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ORIGINAL ARTICLE

Impact of Fc gamma-receptor polymorphisms on the response to rituximab treatment in children and adolescents with mature B cell lymphoma/leukemia

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Abstract Recent studies in adult lymphoma patients have indicated a correlation between polymorphisms of Fc gamma-receptors (FcγRs, encoded by the respective FCGR genes) and the response to rituximab treatment. In vitro, cells expressing FcγRIIIa-158V mediate antibody-dependent cellular cytotoxicity (ADCC) more efficiently than cells expressing FcγRIIIa-158F. The impact of the FCGR2A-131HR polymorphism is unclear. In this study, the FCGR polymorphisms FCGR3A-158VF and FCGR2A-131HR were analyzed in pediatric patients with mature aggressive B cell non-Hodgkin lymphoma/leukemia (B-NHL). Pediatric patients received a single dose of rituximab monotherapy. Response was evaluated on day 5 followed by standard chemotherapy for B-NHL. Among 105 evaluable patients, a response to rituximab was observed in 21 % of those homozygous for FcγRIIa-131RR (5/24) compared to 48 % of patients who were HH and HR FcγRIIa-131 allele carriers (18/34 and 21/47, respectively; $p = 0.044$). Among patients with the FCGR3A-158 polymorphism, those homozygous for the FF genotype had a significantly favorable rituximab response rate of 59 % (22/37) compared to 32 % in patients who were FcγRIIIa-158VV and FcγRIIIa-VF allele carriers (2/9 and 20/59, respectively; $p = 0.022$). A stringent phase II response evaluation of children and adolescents with B-NHL after one dose of rituximab monotherapy showed a significant association between the rituximab response rate and FCGR polymorphisms. These findings support the hypothesis that FCGR polymorphisms represent patient-specific parameters that influence the response to rituximab.

Keywords Lymphoma · Oncology · Rituximab · Fc gamma-receptor · Response

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Introduction

Current chemotherapy regimens result in event-free survival (EFS) rates of 65–100 % for pediatric and adolescent patients with mature B cell non-Hodgkin lymphoma (B-NHL) or leukemia (B-AL) [1–8]. Due to the significant acute toxicities of these treatment regimens and the poor rescue chances at the time of relapse, new treatment strategies with personalized and targeted therapies are needed. Rituximab, a chimeric monoclonal anti-CD20 immunoglobulin G1 (IgG1) antibody widely used in adult lymphoma patients, currently represents one of the most promising candidates to accomplish these goals in pediatric patients.

The efficacy of rituximab treatment in adults is influenced by the characteristics of the lymphoma cells and by those of the host [9]. According to recent studies, genetic polymorphisms of Fc gamma-receptors (FcγRs), which recognize IgGs, are a host parameter with potential prognostic value. FcγRs play an essential role in antibody-dependent cellular cytotoxicity (ADCC), by linking the rituximab-targeted

CD20-positive cells to cytotoxic cells. FcγRIIIa is expressed on natural killer (NK) cells and macrophages, the most important ADCC effector cells. A genetic polymorphism (rs396991) of the encoding gene (*FCGR3A*) results in either a valine (V) or a phenylalanine (F) at amino acid position 158 of FcγRIIIa. Human IgG1 binds more strongly to homozygous FcγRIIIa-158VV NK cells than to homozygous FcγRIIIa-158FF or heterozygous FcγRIIIa-158VF NK cells in vitro [10, 11]. Accordingly, an association between FcγRIIIa-158V and favorable rituximab response compared to FcγRIIIa-158F has been identified in some clinical trials [12–18], although not in others [19–27]. Moreover, the polymorphism (rs1801274) of *FCGR2A*, expressed on neutrophils and mononuclear phagocytes, in which either a histidine (H) or arginine (R) at amino acid position 131 of FcγRIIa has been analyzed for its prognostic impact on rituximab response. Association of rituximab response with FCGR2A-131 could be shown in two trials, while other clinical trials failed to show an association with response to treatment [16, 20–22, 24, 26, 28, 29].

Besides the FCGR genotype, biology of the disease, administration of rituximab as monotherapy or combined with chemotherapy, pre-treatment of patients, time and method of response, and outcome evaluation, impact the evaluated rituximab response. Rituximab serum levels and thus the efficacy of this drug are also influenced by tumor burden and antigenic mass [9].

