**
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53 **Running title:** (<50 characters) ofatumumab PK/PD evaluation in patients with CLL
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Abstract

The purpose of this phase 1-2 study was to investigate the association between pharmacokinetic (PK) properties of ofatumumab, a human monoclonal CD20 antibody, and outcomes in 33 patients with relapsed/refractory chronic lymphocytic leukemia receiving 4 weekly infusions of ofatumumab. The ofatumumab concentration profiles were fitted well by a two-compartment model with different elimination rate constant at first infusion compared to the remaining infusions in line with the observed rapid and sustained B-cell depletion. Exposure to ofatumumab was linked to clinical outcomes: high exposure was associated with higher probability of overall clinical response and longer progression free survival. This association still remained statistically significant even when adjusting for relevant baseline covariates including tumor burden. The trial was registered at www.clinicaltrials.gov (NCT00093314).

Keywords

ofatumumab • monoclonal antibody • chronic lymphocytic leukemia • PK/PD • intravenous infusion

Introduction

Ofatumumab (HuMax-CD20) is an IgG1 κ human monoclonal antibody that uniquely targets a membraneproximal epitope on the CD20 molecule. The CD20 antigen is a transmembrane phosphoprotein expressed on B lymphocytes from pre-B- to mature B-lymphocyte stage and on B-cell tumors. B-cell tumors include non-Hodgkin lymphomas, with high CD20 expression occurring on >90% of tumors, and chronic lymphocytic leukemia (CLL), which generally expresses lower levels of CD20. The CD20 molecule is not shed from the cell surface and is not internalized following antibody binding. The binding of ofatumumab induces relocation of CD20 molecules to so-called lipid rafts, which is considered important for induction of effective complement activation. This efficient recruitment and activation of complement at the cell surface by ofatumumab leads to potent complement-dependent cytotoxicity, resulting in rapid and extensive lysis of tumor cells. Ofatumumab has been shown to induce appreciable cell lysis in complement- and rituximab-resistant cells, even with low levels of CD20 expression and/or high expression levels of complement defense molecules (Teeling *et al*, 2004). In addition, the binding of ofatumumab induces cell death through antibody-dependent cell-mediated cytotoxicity (Teeling *et al*, 2006).

In follicular and mantle cell lymphoma, serum rituximab concentrations have been correlated with clinical response in some studies (Berinstein *et al*, 1998; Igarashi *et al*, 2002; Plosker *et al*, 2003; Tobinai *et al*, 2004), although 2 other studies showed no such correlation (Tobinai *et al*, 1998; Mangel *et al*, 2003). Alemtuzumab, a monoclonal antibody that targets the CD52 antigen, has been characterized with respect to its pharmacokinetics and exposure-response relationship in CLL patients. Alemtuzumab displayed time- and concentration-

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4 dependent pharmacokinetics with large interpatient variability (Mould *et al*, 2007). A
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6 relationship between maximal trough concentrations and clinical outcome was observed, and
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8 increasing alemtuzumab exposure resulted in a greater probability of tumor inhibition.⁹
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10 Recently, Daydé *et al*. showed in a murine model of human CD20⁺ lymphoma that rituximab
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12 exposure was inversely correlated with tumor burden and influenced response and survival
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14 (Daydé *et al*,2009). These observations are in accordance with a study of ofatumumab in a
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16 mouse xenograft model (Bleeker *et al*, 2008).
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21 The pharmacokinetic profile of ofatumumab has been described in patients with
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23 relapsed or refractory follicular lymphoma (Hagenbeek *et al*, 2008). Overall, the AUC
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25 increased with dose in a superproportionate manner, but no association between
26
27 pharmacokinetics and response was found in this phase 1-2 trial.
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31 Here we present the PK profile of ofatumumab in patients with relapsed or refractory
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33 CLL and show by non-compartmental and compartmental analyses that
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35 pharmacokinetic/pharmacodynamic (PK/PD) associations indeed exist. Such relationships
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37 may prove useful for optimizing doses and schedules of CD20 antibodies in CLL and other
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39 B-cell malignancies.
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Methods

Patients

All patients had relapsed or refractory CLL with more than $5 \times 10^9/L$ (5000/ μ L) circulating lymphocytes and were aged 18 years or older with a life expectancy greater than 3 months. Circulating lymphocytes in the screening blood sample had to be CD5⁺, CD20⁺, and CD23⁺. The main exclusion criteria were significant acute or chronic co-morbidity; treatment with rituximab, alemtuzumab, or autologous stem cell transplantation within the last 6 months; and previous treatment with allogeneic stem cell transplantation. A detailed description of the patients has been published (Coiffier *et al*, 2008) see Table I.

The trial was performed in accordance with Good Clinical Practices and local regulatory requirements. The trial protocol was approved by the appropriate national authorities and institutional review boards/ethics committees (see affiliations list). Each patient gave written informed consent in accordance with the Declaration of Helsinki to participate.

Trial design

This open-label phase 1-2 trial was performed between September 2004 and April 2006. The primary objective, which has been published (Coiffier *et al*, 2008), was to evaluate the safety and efficacy of ofatumumab in patients with CLL. The secondary objective, and the focus of the present work, was to determine the pharmacokinetics after a single dose and after 4 weekly doses of ofatumumab in patients with CLL.

Patients received 4 weekly infusions of ofatumumab at 3 different dose levels. The 3 patients in cohort A received a first infusion of 100 mg ofatumumab and 3 subsequent

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4 infusions of 500 mg. The 3 patients of cohort B received a first infusion of 300 mg and 3
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6 subsequent infusions of 1000 mg and the 27 patients of cohort C received a first infusion of
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8 500 mg and 3 subsequent infusions of 2000 mg. Enrollment in cohorts B and C was initiated
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10 when the 3 patients in the previous cohort had received all 4 infusions and had satisfactory
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12 safety evaluations after 1 week follow up (see Coiffier *et al*, 2008), for a detailed description of
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14 the pre-medication given in connection with the ofatumumab infusions). During the treatment
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16 phase, the patients were followed for safety assessments, and samples for efficacy
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18 measurements (flow cytometry analyses) and pharmacokinetics were obtained. Levels of
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20 circulating B cells were assessed via the surrogate marker CD19 and via CD20 (BARC,
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22 Ghent, Belgium). No infection prophylaxis was scheduled in the protocol.
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30 **Pharmacokinetic assessments**

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32 Pharmacokinetic samples were taken on days 0 (before, during, and after infusion), 1, 3, 5, 7,
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34 14, 21 (before, during, and after infusion), 25, week 4, week 7, week 27, month 9, and month
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36 12. The concentration of ofatumumab was determined in serum using a sandwich
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38 immunoassay (Hagenbeek *et al*, 2008).
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42 Serum samples were collected to determine the pharmacokinetics both after a single
43
44 dose and after 4 weekly doses of ofatumumab. Thus the non-compartmental PK parameters
45
46 were derived from the PK samples taken immediately before the first infusion up until the
47
48 second infusion, and from the PK samples taken immediately before the fourth infusion and
49
50 thereafter (software used: WinNonLin version 5.01).
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53 The parameters were: area under concentration-time curve extrapolated to infinity
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55 ($AUC_{0-\infty}$), maximum and minimum concentrations of ofatumumab (C_{max} , C_{trough}), time of C_{max}
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4 (T_{max}), half-life (T_{1/2}), volume of distribution at steady state (V_{ss}), and clearance (CL). Baseline
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6 values for body weight (W) and height (h) were recorded. The body surface area (BSA, m²)
7
8 was calculated using the formula: $W^{0.425} \times h^{0.725} \times 0.007184$ (Du Bois *et al*, 1916), and the
9
10 body mass index (BMI, kg/m²) was calculated using the formula: W/h^2 .
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16 **Pharmacodynamic assessments and PK/PD interaction variables**

