SUPPLEMENTARY APPENDIX

Rationale for optimal obinutuzumab/GA101 dosing regimen in B-cell non-Hodgkin lymphoma

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Supplementary Information

Supplementary Methods

PK assessments and bioanalytical methods

PK assessments In phase 1 and 2 of GAUGUIN, 5 mL of blood was drawn from all patients on D1, C1 (pre-infusion, immediately post-infusion, 3–6 hours post-infusion, 20–28 hours post-infusion, 66–80 hours post-infusion), D8, C1 (pre-infusion, post-infusion, 36–60 hours post-infusion, 144–192 hours post-infusion, 216–312 hours post-infusion), D1, C2–C7 (pre-infusion, post-infusion), and D1, C8 (pre-infusion, post-infusion, 3–6 hours post-infusion, 20–28 hours post-infusion, 66–80 hours post-infusion, 336–384 hours post-infusion, 456–600 hours post-infusion). In the GAUDI study, PK assessments were made during C1 as per the schedule used in GAUGUIN, with blood drawn pre-infusion and immediately post-infusion on D1 of the remaining cycles. At the final cycle, PK was assessed pre-infusion, post-infusion, 3–6 hours post-infusion, 20–28 hours post-infusion, 66–80 hours post-infusion, 336–384 hours post-infusion and 456–600 hours post-infusion.

Bioanalytical methods Obinutuzumab serum concentrations were quantified by enzyme-linked immunosorbent assay. Anti-idiotypic capture and detection antibodies were used in this assay; therefore, only free and active obinutuzumab antibodies were bound and detected. The lower and upper limits of quantification were 4.05 ng/mL and 400 ng/mL, respectively. Quality control samples were tested at the following concentrations: 10 ng/mL, 100 ng/mL and 300 ng/mL.

Calculation of PK parameters A non-compartmental analysis (Phoenix) was performed to derive main PK parameters, including maximum plasma concentration (C_{max}), area under the plasma concentration time curve (AUC), plasma half-life,

clearance and volume of distribution. PK data are presented using descriptive statistics.

Graphical analyses

Factors influencing PK parameters Exploratory graphical analyses were conducted to assess the influence of demographic (age, gender, weight), CD20-antigenic mass-related (B-cell count, stage, tumor burden), and histologic parameters on the PK of obinutuzumab. Demographic factors would be expected to influence the PK of obinutuzumab, while factors associated with tumor burden would be expected to influence the shape of the drug concentration-time curve.

Effect of drug exposure on tumor response Exploratory graphical analyses were performed to examine the relationship between drug exposure and tumor response, which was based on mean obinutuzumab serum concentration during the first 6 weeks of treatment (cumulative AUC at week 6 divided by time) and change in tumor volume (decrease or increase).

Effect of drug exposure on toxicity Exploratory graphical analyses were also conducted to investigate the relationship between obinutuzumab exposure and toxicity, based on occurrences of grade 3/4 neutropenia and grade 3/4 infections.

As the data from phase 2 of the GAUGUIN study were from a relatively small patient population, statistical analyses were not conducted. However, the graphical exploratory analyses were used to guide a full covariate analysis conducted on a larger, pooled clinical dataset from phase 1, 1/2 and 3 studies of obinutuzumab (phase 1 GAUGUIN and GAUDI, phase 1/2 GAUSS and phase 3 CLL11), which has been published separately [1].

PK modelling

Model development Obinutuzumab concentration data in patients with NHL were analyzed by a population PK approach using the software program NONMEM (version 7.1). A structural model that was initially developed for another anti-CD20 antibody, rituximab [1], was used as a reference model and comprised two parallel clearance components; a non-linear time-varying clearance pathway and a linear clearance pathway. The underlying assumption in the modelling of obinutuzumab PK was that the target itself, the CD20 receptor, has a significant impact upon the initial serum concentrations of obinutuzumab when the target is present in high quantities and is considered to be target-mediated drug disposition (TMDD), resulting in non-linearity in obinutuzumab PK with respect to time. This is reflected in the non-linear clearance pathway. This PK model was used as an additional aid to determine an appropriate obinutuzumab dose and regimen for phase 3 assessment.

