FcγRIIIA and FcγRIIA polymorphisms do not predict clinical outcome of follicular non-Hodgkin's lymphoma patients treated with sequential CHOP and rituximab

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ABSTRACT

We analyzed FcγRIIIA-158V/F and FcγRIIA-131H/R polymorphisms in a cohort of 94 newly diagnosed follicular lymphoma (FL) patients sequentially treated with CHOP and Rituximab. With a median follow-up of 5.8 years, the overall survival at 8 years is 83%. Univariate and multivariate analysis showed no correlation between FcγRIIIA-158VV/VF and FcγRIIA-131HH/HR polymorphisms and the overall response rate, the molecular response and the event-free survival obtained after CHOP and Rituximab. By contrast, the achievement of a durable molecular clearance of BCL2/IgH$^+$ cells detectable in the bone marrow is confirmed to be a reliable predictive factor of a better long-term clinical outcome.

Key words: FcγR polymorphisms, follicular lymphoma, BCL2/IgH rearrangements.

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The chimeric anti-CD20 monoclonal antibody Rituximab is nowadays routinely used either as single agent or in combination with chemotherapy for the treatment of non-Hodgkin's lymphomas (NHL). In particular, the addition of Rituximab to chemotherapy significantly improves the clinical outcome in previously untreated follicular lymphoma (FL) patients.[1,2] The precise biological mechanism is still controversial, but several *in vitro* and *in vivo* studies have shown that Rituximab mediates its anti-lymphoma effect by complement-dependent cytotoxicity (CDC)[3] antibody-dependent cellular cytotoxicity (ADCC)[4] and, also possibly, through the induction of apoptosis.[5] In ADCC, natural killer (NK) cells, neutrophils and macrophages bind antibody-coated cells by receptors that recognize the constant region of immunoglobulin (Fc γ receptors, FcγRs). A genomic polymorphism at amino acid 158 of FcγRIIIA has been described whereby the presence of Valine (V) rather than Phenylalanine (F) leads to higher binding affinity to immunoglobulin G (IgG). A polymorphism at position 131 of FcγRIIA has also been described (Histidine/Arginine, H/R). The FcγRIIIA-VV and FcγRIIA-HH genotypes have been associated with a better clinical and molecular response in FL patients treated as first line therapy with Rituximab alone[6,7] and in patients with diffuse large B-cell lymphoma (DLBCL) treated with the concomitant administration of Rituximab and CHOP (R-CHOP).[8] However, this is not the case in patients with FL treated with R-CHOP[9] or in B-cell chronic lymphocytic leukemia (B-CLL) patients.[10] We investigated the influence of FcγRIIIA and FcγRIIA polymorphisms in a cohort of 94 FL patients sequentially treated with CHOP and Rituximab in order to evaluate the effect of these polymorphisms on the clinical and molecular response to treatment, and on the overall outcome.

Design and Methods

Patients

Ninety-four previously untreated patient from six different Italian centers were sequentially treated with 6 cycles of CHOP chemotherapy (cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, vincristine 1.4 mg/m^2, and prednisone 100 mg/m^2) and 4 weekly infusions of Rituximab. At diagno-

Table. 1. Predictive factors of clinical CR after sequential CHOP and Rituximab

	Univariate		*Multivariate* (Logistic analysis)*	
Variable	*CR (%)*	*p value (cz2)*	*O.R. (95% CI)*	*p value*
Sex				
M	39/55 (71)	0.92	0.47 (0.14–1.55)	0.21
F	28/39 (72)			
Age				
≤60	56/79 (71)	0.85	1.11 (0.26 – 4.80)	0.89
>60	11/15 (73)			
B symptoms				
Yes	2/8 (25)	0.003	0.22 (0.03 – 1.62)	0.14
No	64/85 (75)			
LDH				
Elevated	6/15 (40)	0.005	0.21 (0.05 – 0.94)	0.04
Normal	58/76 (76)			
Bulky				
Yes	10/24 (42)	0.000	0.24 (0.07 – 0.81)	0.02
No	55/68 (81)			
BM infiltration (Histology)				
Yes	43/63 (68)	0.40	1.51 (0.43 – 5.36)	0.52
No	23/30 (77)			
Extra-nodal sites				
Yes	15/26 (58)	0.07	0.39 (0.12 - 1.31)	0.13
No	52/68 (76)			
Valine				
VV	14/18 (78)	0.49	0.95 (0.22 – 4.13)	0.95
VF/FF	53/76 (70)			
Histidine				
HH	20/30 (67)	0.49	1.47 (0.47 – 4.65)	0.51
HR/RR	47/64 (73)			