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18 Pharmacodynamics were assessed by objective response and time-to-event endpoints. The
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20 primary efficacy variable was objective response over the period from screening to week 19
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22 and was reported according to the National Cancer Institute Working Group 1988 and 1996
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24 criteria (Cheson *et al*, 1988; Cheson. *et al*, 1996), as complete remission, nodular partial
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26 remission, partial remission, progressive disease, stable disease, and non evaluable.
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28 Response was assessed on the following parameters: (a) lymph node size measured as the
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30 sum of the product of diameters (SPD) of the largest palpable nodes larger than 1 cm; (b)
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32 hepatomegaly and splenomegaly; (c) hemoglobin level and platelet, lymphocyte, and
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34 neutrophil counts; (d) unilateral bone marrow aspirate and biopsy performed at week 19 or 27
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36 for patients with blood lymphocytes less than $4.5 \times 10^9/L$ (4500/ μ L); (e) CT scan of neck,
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38 thorax, abdomen, and pelvis were performed before treatment and at week 19 or 27
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40 depending on the date of the clinical response. CT scans were used to confirm the response
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42 in responding patients classified as complete remission, nodular partial remission or partial
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44 remission maintained for 2 months. For a more complete description of ofatumumab efficacy,
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46 see Coiffier *et al*, 2008.
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54 The objective response data are presented in this paper in order to analyze their
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56 relationship with compartmental and non-compartmental PK parameters.
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Kaplan-Meier estimations were made for time-to-event endpoints: progression-free survival (PFS), response duration and time to next anti-CLL therapy. Progression-free survival was the number of days from baseline to progressive disease (in overall tumor response or assessed in clinical examination) or death related to CLL. Response duration was the number of days from first documentation of a response (complete remission, nodular partial remission or partial remission) to progressive disease (in overall tumor response or assessed in clinical examination). Time to next anti-CLL therapy was the number of days from baseline to when any anti-CLL therapy other than ofatumumab was started. The relationships between time-to-event endpoints and log-transformed PK parameters were investigated.

Toxicity

The analysis of the relationship between toxicity and PK parameters focused on the occurrence of infections, and incidences of toxicity were defined as adverse events coded as belonging to the 'infections and infestations' system organ class in the Medical Dictionary for Regulatory Activities (MedDRA, version 6.0). One incidence of infectious interstitial lung disease was not included in the correlation analysis because the adverse event was classified in the system organ class 'respiratory, thoracic and mediastinal disorders'. For a complete analysis of ofatumumab safety, see Coiffier *et al*, 2008.

Non-compartmental statistical analyses

Analysis and presentation of non-compartmental PK parameters were made for patients with robust parameter estimates (i.e., patients for whom the linear regression model of the elimination phase had an adequate fit ($R^2 > 0.9$)). Two-sided non-parametric tests (Wilcoxon)

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4 were performed to compare the estimates of the PK parameters after infusion 1 with the
5 estimates after infusion 4. The influence on PK parameters of baseline characteristics of the
6 patients were investigated by a linear mixed model including cohort as fixed effect and one
7 covariate at a time (gender, age, height, body weight, BMI, time since CLL diagnosis, Binet
8 stage, modified Rai stage, SPD at screening, and B-cell count at baseline).

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16 For the PK/PD interaction analyses, Wilcoxon tests were performed to test the null
17 hypothesis that there was no difference between responders and non-responders
18 (or infection/no infection) in terms of $AUC_{0-\infty}$, C_{max} , C_{trough} , $T_{1/2}$, or CL. Univariate and
19 multivariate logistic regression analyses were performed to investigate whether the response
20 or toxicity could be explained by $AUC_{0-\infty}$, C_{max} , C_{trough} , $T_{1/2}$, or CL after the fourth infusion, while
21 adjusting for baseline variables (SPD, CD19⁺ counts, Binet stage, modified Rai stage, or
22 gender). The relationship between IgG concentrations in plasma and $T_{1/2}$ was assessed by
23 linear regression.

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35 The relationships between time-to-event endpoints (PFS, duration of response, time to
36 next anti-CLL therapy) and log-transformed PK parameters ($AUC_{0-\infty}$, C_{max} , C_{trough} , $T_{1/2}$, and CL
37 after the fourth infusion) were investigated using Cox regression. In these analyses,
38 time-to-event endpoints were censored at the time of the last relevant assessment.
39 Hazard ratio >1 is associated with increased risk and <1 with decreased risk.

40 41 42 43 44 45 46 47 48 49 **Compartmental pharmacokinetic modeling**

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52 The ofatumumab concentration-time profiles from cohort C were fitted by a first-order, linear
53 two-compartment model as illustrated in Figure 1 (software used: SAS version 9.2) (Kuester
54 *et al*, 2006). The i.v. infusion rate was assumed to be constant over the dosing interval, τ , at

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4 rate given by $D/(V1 \cdot \tau)$, where D was the actual dose (in mg) and $V1$ was the volume of
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6 distribution of the central compartment (in mL/kg). Drug was administered directly into the
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8 central compartment, and the distribution rate constants were denoted k_{12} and k_{21} . The
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10 elimination rate constant was denoted k_{10} and was allowed to differ between the first infusion
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12 and the remaining infusion visits, with the change in rate given by δ_{10} .
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16 The full population data were fitted by a non-linear mixed effect model, where k_{21} was
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18 included as a fixed effect and k_{12} , k_{10} , δ_{10} , and $V1$ were included as random effects and
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20 assumed to be mutually independent and log-normally distributed. A number of concentration
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22 measurements were recorded below the limit of quantification and were included in the
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24 analysis as censored observations. The residual errors were assumed to be mutually
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26 independent and normally distributed with mean zero and variance σ^2 . For model evaluation,
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28 the residuals were plotted against the predicted values. One extreme observation was omitted
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30 from the plot, to keep the axis symmetric.
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37 **Compartmental statistical analysis**

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39 For cohort C the possible association between the estimated PK parameters and
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41 demographic characteristics and baseline information was investigated by ANOVA. The
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43 log-transformed PK parameters, k_{10} , k_{12} , δ_{10} , and $V1$, were analyzed separately, including a
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45 covariate in each analysis. The covariates considered were gender, age, height, body weight,
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47 BMI, time since CLL diagnosis, Binet stage, modified Rai stage, SPD at screening, and B-cell
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49 count at baseline (measured by flow cytometry). The effect of PK parameters on clinical
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51 response was investigated by logistic regression, where also baseline SPD and body weight
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53 were included as covariates.
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Results

Patients

Thirty-three patients took part in the trial. Three patients were allocated to each of cohorts A and B and 27 were allocated to cohort C. All patients completed treatment except one patient in cohort C who was withdrawn after the first infusion.

A detailed description of the demographics and baseline characteristics for all patients is outlined in Table I. In cohort C, the numbers of males/females were 12/15, the median age of the patients was 63 years (range 27 to 82 years), the median body weight was 70.0 kg, (range 48 to 103 kg), the median BMI was 24.7 kg/m² (range 17.0 to 35.6 kg/m²), the median SPD was 14 cm² (range 0 to 274 cm²), and the median number of circulating CD19⁺ B cells in peripheral blood was 47.7 X 10⁹/L (range 5.5 to 220.4 X 10⁹/L).

Pharmacokinetic evaluation

All patients attained measurable concentrations of ofatumumab after the first infusion, and serum concentrations increased during the subsequent treatment course. Serum concentrations were measurable up to 4 weeks after the last infusion in the lower dose cohorts (cohorts A and B) and up to approximately 7 months in a small number of patients in the highest dose cohort (cohort C; Figure 2).

A summary of non-compartmental PK parameters after the first and fourth infusion of ofatumumab is presented in Table II. Values for AUC_{0-∞} and C_{max} tended to increase more than proportionally with dose. T_{1/2} appeared constant across the lower dose cohorts, with longer T_{1/2} in the higher dose cohort. After the first infusion, CL tended to decrease with dose,

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whereas after the fourth infusion, the CL was relatively constant across dose cohorts. As can be seen from Table II, the values for $AUC_{0-\infty}$ and CL showed considerable variability among patients in cohort C. The variation could partly be explained by differences in baseline characteristics between the patients, as gender and SPD at screening were found to be significant predictors of systemic exposure (AUC) and clearance (Cl). At the first infusion baseline SPD was predictive for AUC and clearance ($p < 0.05$). At the fourth infusion both gender and SPD were predictive for AUC and clearance ($p < 0.05$). None of the other covariates (age, height, body weight, BMI, time since CLL diagnosis, Binet stage, modified Rai stage, and B-cell count at baseline) considered were found to be statistical significant predictors of exposure measured by AUC and Cl.