Model evaluation The PK model was evaluated graphically using goodness-of-fit plots and visual predictive checks.

Model simulations PK model simulations were performed to compare obinutuzumab serum concentration profiles following the dose regimen of 1600/800 mg (D1 and D8, C1; D1, C2–C8), as used in the study, and a new fixed-dose regimen of 1000 mg (D1, D8, and D15, C1; D1, C2–C8; 21-day cycle). The population parameter estimates were used to generate the obinutuzumab concentration-time course profiles. One hundred simulations were performed in 100 virtual patients for each administration schedule.

Supplementary Table 1

Demographic and disease characteristics of patients with NHL participating in phase 1 and 2 clinical studies of obinutuzumab [2–5]

Patients, *n*	**GAUGUIN Phase 1 [6] (*n* = 21)**	**GAUGUIN Phase 2 iNHL [4] (*n* = 40)**	**GAUGUIN Phase 2 aNHL [3] (*n* = 40)**	**GAUDI Phase 1 [7] (*n* = 56)**	
				G-CHOP (*n* = 28)	**G-FC (*n* = 28)**
Median age, years (range)	64.0* (39–83)	60.5* (42–79)	71.0* (22–85)	62.5 (32–75)	61.0 (45–77)
Male, %	43	63	68	39	61
Disease stage III–IV at diagnosis, %	62	90	76‡	61†	82*
Tumor histology, *n* (%)					
FL	13 (62)	34 (85)	0 (0)	28 (100)	28 (100)
Other iNHL	3 (14)	6 (15)	0 (0)	0	0
DLBCL	1 (5)	0 (0)	25 (63)	0	0

MCL	4 (19)	0 (0)	15 (38)	0	0
Median tumor burden (SPD), mm^2 (range)	N/A	3178 (288–22801)	3519 (160–20032)	3193 (357–11965)	2391 (160–13661)
Bulky disease,† %	N/A	38	50	32	18
B-cells × 10^9/L (range)	N/A	0.059 (0.002–1.112)	0.04 (0.0–22.47)	0.066 (0.0–0.693)	0.035 (0.0–3.463)
Median previous treatments (range)	5 (1–8)	3 (1–11)	3 (1–17)	1 (1–3)	2 (1–6)
Previous rituximab, *n* (%)	20 (95)	38 (95)	40 (100)	28 (100)	28 (100)
Rituximab-refractory, *n* (%)	9 (43)	22 (55)	25 (63)	4 (14)	10 (36)

Abbreviations are as follows: aNHL, aggressive non-Hodgkin's lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; G-CHOP, obinutuzumab plus cyclophosphamide/doxorubicin/vincristine/prednisone; G-FC, obinutuzumab plus fludarabine/cyclophosphamide; iNHL, indolent non-Hodgkin's lymphoma; MCL, mantle-cell lymphoma; N/A, not available; NHL, non-Hodgkin's lymphoma; SPD, sum of product diameters.

*F. Hoffmann-La Roche 2014. Data on file. †Bulky disease defined as >10 cm in BO20999 phase 1, >5 cm in BO20999 phase 2 and >7 cm in BO21000. ‡Expressed as a percentage of the total number of patients with aNHL in stage 2 of GAUGUIN (n = 40); 38/40 patients with aNHL had staging data available.

Supplementary Table 2

Model parameter values for the basic population pharmacokinetic model.

