

Rituximab serum concentrations during immuno-chemotherapy of follicular lymphoma correlate with patient gender, bone marrow infiltration and clinical response

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for the Arbeitsgemeinschaft Medikamentöse Tumortherapie (AGMT) Investigators

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Online Supplementary Appendix

Design and Methods

The AGMT NHL9 phase II study included 29 adult patients (median age 53 years, range 33-74; 15 male, 14 female) with previously untreated advanced follicular lymphoma grade 1 or 2. Patients' clinical characteristics are shown in Table 1 (main text). Inclusion criteria were a positive *BCL2/IgH* rearrangement in peripheral blood (PB) and/or bone marrow (BM), clinical stage III or IV, requiring treatment with one or more of the following criteria: symptoms related to the disease, hemoglobin less than 12 g/dL, platelets less than $100 \times 10^9/L$, progressive disease, bulky tumor of more than 10 cm. Treatment consisted of 6 cycles of rituximab 375 mg/m² i.v. Day 1, mitoxantrone 10 mg/m² i.v. Day 1, and fludarabine 25 mg/m² i.v. Days 2-4 (R-FM). Cycles were repeated every 28 days. After an interval of 4-12 weeks after initiation of the 6th cycle of R-FM, patients with a complete remission (CR), unconfirmed complete remission (CRu), or partial remission (PR) received maintenance treatment with rituximab 375 mg/m² every two months for two years or until relapse (*Online Supplementary Figure S1*). The protocol was approved by the local ethics committees (IC approval 3/04 NÖ12) and informed consent was obtained from all patients. Between December 2003 and December 2007, 29 patients were enrolled. All patients were evaluated for clinical response, 17 patients for pharmacokinetics (10 of these up to maintenance cycle 6). Twenty-three patients had at least one molecular follow up for *BCL-2/IgH* response in PB (n=21) or BM (n=15) or both (n=13). Data for *BCL-2/IgH* monitoring and PK were available for 14 patients.

Rituximab pharmacokinetics

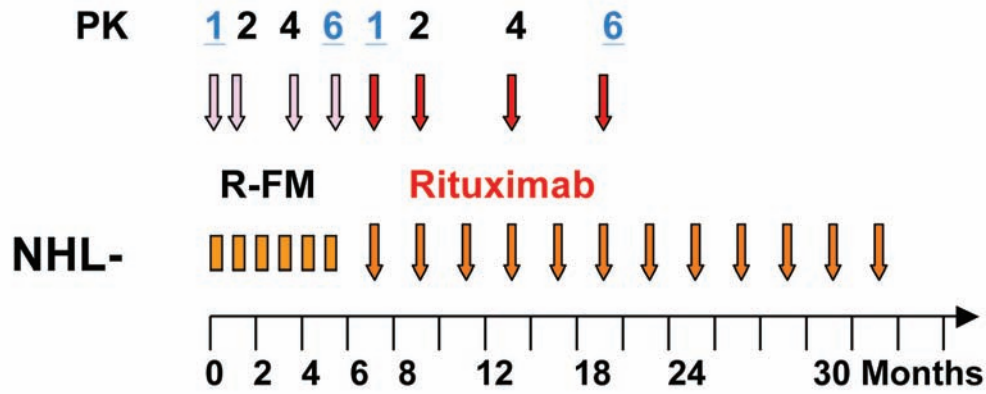
Detailed PK analysis of serum samples was performed in cycles 1 and 6 of induction as well as maintenance (before start of infusion, at the end of infusion, Days 2, 3, 4, 8, 15 and 22). Serum trough levels before start of infusion were determined in cycles 1, 2, 4, and 6 of induction as well as maintenance. The median interval between induction cycle 6 and maintenance cycle 1 was eight weeks (range 4-12 weeks; between maintenance 1 and 2: eight weeks (range 7-12); between maintenance 2 and 4: 15.5 weeks (range 8-36); between maintenance 4 and 6: 15 weeks (range 8-36).

Rituximab serum concentrations were determined by an enzyme-linked immunosorbent assay (QPS Netherlands BV, Groningen, The Netherlands). In brief, high protein affinity 96-well plates are coated with polyclonal goat anti-rituximab antibodies. Samples containing rituximab react with the coated anti-rituximab antibodies. After washing, bound rituximab is detected by incubation with goat antibody to mouse IgG F(ab')₂-conjugated to peroxidase. Bound peroxidase is detected through a chromogenic reaction with TMB. The absorbance ($A_{450} - A_{630}$ value) is proportional to the amount of rituximab present in the sample.