Relationship of non-compartmental pharmacokinetic parameters to clinical response, time-to-event endpoints and infection variables.

The primary efficacy variable, objective response over the period from screening to week 19, is presented in Table III.

The relation between clinical response, exposure to ofatumumab (measured by AUC and Cl) and SPD at screening are illustrated in Figure 3. For example Figure 3A shows AUC versus baseline SPD for responders and non-responders. A clear, vertical separation is seen between responders and non responders, indicating that high systemic exposure is associated with a higher likelihood of being responder, but there also seems to be an increasing trend with SPD at screening, indicating that high SPD at screening is associated with high systemic exposure after forth infusion.

Both $AUC_{0-\infty}$ and C_{max} were higher among patients with a clinical response than among patients without a clinical response ($p=0.001$ and $p=0.006$, respectively; Figures 3A and 3C for AUC). Similarly, responding patients had higher C_{trough} and longer $T_{1/2}$ than patients without a response ($p=0.008$ and $p=0.001$, respectively) (data not shown). Conversely, the CL was lower in responding patients than in non-responders ($p<0.001$; Figure 3B and 3D). The association between these PK parameters and the objective response was still found to be highly significant even when adjusting for baseline variables indicating tumor burden such as SPD, CD19⁺ counts, Binet or Modified Rai disease stage, or on gender (data not shown). No relationship between plasma IgG concentration and ofatumumab $T_{1/2}$ was found (data not shown).

It is of note that responders had higher $AUC_{0-\infty}$ and C_{max} and lower CL than non-responders already after the first infusion (Figure 4). It seems that the relative increase between the first and fourth infusion in $AUC_{0-\infty}$ and C_{max} in individual patients (whether non-responders or responders) was similar, as shown with log-transformed data in Figures 4A and 4B. This was also the case for $T_{1/2}$, again with higher $T_{1/2}$ in responding patients compared to non-responders (data not shown). A similar pattern for the reduction of CL was also seen (Figure 4C).

Kaplan-Meier estimates of PFS (all patients and responders), median duration of response and time to next anti-CLL therapy are presented in Figure 5(A-D). The relationships between these time-to-event endpoints and PK parameters ($AUC_{0-\infty}$, C_{max} , C_{trough} , $T_{1/2}$, and CL after the fourth infusion) were tested for statistical significance. $AUC_{0-\infty}$ and CL correlated with all the endpoints, and C_{max} , C_{trough} , and $T_{1/2}$ correlated with PFS and time to next anti-CLL therapy (Table IV). Kaplan-Meier estimates for time-to-event endpoints for patients with

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4 clearance above and below the median clearance are presented in Figure 5A'-5D'. The
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6 relation between time-to-event endpoints and clearance, now evaluated by a log-rank test,
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8 was highly significant for time to progression and time to next therapy ($p < 0.0001$ figure 5A'
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10 and $p < 0.007$ figure 5D').
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14 To investigate a possible relationship between reports of infections and exposure to
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16 ofatumumab, infections were plotted together with $AUC_{0-\infty}$, C_{max} , C_{trough} , $T_{1/2}$, and CL, and the
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18 potential relationship was statistically tested. No such relationship was demonstrated, even
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20 after adjustment for the baseline variables of SPD, CD19⁺ counts, Binet stage or modified
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22 Rai stage, or gender (data not shown).
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28 **Compartmental model evaluation and qualification**

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30 The compartment model was evaluated graphically. The plots of the first six patients included
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32 in cohort C with individual observed and predicted PK profiles are presented in Figure 6A.
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34 The circles represent observed concentrations, the dots represent observations below lower
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36 level of quantification (LLOQ), and the full line represents the predicted profile (by empirical
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38 Bayes prediction). The predicted random effects were plotted in Quantile-Quantile (QQ)-plots
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40 to evaluate the normality assumption. A QQ-plot of $\log(k_{10})$ is shown in Figure 6B. If the
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42 assumptions are fulfilled, the predicted random effects should form a straight line.
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44 Quantile-Quantile-plots of $\log(k_{12})$, $\log(\delta_{10})$, and $\log(V1)$ showed similar patterns (data not
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46 shown). Finally, a residual plot for all observations is presented in Figure 6C. One extreme
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48 observation was omitted from the plot to keep the axis symmetric.
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54 The graphical model evaluation showed good fit of the individual predictions to the
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56 observed profiles. Similarly, the assumption of normality of the random effects and the
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4 residual errors seemed to be fulfilled. The model results in estimated parameters with good
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6 precision (coefficient of variation less than 26% for all fixed effect parameters).
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10 11 **Pharmacokinetic evaluation by compartmental modeling**

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14 The distribution rate constant k_{12} was approximately half the size of the distribution rate
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16 constant k_{21} ($k_{12}=0.011 \text{ h}^{-1}$ [0.0060;0.016]; $k_{21}=0.022 \text{ h}^{-1}$ [0.013;0.031]; $p=0.0005$), The
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18 individual predicted concentration and amount profiles in central and peripheral compartments
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20 are plotted in Figure 6A, indicating that between $\frac{1}{4}$ and $\frac{1}{2}$ of the drug was distributed to the
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22 peripheral compartment during the distribution phase. The parameter estimates correspond to
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24 a distribution process with a half-life of approximately 20 hours. The elimination rate constant
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26 following first infusion was found to be faster compared to that following fourth infusion
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28 ($k_{10, \text{first}}= 0.0033 \text{ h}^{-1}$ [0.0026;0.0041]; $k_{10, \text{fourth}}= 0.016 \text{ h}^{-1}$ [0.0091;0.023]), corresponding to
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30 elimination processes after first and fourth infusion with half-lives of approximately 3 and 14
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32 days, respectively.
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40 **Relationship of compartmental PK parameters to patient covariates and objective** 41 **response**

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44 The statistically significant associations between baseline characteristics and compartmental
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46 model-based PK parameters are shown in Table V. The analyses showed that male patients
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48 had faster elimination compared to female patients and that high SPD at baseline was
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50 associated with faster elimination, after both first and fourth infusion. The gender, body weight
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52 and BMI of the patients were associated with the estimated volume of distribution, since male
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54 patients were seen to have larger volume of distribution compared to female patients and high
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4 body weight and BMI resulted in larger volume of distribution when expressed in mL.
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6 Furthermore, low age and low BMI were related to faster distribution between the central and
7
8 the peripheral compartment. Also male patients had faster distribution compared to female
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10 patients. The covariates height, time since CLL diagnosis, Binet stage, modified Rai stage,
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12 and B-cell count at baseline had no statistically significant association with the model-based
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14 PK parameters.
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18 The compartmental analyses also showed statistically significant associations between
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20 estimated elimination rate constants after first and fourth infusion and objective response
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22 (Table VI). The finding that fast elimination predicts a lower probability of objective response
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24 was also valid when adjusted for body weight and baseline SPD. The estimated volume of
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26 distribution was seen to be significantly associated with objective response, since larger
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28 volume of distribution predicts lower probability of objective response. There was no
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30 indication of an effect of distribution rate constants on objective response.
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Discussion

This work is based on a trial of ofatumumab, a human monoclonal CD20 antibody, in CLL patients showing an objective response in half of the patients with a median PFS of 15 weeks (Coiffier *et al*, 2008). In this trial, a dose-intensive strategy was selected following studies with rituximab used as monotherapy in CLL (Huhn *et al*, 2001; Itala *et al*, 2002; O'Brian *et al*, 2001).