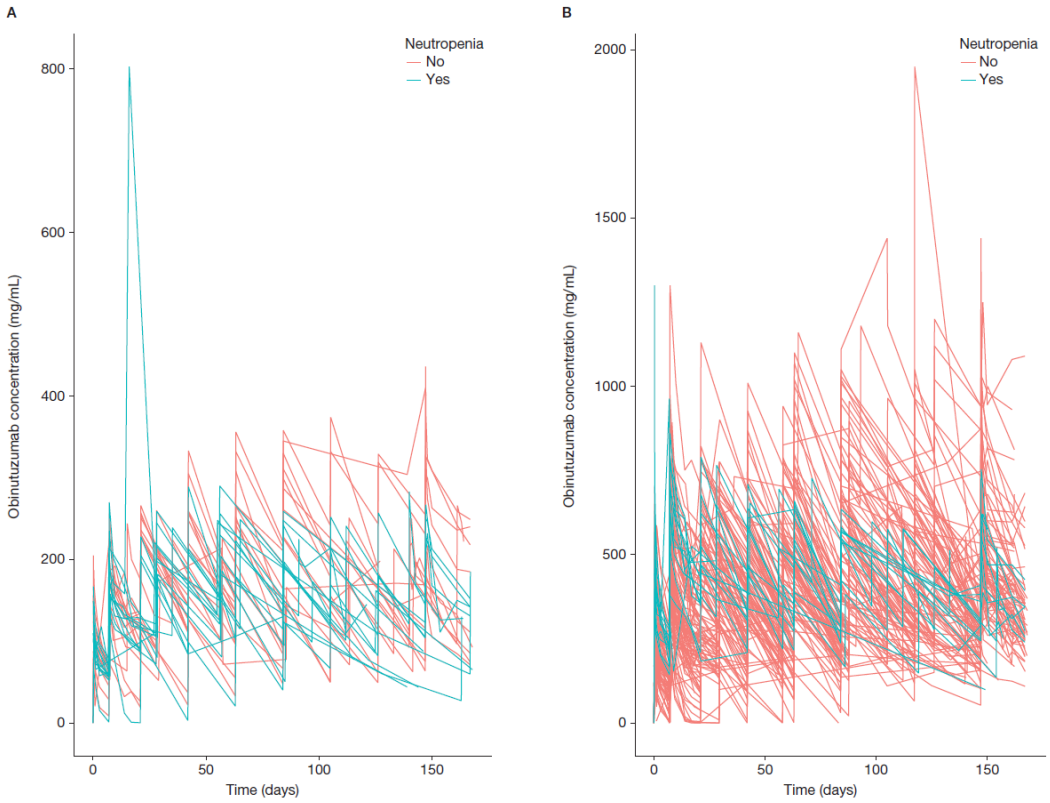
Parameters		Unit	Estimate	Relative SE (%)
Fixed Effect Parameters				
θ_1	K_{des}	Day^{-1}	0.0923	22.0
θ_2	CL_2 at time zero	mL/day	126.0	30.8
θ_3	CL_1	mL/day	95.1	5.75
θ_4	V_1	mL	3610.0	3.97
θ_5	V_2	mL	1710.0	13.3
θ_6	Distribution clearance	mL/day	499.0	9.13
θ_7	Proportional residual error	%	23.6	0.61
Random Effect Parameters				
ω_1^2	BSV on K_{des}	% CV	108.6	13.7
ω_2^2	BSV on CL_2	% CV	189.7	11.0
ω_3^2	BSV on CL_1	% CV	63.0	6.2
ω_4^2	BSV on V_1	% CV	33.7	6.3
ω_5^2	BSV on V_2	% CV	96.0	19.6
ω_6^2	BSV on distribution clearance	% CV	127.3	30.2
OFV: 71799.021 *n* = 164 patients and 3119 concentrations				

BSV, between-subject variability; CL_1, non-specific clearance; CL_2, specific clearance; CV, central volume; K_{des}, rate of specific clearance decay; OFV: NONMEM objective function; SE, standard error of estimate; V_1, volume of

distribution in the central compartment; V_2, volume of distribution in the peripheral compartment