Taken together, in vitro and in vivo data indicate that FCGR2A-158 and FCGR3A-131 polymorphisms influence the therapeutic activity of rituximab [30, 31]. The objective of this study was to evaluate the prognostic implications of FCGR2A-158 and FCGR3A-131 polymorphisms in pediatric and adolescent patients with newly diagnosed mature B-NHL treated in a phase II clinical trial in which a single dose of rituximab was administered 5 days prior to standardized response evaluation and the start of standard chemotherapy. The advantage of the study was its well-standardized clinical setting, which allowed systematic investigations with potential implications for future treatment. The confirmation of FCGR polymorphisms as a relevant patient-specific prognostic parameter for rituximab response could allow patient-specific dosing schedules of rituximab in a personalized medicine approach to treatment.

Methods

Patients, treatment, and response evaluation

All analyzed patients were enrolled after written informed consent for participation in the B-NHL BFM (Berlin-Frankfurt-Muenster) rituximab trial [32, 33]. This was obtained from the parents of the patients or from their legal guardians. Eligibility criteria were newly diagnosed CD20-positive mature B-NHL or B-AL, age below 19 years, and at least one index lesion available for response evaluation within 24 h prior to rituximab administration. Material for FCGR genotyping was available for 128 patients. B-NHL subtypes were classified according to the classification system of the World Health Organization and reviewed centrally. Initial staging was performed according to the St. Jude staging system [34]. Studies associated with the B-NHL BFM rituximab trial were approved by the Ethical Board of the Justus Liebig University, Giessen, Germany. The research was conducted in accordance with the Declaration of Helsinki.

Patients received one dose of rituximab, either 375 mg/m^2 (88 patients) or 700 mg/m^2 (40 patients), intravenously on day 1 of treatment. The dose of rituximab was escalated from 375 to 700 mg/m^2 after an interim analysis of the phase II trial [33, 32]. For initial staging, the most appropriate imaging method was used, either ultrasound, magnetic resonance imaging, or computed tomography. Within 24 h before starting rituximab administration, at least one index lesion was identified and then measured by determining the product of the two largest perpendicular diameters. In addition, the ratio of blasts in bone marrow (BM) to those in peripheral blood (PB) was determined. Re-staging after rituximab treatment was performed with identical imaging methods and instrument settings and by the same investigator on day 5 and prior to the beginning of chemotherapy. In patients with initial BM/PB blasts, this ratio was re-examined as well. Evaluation records were reviewed centrally to assess the rituximab response. Patients with stable or progressive disease were classified as non-responders, defined as a <25 % reduction of the tumor or disease progression. For the early detection of tumor lysis syndrome (TLS), serum lactate dehydrogenase (LDH) levels were evaluated prior to and 4, 8, 12, 16, and 20 h after the start of rituximab infusion and on days 2, 3, 4, 5, and 10. Following this window, the patients were treated according to the B-NHL BFM 04 trial. Patients did not receive any form of chemotherapy and/or corticosteroids during the window, except for patients with central nervous system (CNS) disease, who received intrathecal therapy on days 1 and 3. Patients requiring corticosteroid therapy during the rituximab window (due to anaphylactic reactions to rituximab) were excluded from the response analyses.

Determination of FCGR2A-131 and FCGR3A-158 genotypes

PB and BM samples with sufficient quality and quantity were available for 128 patients. Mononuclear cells were isolated from PB or BM with Lymphoprep (Axis Shield, Oslo, Norway) using standard protocols. Genomic DNA was isolated from mononuclear cells using the QIAmp DNA blood mini-kit (Qiagen, Hilden, Germany). FCGR3A-158VF

(rs396991) was genotyped using a Taqman-based assay for rs396991 (Life Technologies, Darmstadt, Germany). The probe specific for the V allele was labeled with VIC, and the probe for the F allele with FAM. Genotyping was carried out using an Applied Biosystems 7500 real-time PCR system. Allelic discrimination was performed using SDS software v1.4 (Life Technologies).