The purpose of the present work was to determine whether or not there was an association between the exposure of ofatumumab and the probability of obtaining a clinical response and long time-to-event endpoints. Such analyses may contribute to further optimization of the dose and schedules of ofatumumab in future clinical trials.

Monoclonal antibodies, such as clenoliximab, trastuzumab and alemtuzumab, have shown evidence of non-linear behavior in humans (Mould *et al*, 2007; Mould *et al*, 1999; Herceptin[®] Product Label, 2001). The non-linear pharmacokinetics of these types of antibodies may in part be explained by binding of the antibodies to their respective targets, with a large component of target-mediated elimination after the first dose that is decreased after subsequent infusions due to a reduction in available target. In the present trial, a marked reduction of circulating leukemic B cells and of the SPD was noted after the first infusion (Coiffier *et al*, 2008), indicating rapid binding of ofatumumab to the target cells. When comparing the results from the fourth infusion of ofatumumab to those of the first, C_{\max} and $AUC_{0-\infty}$ seemed to increase more than could be expected from linear accumulation of drug when considering weekly dosing and $T_{1/2}$, suggesting non-linear pharmacokinetics. In addition, the results suggested that $AUC_{0-\infty}$ and C_{\max} increased more than proportionally with dose at

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4 both first and fourth infusion. However, the number of patients in the lower dose cohorts (A
5 and B) was small and the variability in parameter values was considerable, making a firm
6
7 conclusion regarding dose linearity or non-linearity difficult.
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11 Statistically significant positive correlations between objective response and $AUC_{0-\infty}$,
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13 C_{max} , C_{trough} , and $T_{1/2}$ were observed in this trial, where an inverse correlation was observed for
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15 CL. The analysis of the association between time-to-event endpoints and PK parameters
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17 suggests that high exposure and low CL of ofatumumab are important not only for obtaining a
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19 clinical response, but also for the duration of clinical responses. Thus, a high exposure may
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21 be able to effectively remove CLL cells from distant sites to prevent early repopulation
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23 (Chiorazzi *et al*, 2005), and disease progression. In addition, a long $T_{1/2}$ combined with low CL
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25 may provide sufficient ofatumumab concentrations to saturate circulating CLL cells for several
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27 months (Bleeker *et al*, 2008). Follicular lymphoma trials with rituximab (Berinstein *et al*, 1998;
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29 Plosker *et al*, 2003; Mangel *et al*, 2003) have not consistently shown a relationship between
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31 exposure and objective response, and in the recent FL trial with ofatumumab (Hagenbeek *et*
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33 *al*, 2008), no such correlation could be demonstrated. The large variability of PK parameters in
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35 patients treated with monoclonal antibodies has been observed with other compounds
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37 (Berinstein *et al*, 1999; Mangel *et al*, 2001; Mould *et al*, 2001. In the present work anti-drug
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39 antibodies were not demonstrated (Coiffier *et al*, 2008) and an effect mediated via the FcRn is
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41 not likely due to the absence of correlation of $T_{1/2}$ with baseline IgG levels. The variability
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43 might rather be explained by variation of covariates, such as tumour burden weight, or BSA,
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45 or combinations thereof. The data presented here show that responding patients already have
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47 significantly higher C_{max} and AUC after the first dose and that the increase of these
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49 parameters after the fourth dose was similar in responding and non-responding patients.
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4 However, the association observed in our trial between non-compartmental PK parameters
5 and the objective response was not found to depend significantly on surrogate parameters for
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7 baseline tumor burden such as SPD, CD19⁺ counts, Binet or Modified Rai disease stage, or
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9 for gender. With ofatumumab, the correlation between objective response and the PK
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11 parameters were strong whether the parameters were unadjusted, were adjusted for body
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13 weight, or were adjusted for BSA. Thus, with respect to objective response, adjustment of
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15 dosage for body weight or BSA is not supported.
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21 The associations between PK parameters and objective response or time-to-event
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23 endpoints might reflect the tumor burden and the subsequent target-mediated elimination of
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25 ofatumumab. A recent study with rituximab in a syngeneic murine model of lymphoma
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27 expressing human CD20 analyzed the relationship between PK parameters and outcome.¹⁰
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29 The tumor burden in mice receiving the same dose of rituximab influenced the efficacy, and
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31 rituximab exposure subsequently affected both response and survival. These observations
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33 are in accordance with a study of ofatumumab in a mouse xenograft model where tumor load
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35 had a major impact on ofatumumab plasma concentrations (Bleeker *et al*, 2008)..However, in
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37 CLL patients, the tumor burden can only be measured indirectly by surrogate parameters,
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39 such as SPD and lymphocyte counts, and the tumor load in organs such as bone marrow,
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41 liver, and spleen can not be quantified.
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47 In the compartmental analysis, a high AUC, high C_{max} , and a low CL is reflected in a
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49 slow elimination rate constant. In the present work, the compartmental PK analyses showed
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51 that SPD, a surrogate for tumor burden, was a significant predictor for the elimination rate
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53 constant. High SPD at baseline was associated with faster elimination both at the first and
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55 fourth infusion. In turn, the elimination rate constant was a significant predictor for clinical
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4 response; however, the prediction of a lower probability for clinical response with a faster
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6 elimination rate was significant even when adjusted for baseline SPD. Thus, SPD only
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8 partially explained the associations between elimination rate constant (and thus exposure)
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10 and clinical outcome. Other surrogates for tumor burden, such as lymphocyte counts, Rai and
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12 Binet stages were not related to the elimination rate constant.
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16 In conclusion, an association between the exposure to the antibody (determined by
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18 $AUC_{0-\infty}$ and CL) and both objective response and the time-to-event endpoints (PFS,
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20 duration of response, and time to next CLL treatment) was found in patients with relapsed or
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22 refractory CLL. This finding suggests that enhanced exposure (e.g., prolonged dosing of
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24 ofatumumab) may increase response in patients with CLL. Future trials to measure
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26 ofatumumab exposure as well as clinical outcome in CLL patients with varying tumor burden
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28 are needed to explore this association further.
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Authorship

Contribution: B.C., N.L., J.P. conceived and designed the trial; B.C., S.L., L.M.P., O.G., H.F., M.H.J.O., J. Wooldridge, J.K., J.H., A.H., J. Walewski., and T.R. provided trial materials or patients; N.L. and J.P. collected and assembled data; B.C., N.L., J.P. and B.B.R. analyzed and interpreted data; B.C. wrote the paper; B.C., N.L., J.P., B.B.R., S.L., L.M.P., O.G., H.F., M.H.J.O., J. Wooldridge., J.K., J.H., A.H., J. Walewski., and T.R. approved the final paper.

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Tables and figures

Table I. Demographics and baseline characteristics

Parameters	Median (Min-Max) or %
Demography	
Age (years)	61 (27-82)
Male (%)	19 (58%)
Caucasian (%)	33 (100%)
Disease characteristics	
Duration of CLL (years)	6.3 (1.2-14)
Time from latest treatment (years)	0.8 (0.1-5.3)
Number of previous treatments	3 (1-9)
Modified Rai stage at screening (%)	
0	1 (3%)
I	13 (39%)
II	15 (45%)
III	1 (3%)
IV	3 (9%)
Modified Binet stage at screening (%)	
A	7 (21%)
B	22 (67%)
C	4 (12%)
Constitutional symptoms	
Extreme fatigue	2 (6%)
Night sweats	5 (15%)
Hematologic symptoms (%)	
Anemia (hemoglobin < 12 g/dL)	7 (21%)
Neutropenia (neutrophils < 1,500/ μ^3)	0
Thrombocytopenia (platelets < 120,000/ μ^3)	9 (27%)
Lymphocytes (/ μ^3)	51.3 (8.2-227.2)
Lymph nodes	
None	5 (15%)
>5 cm in diameter	4 (12%)
SPD >50 cm ²	4 (12%)
Splenomegaly	
No	22 (67%)
>5 cm below diaphragm	2 (6%)