Online Supplementary Table S1. Detailed pharmacokinetic analysis of rituximab.

	C_{max} (mg/L)	AUC _{total} (mg/L*days)	T _{1/2} (days)	Cl (L/day)	Vd (L)
Overall population					
Induction Cycle 1 (n=16)	174 (110; 237)	1409 (154; 2939)	9.7 (1.3; 20.2)	0.53 (0.22; 4.47)	6.4 (4.2; 14.3)
Induction Cycle 6 (n=14)	249 (196; 370)	6102 (3692; 11613)	21.1 (13.5; 43.8)	0.11 (0.06; 0.18)	3.7 (1.9; 5.1)
Maintenance Cycle 1 (n=15)	240 (137; 365)	5736 (1540; 12025)	23.3 (5.9; 54.7)	0.12 (0.06; 0.41)	3.9 (2.3; 6.5)
Maintenance Cycle 2 (n=7)	242 (173; 271)	4585 (2872; 7395)	23.5 (13.1; 35.3)	0.14 (0.09; 0.24)	4.3 (3.3; 7.6)
Male					
Induction Cycle 1 (n=8)	149 (110; 226)	959 (154; 2182)	7.7 (1.3; 19.3)	0.84 (0.33; 4.47)	8.6 (6.3; 14.3)
Induction Cycle 6 (n=5)	249 (196; 365)	4630 (4432; 8079)	21.3 (21.1; 28.8)	0.15 (0.09; 0.18)	4.6 (2.8; 5.1)
Maintenance Cycle 1 (n=6)	207 (137; 278)	5168 (3194; 7496)	24.2 (19.7; 35.7)	0.14 (0.10; 0.22)	5.5 (3.9; 6.5)
Maintenance Cycle 2 (n=3)	198 (187; 271)	4585 (3197; 5431)	19.8 (17.0; 24.0)	0.15 (0.13; 0.24)	4.3 (3.3; 7.6)
Female					
Induction Cycle 1 (n=8)	178 (160; 237)	1830 (653; 2939)	11.5 (4.1; 20.2)	0.37 (0.22; 0.98)	5.3 (4.2; 8.7)
Induction Cycle 6 (n=9)	257 (206; 370)	6206 (3692; 11613)	19.0 (13.5; 43.8)	0.10 (0.06; 0.17)	3.4 (1.9; 4.4)
Maintenance Cycle 1 (n=9)	258 (209; 365)	6073 (1540; 12025)	23.3 (5.9; 54.7)	0.11 (0.06; 0.41)	3.5 (2.3; 4.4)
Maintenance Cycle 2 (n=4)	249 (173; 261)	4876 (2872; 7395)	25.7 (13.1; 35.3)	0.13 (0.09; 0.22)	4.3 (4.1; 5.2)
Bone marrow infiltration negative					
Induction Cycle 1 (n=7)	191 (156; 237)	1550 (809; 2939)	10.4 (5.0; 20.2)	0.46 (0.22; 1.02)	6.3 (4.2; 9.8)
Induction Cycle 6 (n=5)	287 (210; 370)	6879 (4137; 11613)	21.1 (13.5; 43.8)	0.09 (0.06; 0.14)	2.8 (1.9; 3.9)
Maintenance Cycle 1 (n=7)	240 (137; 365)	7086 (3194; 12025)	27.8 (21.0; 54.7)	0.10 (0.06; 0.22)	4.0 (2.3; 6.5)
Maintenance Cycle 2 (n=2)	256 (242; 271)	4997 (4564; 5431)	20.3 (17.0; 23.5)	0.13 (0.13; 0.13)	3.7 (3.3; 4.1)
Bone marrow infiltration positive					
Induction Cycle 1 (n=9)	160 (110; 205)	1267 (154; 2022)	9.0 (1.3; 19.3)	0.55 (0.32; 4.47)	7.9 (5.0; 14.3)
Induction Cycle 6 (n=9)	249 (196; 285)	4630 (3692; 8539)	21.2 (16.6; 32.1)	0.15 (0.07; 0.18)	4.4 (2.9; 5.1)
Maintenance Cycle 1 (n=8)	244 (186; 301)	5196 (1540; 10677)	20.4 (5.9; 37.8)	0.14 (0.06; 0.41)	3.6 (3.2; 5.6)
Maintenance Cycle 2 (n=5)	198 (173; 261)	4585 (2872; 7395)	24.0 (13.1; 35.3)	0.15 (0.09; 0.24)	4.3 (4.3; 7.6)