The FCGR2A-131HR polymorphism (rs1801274) was analyzed according to a PCR-based approach. PCR amplification was carried out using the following oligonucleotides: 5′-GGAAAATCCCAGAAATTCTCGC-3′ (forward) and 5′-CAACAGCCTGACTACCTATTACGCGGG-3′ (reverse) [35]. The forward primer introduced a *Bst*UI restriction site if the nucleotide at the polymorphic site was G. The reverse primer introduced a *Bst*UI site in all PCR products. PCR was performed with 200 ng of DNA, 1 unit (U) of AccuPrime *Taq* high fidelity DNA polymerase (Life Technologies), 10 pmol of each primer, and 1× AccuPrime PCR buffer II in a volume of 50 μl and standard amplification conditions. The PCR products were purified using the Illustra GFX PCR DNA and gel band purification kit (GE Healthcare, Freiburg, Germany) and digested with 20 U BstUI (New England Biolabs, Frankfurt, Germany) overnight at 60 °C. Samples were analyzed on 3 % agarose gels and visualized by ethidium bromide staining. Subgroups of FCGR2A-131 and FCGR3A-158 genotypes were evaluated according to tumor histology and risk group. B-NHL histology was classified as Burkitt's lymphoma or leukemia (BL, B-AL) or diffuse large B cell lymphoma (DLBCL). Risk group was dichotomized as low-risk (R2 patients) or high-risk (R3/R4 patients). The R2 risk group was defined by the absence of CNS and BM involvement and an initial LDH serum level < 500 U/l. The higher risk groups, R3 and R4, consisted of patients with stage III or IV disease and an initial LDH level > 500 U/l.

Statistical analyses

Associations of FCGR genotypes with rituximab response, B-NHL subtypes, involved compartments and LDH level were analyzed using the χ^2 test or Fisher's exact test. Cumulative incidences of relapse were calculated according to Kalbfleisch and Prentice [36]. The association of FCGR2A-131 and FCGR3A-158 genotypes with rituximab response was calculated using the Cochran-Armitage trend test for analyzing the association of two variables, the second of them with more than two categories. This modified Pearson χ^2 test is able to incorporate a suspected ordering in the effects of the categories of the second variable [37]. The areas under the curves (AUCs) of the logarithmic values of the individual changes in LDH serum level compared to the initial LDH level were also determined. The values were calculated with the rectangle method. Missing values were replaced by the previous value (last value carried forward) or, in case only one value was missing, by the mean of the neighboring values. Data were updated as of July 2015.

Results

Material for genotyping was evaluable in 128 pediatric B-NHL patients with the FCGR2A-131 polymorphism and in 127 pediatric patients with the FCGR3A-158 polymorphism. The patients characteristics are summarized in Table 1. Their comparison according to FCGR status revealed no significant associations except for a higher proportion of female patients in the FCGR2A-131RR subgroup (Table 1). The distribution of FCGR genotypes was similar to that described for other populations of patients with lymphoma [38].

Clinical response

After the exclusion of 23 patients whose response evaluation was not according to protocol guidelines or who received steroids during the 5-day window, a total of 105 patients with evaluable response data and known FCGR status were available for analyses of the prognostic impact of FCGR polymorphisms on rituximab response. The response rate was better in patients with the low-affinity FCGR3A-158FF genotype than in patients carrying the high-affinity valine allele. Patients with the former genotype had a response rate of 59 % (22/37) compared to 22 % for patients homozygous for the valine isotypes (2/9) and 33.9 % for heterozygous patients (20/59 patients; $p = 0.02$) (Table 1). Additional parameters with potential impact on rituximab response were analyzed within each patient subgroup defined by their FCGR3A genotype. FCGR3A-158VF patients had a significantly better response rate when the fluid compartment rather than solid lymphoma was used for response evaluation ($p = 0.0005$). Neither histological B-NHL subtype nor rituximab dose was significantly associated with rituximab response in these subgroups (Table 2).

In addition to FCGR3A, we analyzed the prognostic impact of FCGR2A genotype status. A response to rituximab was observed in 21 % of patients with homozygous for FCGR2A-131RR compared to 53 % and 45 % of the FCGR2A-131 histidine allele carriers (HH and HR respectively; $p = 0.04$). Superior rituximab response rates were significantly associated with the R3/R4 risk group of FCGR2A-131HR patients [65 % (13/20) vs. 31 % (8/26) in the R2 risk group; $p = 0.04$] and

Table 1 Patient characteristics according to Fc gamma-receptor IIIa and IIa polymorphisms