Table II. Pharmacokinetics after first and fourth ofatumumab infusion**(non-compartmental analysis)**

PK parameter	First infusion			Fourth infusion [†]		
	Cohort A 500 mg (n=2*)	Cohort B 1000mg (n=3)	Cohort C 2000 mg (n=27)	Cohort A 500 mg (n=3)	Cohort B 1000 mg (n=3)	Cohort C 2000 mg (n=23)
Median [range]						
AUC _{0-∞} , h*mg/L	455 [447-463]	1571 [485-1922]	7654 [446-117549]	32939 [1703-88519]	35393 [13857-51503]	367832 [62709-1845398]
C _{max} , mg/L	18 [17-18]	48 [27-57]	131 [42-714]	167 [107-211]	307 [204-381]	1067 [540-1830]
C _{trough} , mg/L	0 [0-0]	0 [0-0]	0 [0-0]	39 [0-87]	91 [1-287]	384 [104-785]
T _{max} , h	6.8 [6.6-7.0]	10.4 [7.1-11.8]	7.7 [5.3-14.7]	7.6 [5.8-9.3]	5.5 [5.2-6.0]	6.3 [4.9-10.2]
T _{1/2} , h	12.6 [9.7-15.5]	11.6 [10.8-13.9]	30.4 [3.8-143.1]	68.0 [10.0-246.6]	81.8 [25.0-103.4]	326.5 [55.2-735.1]
V _{ss} , mL	3812 [3066-4557]	3487 [2973-10519]	3220 [870-7902]	1765 [1762-4432]	3170 [2781-3310]	1785 [563-3589]
CL, mL/h	215 [215-216]	191 [156-619]	65 [4-1122]	17 [9-294]	28 [26-72]	10 [1-42]

*for one patient it was not possible to estimate the PK parameters since only 2 observations were above lower level of quantification.

†comparison of first and fourth infusion PK parameters showed statistically significant differences for AUC_{0-∞}, C_{max}, C_{trough}, T_{max}, T_{1/2} and CL (p<0.0001) for cohort C and in total.

Table III. Primary end point: objective response from screening to week 19

	Cohort A, (n=3)	Cohort B, (n=3)	Cohort C, (n=27)	Total, (n=33)
Non evaluable patients	0	0	1	1
Objective response (%)	1 (33)	0	13 (50)	14 (44)
95% CI, %	1-91	NA	30-70	29-67

NA = not applicable

Table IV. Relationship between time-to-event endpoints and log-transformed AUC_{0-∞}, C_{max}, C_{trough}, T_{1/2} and CL after fourth infusion (non-compartmental analysis)

PK parameter	Progression free survival (n=28)		Duration of response (n=20)		Time to next anti-CLL therapy (n=28)	
	Hazard ratio	p-value	Hazard ratio	p-value	Hazard ratio	p-value
AUC _{0-∞} , g/L*h	0.337	<0.0001	0.423	0.0023	0.387	0.0002
C _{max} , g/L	0.355	0.0023	0.476	0.0959	0.455	0.0194
C _{trough} , g/L	0.364	0.0005	0.496	0.0808	0.436	0.0010
T _{1/2} , h	0.995	0.0010	0.997	0.0518	0.004	0.0094
CL, mL/h	6.041	<0.0001	3.518	0.0057	9.061	0.0003

n = number of observations

Table V. Effect of baseline characteristics on PK parameters, cohort C**(compartmental analysis)**

PK parameter	Gender p-value	Age p-value	Body weight p-value	BMI p-value	Baseline SPD p-value
Elimination rate constant (k_{10}), first infusion	0.034	0.72	0.68	0.91	0.0034
Elimination rate constant (k_{10}), fourth infusion	0.019	0.60	0.38	0.67	0.014
Distribution rate constant (k_{12})	0.046	0.037	0.15	0.012	0.83
Volume of distribution of central compartment (V_1 , mL)	0.047	0.56	0.0057	0.026	0.11

**Table VI. Effect of estimated PK parameters on clinical response, cohort C
(compartmental analysis)**

PK parameter	No adjustment p-value	Adjusted for body weight p-value	Adjusted for baseline SPD p-value
Elimination rate constant (k_{10}), first infusion	0.0051	0.0091	0.018
Elimination rate constant (k_{10}), fourth infusion	0.0022	0.0057	0.0071
Distribution rate constant (k_{12})	0.11	0.27	0.15
Volume of distribution of the central compartment (V_1 , mL)	0.0056	0.052	0.012

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4 **Figure 1.**

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6 **Linear two-compartment model with zero-order input, first-order distribution between**
7 **the central and peripheral compartments, and first-order elimination from the central**
8 **compartment.**

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14 The elimination rate constant (k_{10}) was allowed to change between first and second infusion;
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16 t = time, τ = dosing interval, D = dose, V_1 = volume of distribution of the central compartment,
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18 and k_{12} and k_{21} = distribution rate constants.
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23 **Figure 2.**

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25 **Ofatumumab serum concentrations during trial in cohort A (A), cohort B (B), and**
26 **cohort C (C).**

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30 Pharmacokinetic samples were taken on days 0 (before, during, and after infusion), 1, 3, 5, 7,
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33 14, 21 (before, during, and after infusion), 25, week 4, week 7, week 27, month 9, and month
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35 12.
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38 The limit of quantification was 0.1 $\mu\text{g/mL}$.
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42 **Figure 3.**

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44 **PK parameters $\text{AUC}_{0-\infty}$ (A and C) and CL (B and D) after first and fourth infusion versus**
45 **baseline SPD and CD19⁺ counts by overall clinical response.** Letters A, B and C inside the
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47 figures indicate cohort and individual patient values. Bold-faced red letters: responders, thin
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49 blue letters: non-responders.
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Figure 4.

Objective response (screening to week 19) versus log-transformed (natural logarithm) $AUC_{0-\infty}$ (A), C_{max} (B), and CL after first and fourth infusion. Robust parameter estimates; non-compartmental analysis.

The p-values are taken from the 2-sided Wilcoxon test. Letters A, B and C inside the figures indicate cohort and individual patient values from first to fourth infusion. Boxes illustrate quartiles and whiskers range. Asterisks denote mean.

Figure 5.

Kaplan-Meier estimates of progression free survival (all patients) (A), progression free survival (responders) (B), duration of response (C), and time to next anti-CLL therapy (D). In addition, the same parameters divided by higher and lower clearance are shown (A', B', C' and D')

Based on Kaplan-Meier estimates, at a median/maximum follow-up of 7.1/33.3 months, the median progression free survival (PFS) (all patients) for all cohorts/cohort C was 3.6 months (95% CI; 3.2–4.6)/4.4 months (95% CI; 3.4–5.2); the median PFS (responders) for all cohorts and cohort C was 5.3 months (95% CI; 4.6–7.2); the median duration of response for all cohorts/cohort C was 4.3 months (95% CI; 3.5–5.7)/4.4 months (95% CI; 3.7–5.7); and the median time to next anti-CLL therapy for all cohorts/cohort C was 12.0 months (95% CI; 6.2 to not estimable)/12.1 months (95% CI; 8.5 to not estimable).