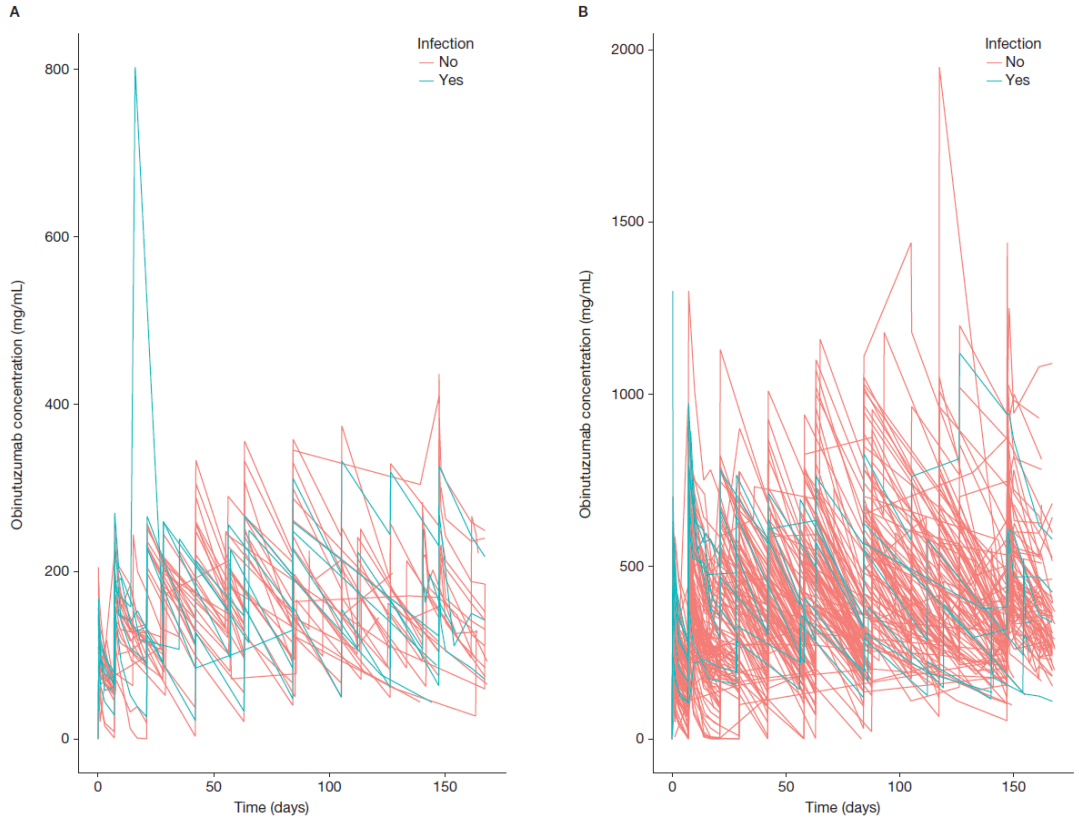
Supplementary Figure 1

Time course of inter-individual serum concentrations of patients participating in GAUDI and phase 2 of GAUGUIN who received obinutuzumab (A) 400/400 mg or (B) 1600/800 mg, stratified by experience of grade 3/4 neutropenia.