Fc gamma-receptor IIIa polymorphisms		FF (n = 45)		VF (n = 69)		VV (n = 13)		p value
		n	%	n	%	n	%	
Gender	Female	7	15.6	15	21.7	3	23.1	0.68246
	Male	38	84.4	54	78.3	10	76.9	
Age (years)	<10	25	55.6	38	55.1	4	30.8	0.42844
	10–14	15	33.3	20	29.0	7	53.8	
	≥15	5	11.1	11	15.9	2	15.4	
Stage	I	4	8.9	2	2.9	–	–	0.88133
	II	10	22.2	16	23.5	4	33.3	
	III	19	42.2	32	47.1	4	33.3	
	IV CNS−	3	6.7	2	2.9	1	8.3	
	IV CNS+	1	2.2	3	4.4	–	–	
	B-AL CNS−	3	6.7	7	10.3	1	8.3	
	B-AL CNS+	5	11.1	6	8.8	2	16.7	
LDH (U/l)	<500	28	62.2	35	50.7	8	61.5	0.29671
	500–<1000	5	11.1	14	20.3	–	–	
	≥1000	12	26.7	20	29.0	5	38.5	
Risk group	R2	27	60.0	31	45.6	6	46.2	0.64994
	R3	4	8.9	11	16.2	2	15.4	
	R4	14	31.1	24	35.3	5	38.5	
	PMLBL (LDH <500 U/l)	–	–	2	2.9	–	–	
Histology	BL/B-AL	34	77.3	55	84.6	10	76.9	0.69422
	DLBCL	8	18.2	9	13.8	3	23.1	
	Mature B-NHL, n.f.c.	2	4.5	1	1.5	–	–	
Rituximab response	Responder	22	59.5	20	33.9	2	22.2	0.02161*
	Non-responder	15	40.5	39	66.1	7	77.8	
Cumulative incidence of NR/relapse		0.022 ± 0.022		0.116 ± 0.039		0.000 ± 0.000		0.10#
Fc gamma-receptor IIa polymorphisms		HH (n = 41)		HR (n = 61)		RR (n = 26)		p value
		n	%	n	%	n	%	
Gender	Female	6	14.6	9	14.8	10	38.5	0.02424
	Male	35	85.4	52	85.2	16	61.5	
Age (years)	<10	21	51.2	31	50.8	16	61.5	0.81462
	10–14	15	36.6	21	34.4	6	23.1	
	≥15	5	12.2	9	14.8	4	15.4	
Stage	I	3	7.5	2	3.3	1	3.8	0.99341
	II	8	20.0	17	28.3	5	19.2	
	III	17	42.5	26	43.3	13	50.0	
	IV CNS−	2	5.0	3	5.0	1	3.8	
	IV CNS+	2	5.0	1	1.7	1	3.8	
	B-AL CNS−	4	10.0	5	8.3	2	7.7	
	B-AL CNS+	4	10.0	6	10.0	3	11.5	
LDH (U/l)	<500	23	56.1	34	55.7	14	53.8	0.68927
	500–<1000	6	14.6	7	11.5	6	23.1	
	≥1000	12	29.3	20	32.8	6	23.1	
Risk group	R2	20	48.8	31	51.7	13	50.0	0.87108
	R3	6	14.6	7	11.7	4	15.4	
	R4	14	34.1	22	36.7	8	30.8	
	PMLBL (LDH <500 U/l)	1	2.4	–	–	1	3.8	
Histology	BL/B-AL	34	78.2	44	74.6	22	88.0	0.43147
	DLBCL	4	10.3	13	22.0	3	12.0	
	Mature B-NHL, n.f.c.	1	2.6	2	3.4	–	–	
Rituximab response	Responder	18	52.9	21	44.7	5	20.8	0.04444*
	Non-responder	16	47.1	26	55.3	19	79.2	
Cumulative incidence of NR/relapse		0.073 ± 0.041		0.082 ± 0.035		0.038 ± 0.038		0.77#

Presented are absolute numbers and rates for patients fulfilling certain criteria as well as p (χ^2) values evaluating the differences. The given data refer to patients with successful investigation of the respective criterion

CNS central nervous system, *B-AL* Burkitt (L3) leukemia, *PMLBL* primary mediastinal large B cell lymphoma, *DLBCL* diffuse large B cell lymphoma, *n.f.c.* not further classified, *cum.* cumulative, *NR* non-response

*Only evaluable patients are analyzed using the Cochran-Armitage trend test

The p value is calculated according to Gray

fluid lymphoma compartment of FCGR2A-131HH patients. A response rates of 100 % (8/8) within the BM compartment contrasted with the 38 % (10/26) response rate of nodal lesions (p = 0.003) (Table 3).