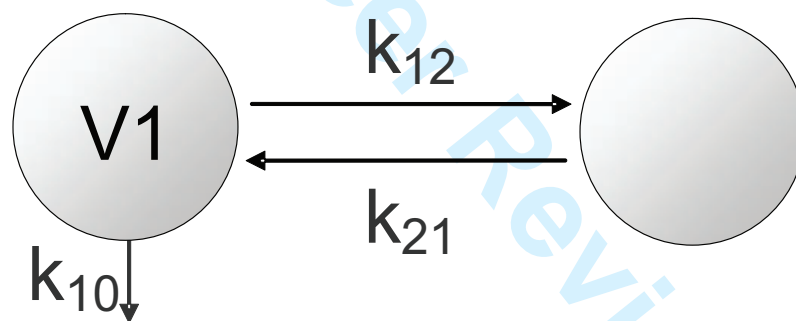
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4 **Figure 6. Model evaluation: Individual observed profiles (A), normality plot for random**
5 **effects (B) and residual plot (C); compartmental analysis.**
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9 A: circles = observed ofatumumab concentrations; dots = observations below LLOQ; solid
10 blue line = predicted concentration/amount of ofatumumab in plasma (central compartment)
11 and solid green line = predicted amount of ofatumumab in peripheral compartment. B: linear
12 relationship indicates correspondence with normality assumption. C: no systematic pattern
13 indicates correspondence with normality assumption on residual errors. One extreme
14 observation was omitted to ensure a symmetric axis.
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Figure 1

i.v. Infusion, $0 \leq t \leq \tau$

$D/V \cdot \tau$



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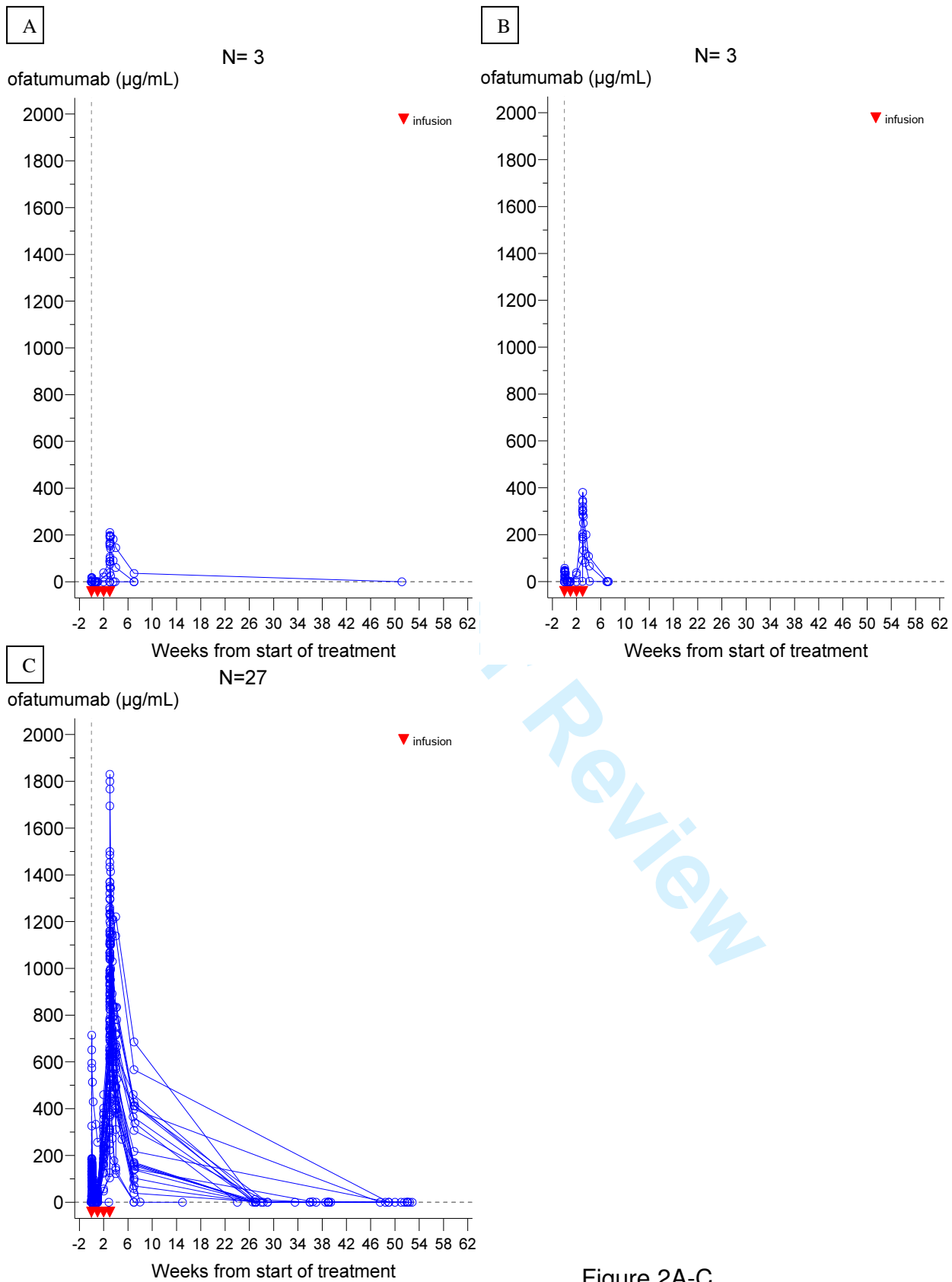
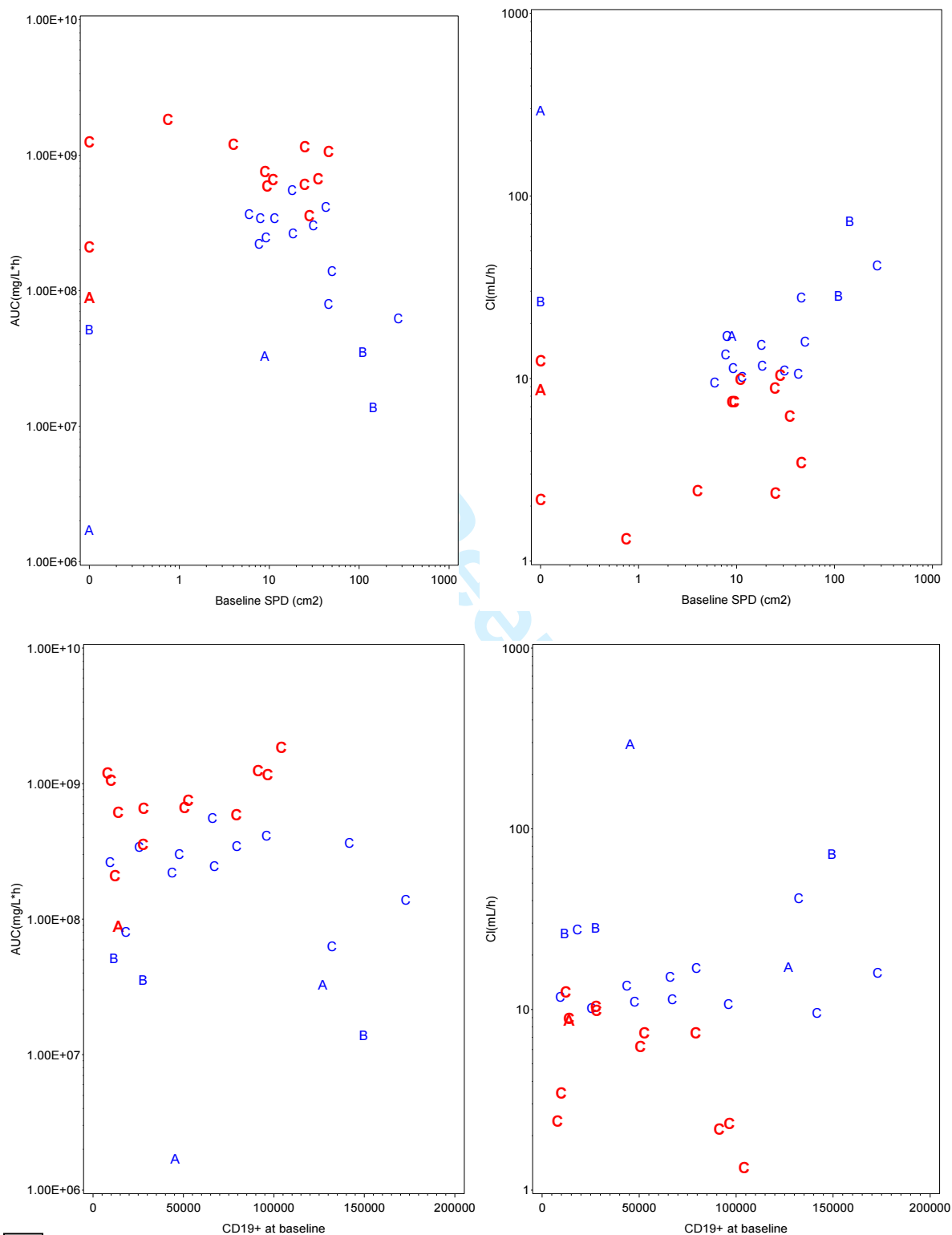