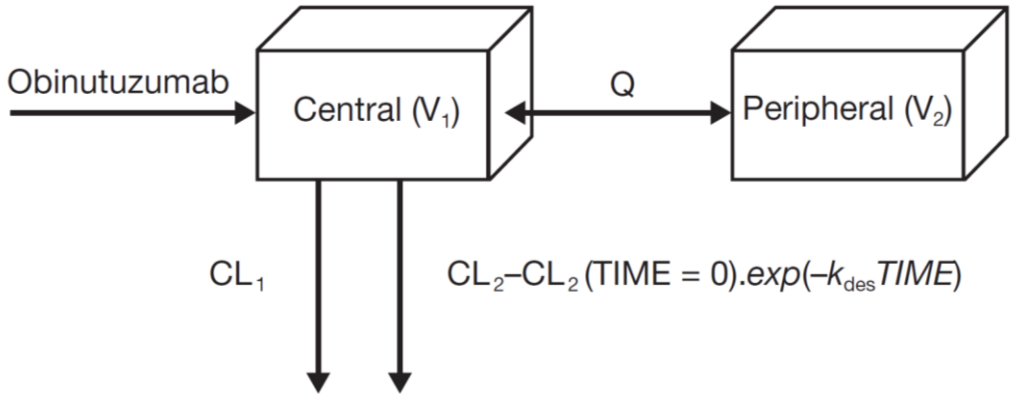
Supplementary Figure 2

Time course of inter-individual serum concentrations of patients participating in GAUDI and phase 2 of GAUGUIN who received obinutuzumab (A) 400/400 mg or (B) 1600/800 mg, stratified by experience of grade 3/4 infection.



Supplementary Figure 3

Basic structural model for obinutuzumab pharmacokinetics.

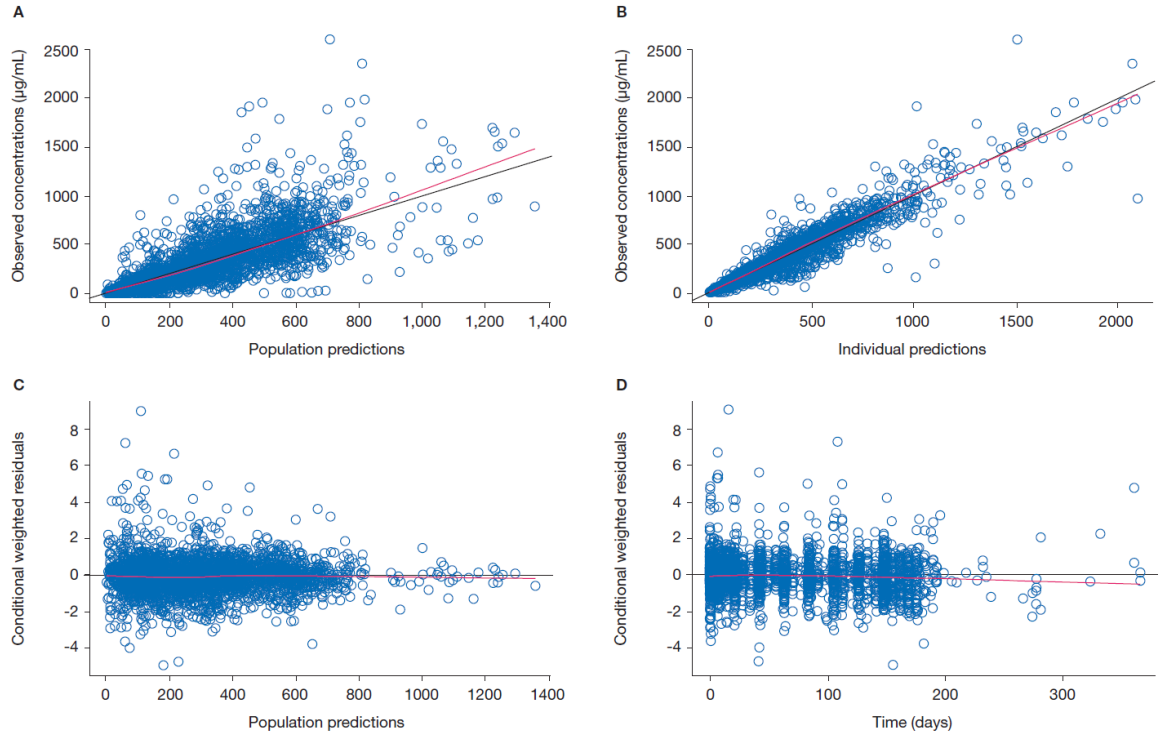


CL_1, non-specific clearance; CL_2, specific clearance; k_{des}, rate of specific clearance decay; Q, intercompartmental clearance