Table 2 Prognostic parameters for rituximab response according to FCGR3A genotype in 105 patients evaluable for rituximab response on day 5

	FF (n = 37)			VF (n = 59)			VV (n = 9)		
	Responders	Non-responders		Responders	Non-Responder		Responders	Non-responders	
	n	n	p value*	n	n	p value*	n	n	p value*
Histology									
BL/B-AL	17	12		18	28		2	5	
DLBCL	4	2	1.00	1	8	0.14	0	2	1.00
Risk group									
R2	12	10		6	22		1	4	
R3/R4	10	5	0.51	14	15	0.05	1	3	1.00
Evaluated compartment									
Solid lymphoma manifestation	18	14		11	37		1	7	
Bone marrow	4	1	0.63	9	2	0.0005	1	0	0.22
Gender									
Male	19	11		18	28		1	5	
Female	3	4	0.41	2	11	0.18	1	2	1.00
Rituximab dose (mg/m^2)									
375	17	11		14	25		1	6	
700	5	4	1.00	6	14	0.77	1	1	0.42

If both compartments (nodal lesions and bone marrow) were evaluated, the one showing the better response was considered

*The p value was calculated according to Fisher's test

Association of rituximab response and LDH serum level changes

For the early detection of TLS, serum LDH levels were closely monitored, with control carried out at 4 h intervals on the day of rituximab infusion followed by daily controls until day 5. Follow-up data on serum LDH levels were available for 119 patients, with 104 also evaluable for rituximab response. In 34 out of 119 patients (29 %), follow-up serum LDH levels did not exceed the individual upper normal limit until day 5. In the remaining 85 patients, the maximum LDH level was reached between 4 h and 5 days following rituximab infusion. Since the majority of LDH changes occurred within 48 h, this 48 h interval was analyzed in greater detail. Changes in the LDH serum level compared to the initial LDH level were quantified by calculating the AUC of all 96 patients for whom initial LDH levels and follow-up levels on day 2 were available, together with a sufficient rituximab response evaluation on day 5 and FCGR genotyping. Serum LDH changes were more prominent in patients who responded to rituximab than in rituximab non-responders (Table 4). This difference was seen in all genetic subgroups and reached statistical significance in FCGR3A-158VF, FCGR2A-131HR, and FCGR2A-131-HH patients.

The changes in LDH serum level according to rituximab response within the subgroup of 36 patients with FCGR3A-158FF genotype is exemplarily shown in Fig. 1, which compares the LDH changes to both the initial LDH levels, prior to the start of rituximab infusion (Fig. 1**a**, **b**), and the individual upper normal limit of each patient (Fig. 1**c**, **d**).

Multivariate analysis was carried out with the co-variables rituximab dose, evaluated compartment, disease extent, FCGR2A-131 and FCGR3A-158 genotypes, and quantitative changes in serum LDH levels. FCGR2A-131 and FCGR3A-158 genotypes and changes in serum LDH levels remained statistically significant prognostic parameters for rituximab response on day 5 (Table 5).

Outcome

Among the evaluated patient cohort, nine patients suffered disease relapse (7 % of evaluated patients). The incidences of the FCGR3A-158 variants VV, VF, and FF for these nine patients were 0 (0 %), 8 (89 %), and 1 (11 %) compared to 13 (11 %), 61 (52 %), and 44 (37 %) in the 118 patients without relapse, respectively as illustrated in Fig. 2. The VF polymorphism was not evaluable in one patient without relapse. The RR, HR, and HH FCGR2A-131 genotypes were detected in 1 (11 %), 5 (56 %), and 3 (33 %) of patients with disease relapse compared to 25 (21 %), 56 (47 %), and 38 (32 %) of the 119 patients without relapse. The cumulative incidences of relapse were similar for all genetic subgroups.