Figure 2A-C



C

Figure 3A-D

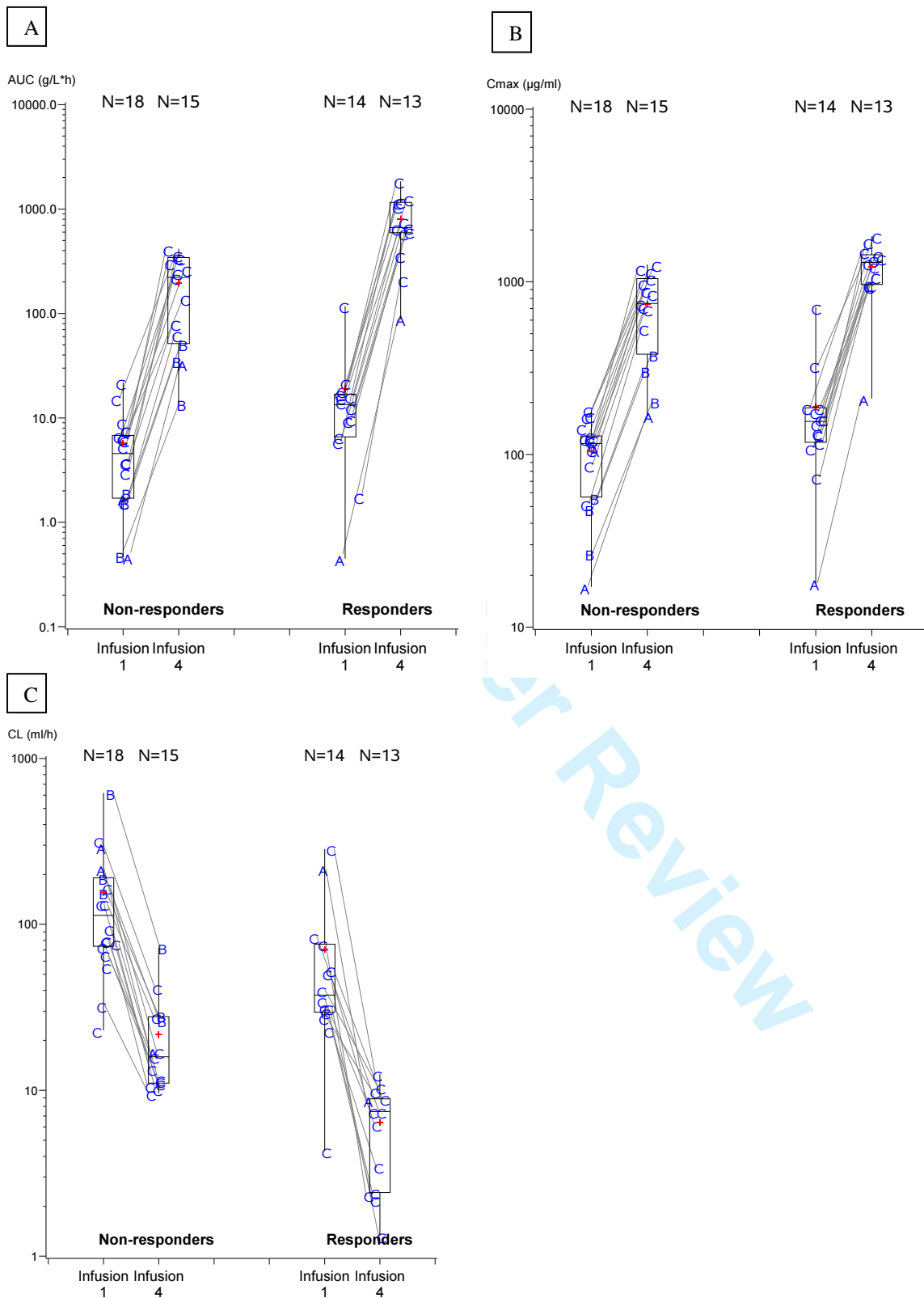


Figure 4A-C

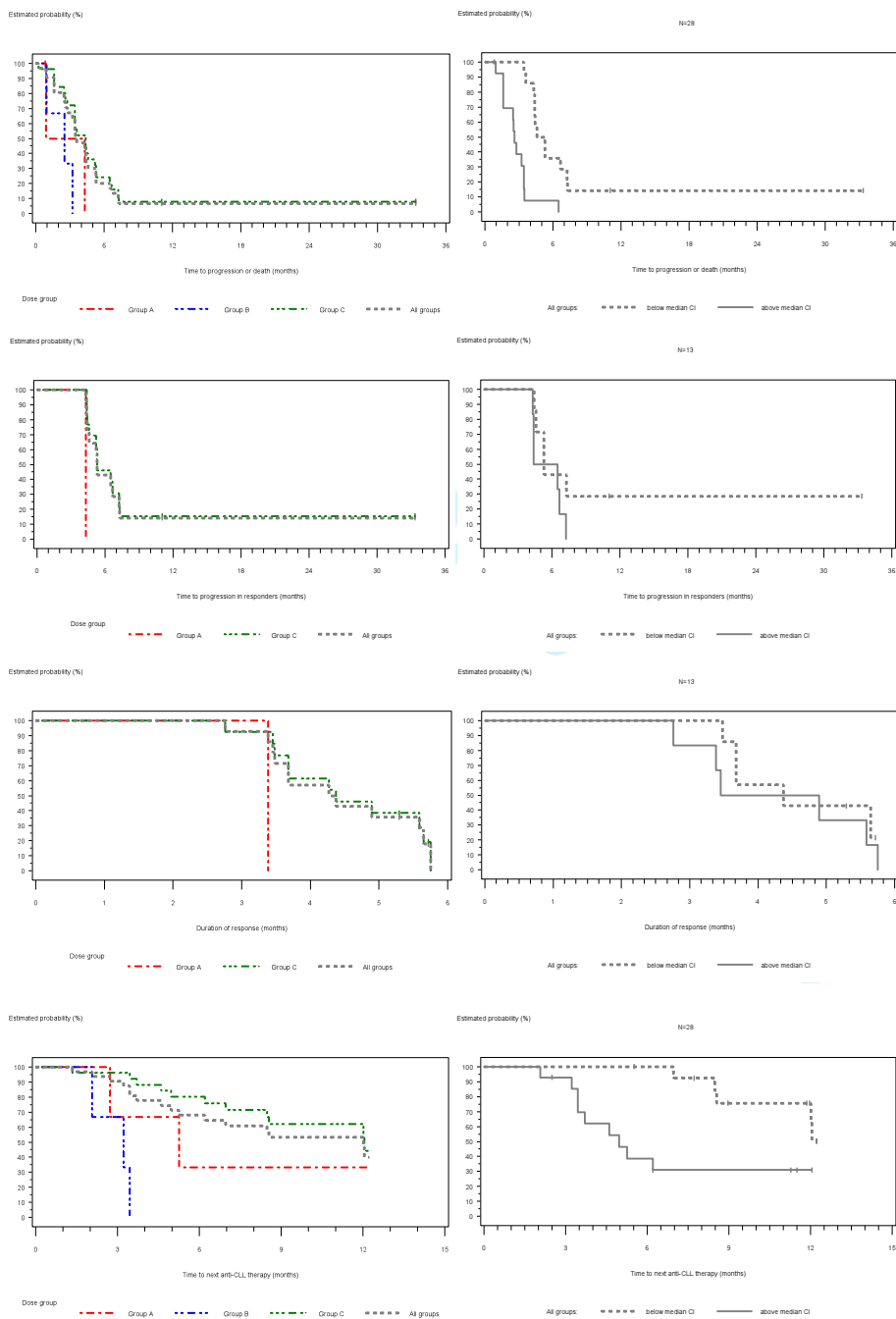
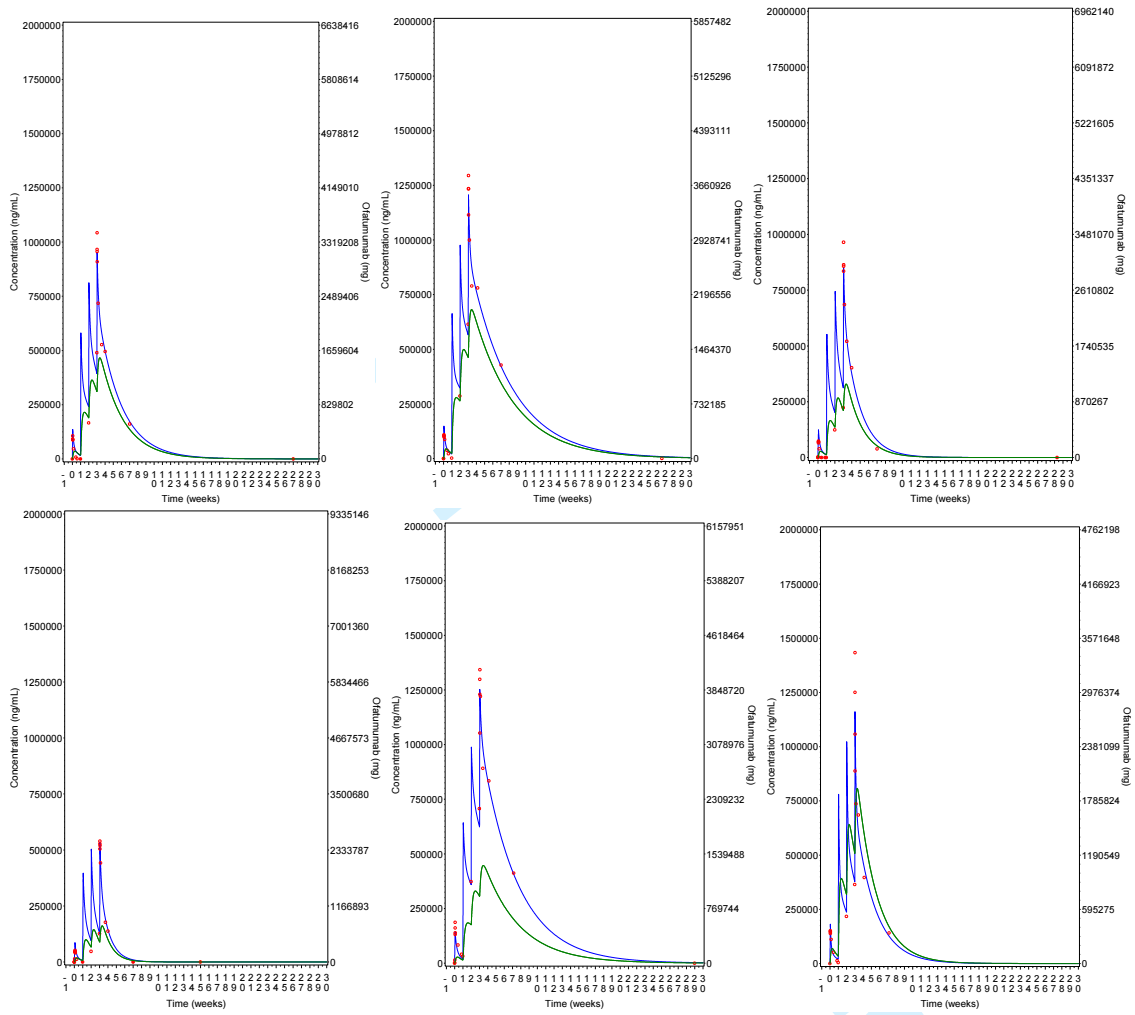