Supplementary Figure 4

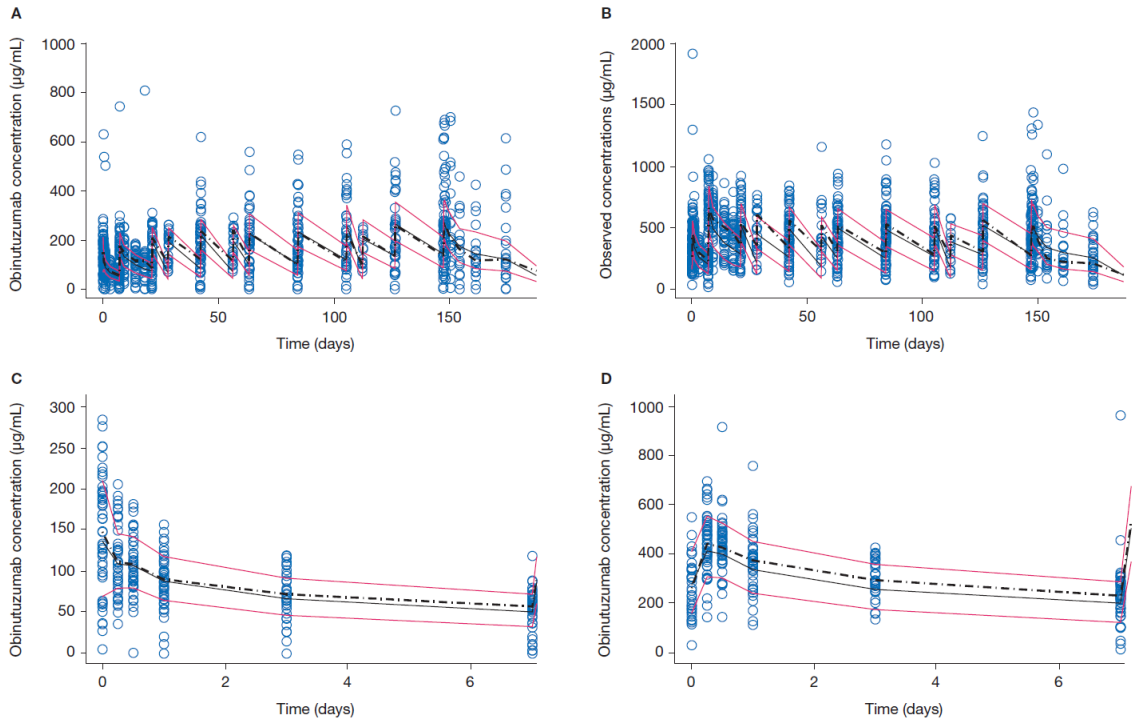
Goodness-of-fit plots for the obinutuzumab pharmacokinetic model.

(A) Population predictions versus observed concentrations of obinutuzumab. (B) Individual predictions versus observed concentrations of obinutuzumab. (C) Conditional weighted residuals versus population predictions. (D) Conditional weighted residuals versus time after first dose of obinutuzumab. The solid black line is the identity line. Smoother lines are shown in blue.



Supplementary Figure 5

Visual predictive checks for the obinutuzumab pharmacokinetic model. (A) Visual predictive check for the low dose group treated with 400 mg on cycle 1 days 1 and 8 and 400 mg on day 1 of cycles 2–8 (each cycle was 21 days in length) (*n* = 50). (B) Visual predictive check for the high dose group treated with 1600 mg on cycle 1 days 1 and 8 and 800 mg on day 1 of cycles 2–8 (*n* = 53). (C) Focus of the visual predictive check on the first dose for the low dose group (400/400 mg) (*n* = 50). (D)Focus of the visual predictive check on the first dose for the high dose group (1600/800 mg) (*n* = 53). Blue circles represent the observed data. The black and red solid lines represent the median and the 25th and 75th percentiles of the simulated data, respectively. The black dotted line represents the median of the observed data.



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