Table 3 Prognostic parameters for rituximab response according to FCGR2A genotype in 105 patients evaluable for rituximab response on day 5

	RR (n = 24)			HR (n = 47)			HH (n = 34)		
	Responders	Non-responders		Responders	Non-responders		Responders	Non-responders	
	n	n	p value*	n	n	p value*	n	n	p value*
Histology									
BL/B-AL	4	16		17	16		16	13	
DLBCL	1	2	0.54	3	8	0.29	1	2	0.59
Risk group									
R2	3	9		8	18		8	9	
R3/R4	2	9	1.00	13	7	0.04	10	7	0.73
Evaluated compartment									
Solid lymphoma manifestation	4	17		16	25		10	16	
Bone marrow	1	2	0.52	5	1	0.08	8	0	0.003
Gender									
Male	3	11		20	19		15	14	
Female	2	8	1.00	1	7	0.06	3	2	1.00
Rituximab dose (mg/m^2)									
375	1	11		17	19		14	12	
700	4	8	0.32	4	7	0.73	4	4	1.00

If both compartments (nodal lesions and bone marrow) were evaluated, the one showing the better response was considered

*The p value was calculated according to Fisher's test

Discussion

With the goal of individualized treatment of patients with B-NHL, relevant efforts have been undertaken to elucidate the mechanisms responsible for the interindividual variability in the response to rituximab, with most evidence pointing to FCGR polymorphisms. However, according to the published data in the literature, the impact of these polymorphisms on the effectiveness of rituximab treatment is unclear [12–24]. An important question is whether data obtained in adult patients can be transferred to pediatric patients because of relevant differences in the treatment, response evaluation, outcome, and biology of the disease between the two age groups. In fact, there are as yet no data on the role of FCGR polymorphisms in the rituximab response of children and adolescents with B-NHL.

Table 4 Quantitative lactate dehydrogenase (LDH) changes until day 2 in 96 patients with available follow-up LDH levels and FCGR3A and FCGR2A genotyping

Genotype	Response	n	Median AUC	First quartile AUC	Third quartile AUC	p value
Discrimination according to FCGR3A genotype						
FF	Responder	20	3.37	−0.4	13.1	0.5992
FF	Non-responder	12	1.26	0.7	4.0	
VF	Responder	19	5.80	2.1	11.6	0.0007
VF	Non-responder	37	1.02	−0.1	2.7	
VV	Responder	2	7.72	−2.0	17.4	0.8676
VV	Non-responder	6	−0.20	−1.5	0.4	
Discrimination according to FCGR2A genotype						
RR	Responder	4	10.78	0.7	19.6	0.3470
RR	Non-responder	17	2.71	0.3	6.5	
HR	Responder	19	4.73	0.1	12.5	0.0157
HR	Non-responder	25	1.06	0.3	1.8	
HH	Responder	18	4.28	−0.2	11.6	0.0192
HH	Non-responder	13	−0.01	−0.7	0.5	