Figure 5A-D

Figure 5A'-D'

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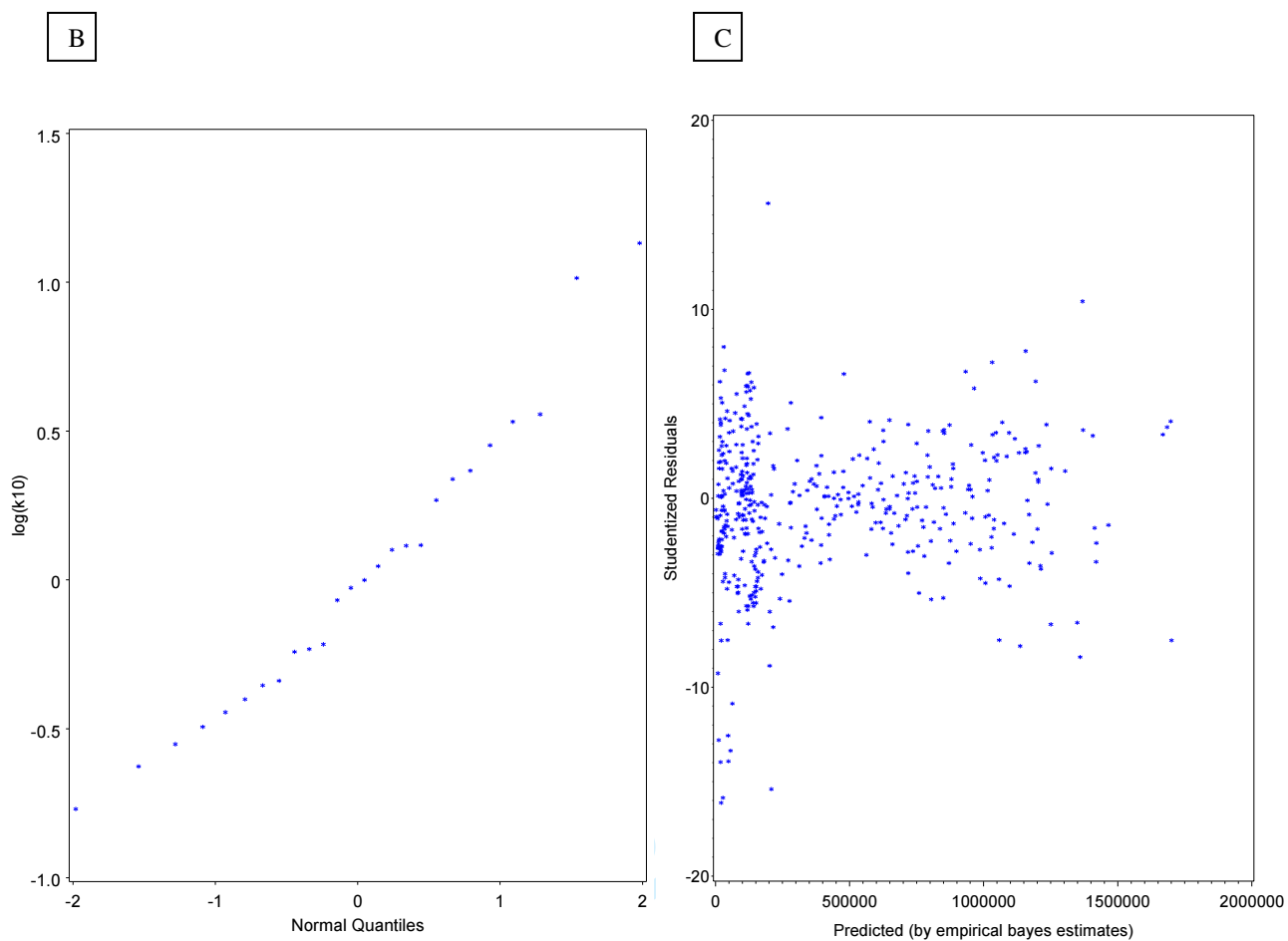


Figure 6A-C

Review