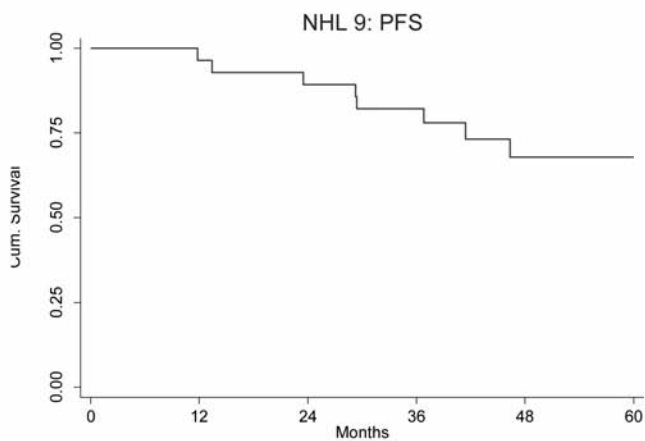
C_{max}: maximum concentration; *AUC_{total}*: area under the curve; *T_{1/2}*: serum half life; *Cl*: clearance; *Vd*: volume of distribution.



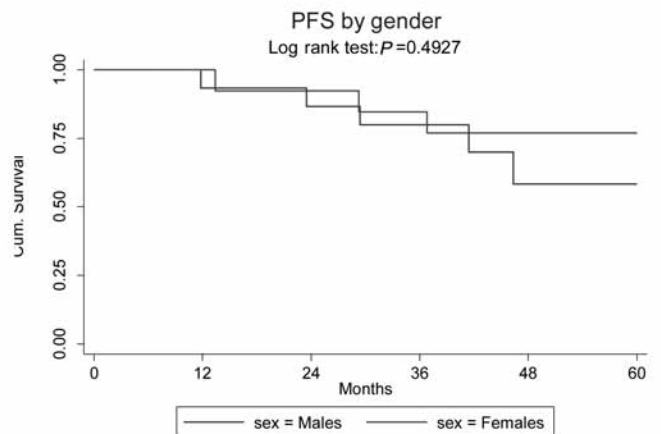
Complete PK: Cycles 1 and 6 of R-FM induction; cycles 1 and 6 of R maintenance
 Serum trough levels: Before cycles 1, 2, 4, 6 of R-FM induction; cycles 1, 2, 4, 6 of R

Online Supplementary Figure S1. NHL9 study scheme.

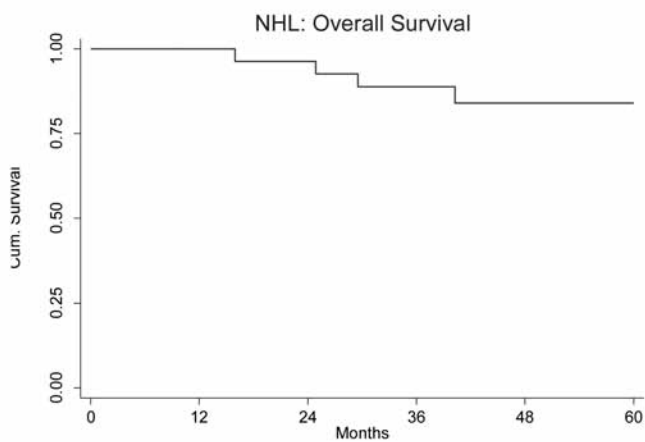
A Progression free survival of all patients



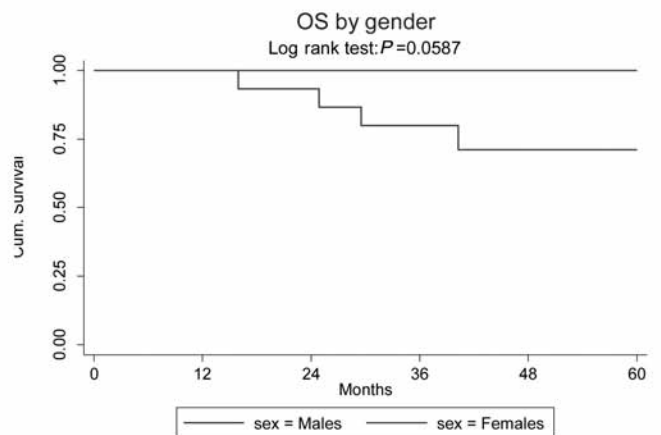
C Progression free survival by gender



B Overall survival of all patients



D Overall survival by gender



Online Supplementary Figure S2. Survival analysis of all 29 patients.