AUC defines the area under the curve

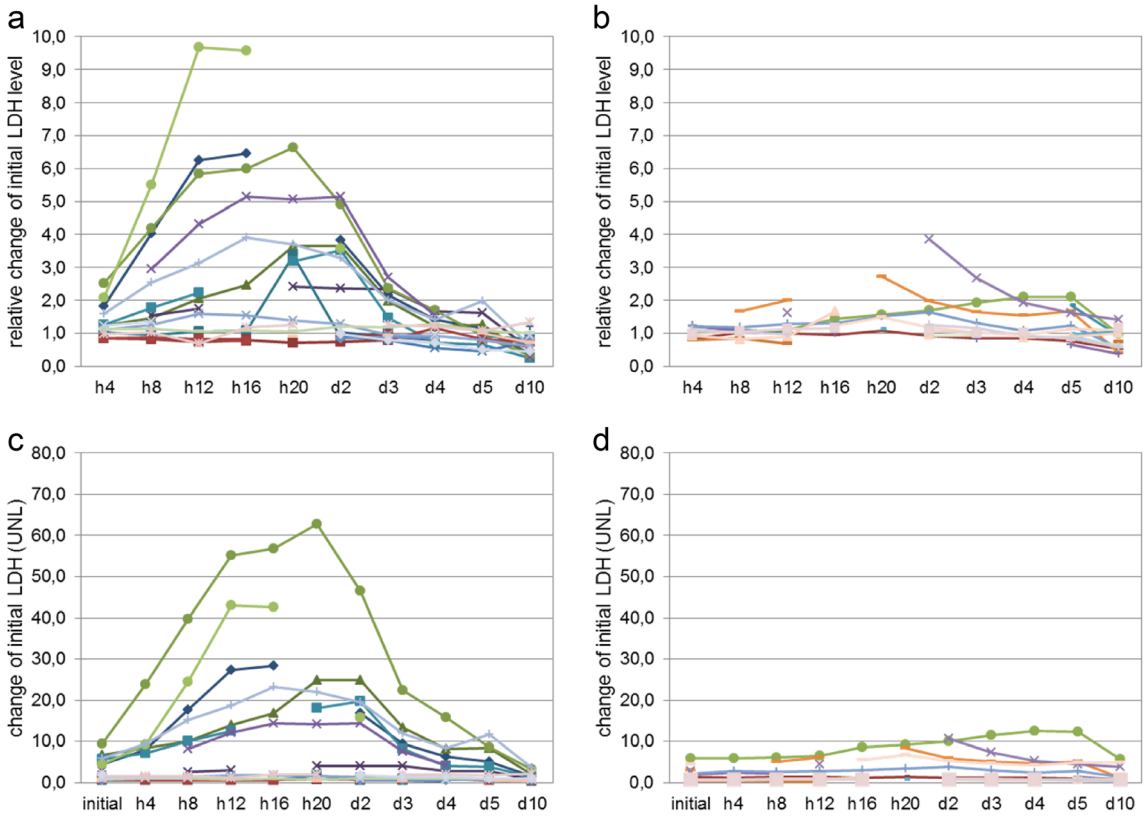

Fig. 1 Changes in serum LDH levels exemplarily shown for patients with the FCGR3A-158FF genotype, available initial and follow-up LDH levels, and sufficient rituximab response evaluation on day 5. Shown are the changes in the LDH serum levels compared to the initial LDH level in rituximab responders (**a**) and non-responders (**b**) and according to the individual upper normal limit (UNL) in rituximab responders (**c**) and non-responders (**d**). The hours (*h*) or days (*d*) after the start of rituximab infusion are indicated

The rituximab window-trial, described herein, was conducted by the NHL-BFM group with the aim of systematically investigating the rituximab response in pediatric mature B-NHL patients. The major strengths of the study were the large number of patients with uniform treatment and the stringent response evaluation after the rituximab window. All patients in the study were simultaneously enrolled in a phase II trial with the endpoint rituximab response, the response evaluation of our study was highly standardized concerning timelines and procedure. All cases were centrally reviewed, with the strict exclusion of patients in case of protocol violations. Almost 130 of the patients enrolled in the trial were genotyped for FCGR2A and FCGR3A status, which was then correlated with clinical characteristics, rituximab response, and treatment outcome.

Patient characteristics were similar among the subgroups defined by FCGR2A and FCGR3A genotypes, indicating no association of specific genotypes with specific patient characteristics. However, the rituximab response rates of the genetic subgroups significantly differed, with a much higher response rate in patients with the FCGR2A-131HH and FCGR2A-131HR genotypes than in those with the RR genotype. This finding is in line with the clinical observations of two other trials [13, 28], although seven clinical trials failed to show an association with response to treatment [16, 18–22, 24]. The homozygous low-affinity FCGR3A-158FF genotype was significantly associated with a favorable rituximab response compared to patients carrying the high-affinity valine allele. This is in contrast to the clinical observations reported for adult lymphoma patients, in whom either no prognostic impact or a lack of a favorable response was determined in carriers of the valine allele [12, 13, 15–22, 24, 25, 27, 38]. An association of the FCGR3A-158FF genotype with unanticipated clinical findings was noted in three other studies: Two of those studies, on indolent and aggressive lymphoma or chronic lymphocytic leukemia (CLL), reported a trend towards a favorable rituximab response and/or outcome in FCGR3A-158FF patients [20, 23]. The third study analyzed a series of 24 lymphoma patients and found that the FCGR3A-158FF genotype correlated significantly with lower immunoglobulin levels after autologous stem cell transplantation and adjuvant rituximab [39]. The precise mechanisms underlying these lower immunoglobulin levels are unknown.

Capuano and colleagues showed that NK cell interaction with Rituximab opsonized target cells resulted in a downmodulation of FcγRIIIa. Furthermore the ability of NK cells to kill target cells was impaired after the interaction with the rituximab opsonized target cells [40]. In this context the

Table 5 Multivariate analysis of the prognostic parameters for rituximab response

	Odds ratio	Lower limit 95 % CI	Upper limit 95 % CI	p (X^2)
R3/R4	2.1	0.5	8.4	0.2885
Dose 700 mg/m^2	0.7	0.2	2.3	0.5768
FCGR2A	3.9	1.7	9.2	0.0015
FCGR3A	3.2	1.2	8.3	0.0180
LDH change*	0.8	0.7	0.9	0.0078
BM evaluation	0.2	0.03	1.3	0.0961

*LDH changes as area under the curve until day 2

CI confidence interval, *BM* bone marrow, *R3/R4* risk group R3 and risk group R4 including patients with stage III/IV disease and initial LDH serum level > 500 U/L, *FCGR2A* the gene encoding the Fc gamma-receptor IIa, *FCGR3A* the gene encoding the Fc gamma-receptor IIIa

low-affinity homozygous FcγRIIIa-F receptor may contribute to a more favorable NK cell responsiveness compared to the FcγRIIIa-158VF or VV receptors.

In terms of the prognostic impact of FCGR polymorphisms in the context of lymphoma biology and disease extent, the response rates of patients homozygous for the FCGR3A-158FF genotype were favorable throughout all analyzed subgroups with no significant differences according to lymphoma histology, risk group stratification, and analyzed compartment (Table 2).

Our analyses also indicated a potential prognostic impact of FCGR2A-131 polymorphisms on rituximab response. In the group of favorably responding patients with FCGR2A-131HH genotypes, a good rituximab response was achieved in those with a BM response. This is in line with earlier reports of a faster and superior response in fluid compartments compared to solid lymphoma [33, 41]. Increasing the dose of rituximab from 375 mg/m^2 to 700 mg/m^2 did not enhance the rituximab response rates, except in FCGR2A-131RR patients. Larger series will be necessary to evaluate the role of the rituximab dose in the context of FCGR2A-131 and FCGR3A-158 polymorphisms. The small number of patients with disease relapse (n = 9) precluded detailed outcome analyses according to genetic subgroups; thus, larger patient series are required here as well. Therefore, the current analyses will be continued in the upcoming clinical trial of the NHL-BFM group.

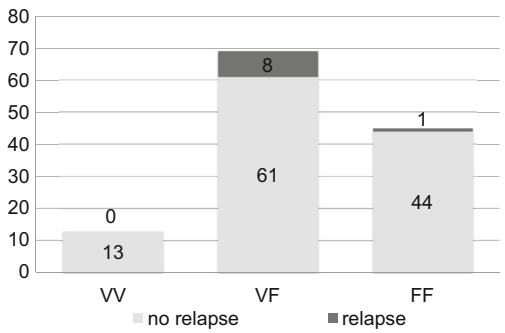

Fig. 2 Outcome of the evaluable patients according to FCGR3A-158 genotypes illustrated in absolute numbers of patients. Shown is the distribution of relapsed and non-relapsed patients according to FCGR3A-158-genotype. The number of patients with relapse is indicated in *dark gray*, the number of patients without relapse is shown in *light gray*

Analysis of early changes in LDH serum levels revealed larger increases in patients with a clinical response to rituximab than in non-responders. Thus, a sufficient rituximab response might correlate with cell lysis and the liberation of cytoplasmic LDH.

Other relevant mechanisms of action of monoclonal antibodies besides ADCC are complement-dependent cytotoxicity (CDC), phagocytosis, and direct apoptosis induction [42]. As a type I anti-CD20 antibody, rituximab demonstrates efficient phagocytosis, ADCC, and CDC but it does not directly induce apoptosis. This contrasts with type II antibodies, which strongly induce apoptosis but only weakly activate CDC [42]. Thus, the most important mechanisms of action of rituximab seem to be ADCC and CDC, while a direct induction of apoptosis is probably negligible. None of the available parameters in the current study allowed the observed response to be attributed to CDC vs. ADCC. But in line with the hypothesis that CDC occurs immediately after the start of rituximab therapy while ADCC requires a certain latency period, the observed kinetics of the LDH levels, as an indirect parameter of tumor cell lysis, support ADCC as the relevant mechanism of rituximab response in patients with newly diagnosed B-NHL. However, the experimental setup of this study might also have measured mechanisms other than ADCC.

Our results show that a detailed evaluation of host genotype characteristics in combination with disease parameters, such as histology, disease extent, and/or involved compartments, can identify subgroups likely to exhibit a specific response to rituximab. Analyses of larger patient series are necessary to allow correlation with disease outcome and to achieve the long-term goal of personalized treatment, by modifying rituximab administration according to the individual response profile.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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