**88 observations were used for multivariate analysis.*

sis, 88 out of 94 patients proved positive for the rearrangement of the Bcl-2 proto-oncogene on chromosome 18 with the immunoglobulin heavy chain (IgH) region on chromosome 14 (BCL2/IgH). Among these patients, 68 had been previously enrolled in a published study designed to investigate whether the sequential infusion of CHOP and Rituximab could induce a durable eradication of Bcl2/IgH$^+$ cells from the bone marrow (BM) or peripheral blood (PB).[11] The study was conducted according to good clinical and laboratory practice rule and the principles of the Declaration of Helsinki. The local Ethics Review Committees approved the protocol at each center.

Molecular analysis

A qualitative molecular evaluation of Bcl2/IgH$^+$ cells was performed on 68/94 BM samples 1 year after the end of Rituximab administration as previously described.[11] Genotyping of FcγRIIIA and FcγRIIA polymorphisms was performed on 94 BM or PB samples amplifying 100 ng of DNA on a Real-Time platform (LightCycler, Roche Molecular Biochemicals, Mannheim, Germany), in 20 μL with 1X LightCycler DNA Master Hybridization Probes buffer (Roche) for 50 cycles (1" at 95°C, 5" at 62°C, 7" at 72°C) and subsequent melting. The 3' fluorescent labeled sensor probes and 5' RED 640 labeled anchor probes (TIB MOLBIOL, Genoa, Italy) employed were: 5'-GGAGAAACCATCATGCTGAG-3', 5'-CATACCTTGGACAGTGATGGTC-3', 5'-GTGGGATCCAAACGGGAGAA-3', 5'- TCTGGGATTTTCCATTCTGGAAGAATGTGACC-3' for FcγRIIA and 5'-CATATTTACAGAATGGCAAAGGC-3', 5'-TGAGTGATGGTGATGTTCACAGT-3', 5'-CATTTTTACTCCCAAAAAGCCC-3', 5'-CTGCAGAAGTAGGAGCCGCTGT-3' for FcγRIIIA. Melting curve analysis was performed by LightCycler 3.5 software. The genotype was confirmed by single base sequencing on an ABI Prism 310 Genetic Analyzer (Applera, Foster City, CA, USA), with the ABI Prism SNaPshot Multiplex Kit (Applera) according to the manufacturer's instructions using 100 ng of genomic DNA and the sequencing primers 5'-GATGGAGAAGGTGGGATCCAAA-3' for FcγRIIA and 5'-TTTTTTTTTCCTACTTCTGCAGGGGGCTT-3' for FcγRIIIA.

Statistical analysis

Patients were analyzed for overall survival (OS) and for event-free survival (EFS) according to the Kaplan-Meier method. The log-rank test was used to analyze differences between curves. Univariate analysis was performed using a χ^2 test and multivariate analysis by a multiple regression model. The following variables were evaluated: age, sex, serum LDH, bulky disease, BM infiltration, number of extra-nodal sites, FcγRIIA (HH, HR, RR) and FcγRIIIA (VV, VF, FF) genotypes.

Discussion and Results

The correlation between FcγRIIIA-158VV and FcγRIIA-131HH genotypes and clinical response in FL after Rituximab immunotherapy is still controversial.[6,7,9] We investigated 94 FL patients sequentially treated with CHOP and Rituximab to determine the influence of these two receptors on the clinical and molecular response and the long-term outcome. The distribution of genotypes in our cases was similar to that previously reported: 32% HH, 49% HR and 19% RR for FcγRIIA and 19% VV, 49% VF and 32% FF for FcγRIIIA. There was no significant difference in the distribution of FcγRIIA and FcγRIIIA polymorphisms and age, sex, B symptoms, LDH, bulky disease, number of extra-nodal sites, bone marrow infiltration and stage (*data not shown*). In our FL patients who received a sequential CHOP and Rituximab treatment, there was no difference in the overall response rate (complete and partial response) between patients with FcγRIIIA-VV or FcγRIIIA-VF/FF

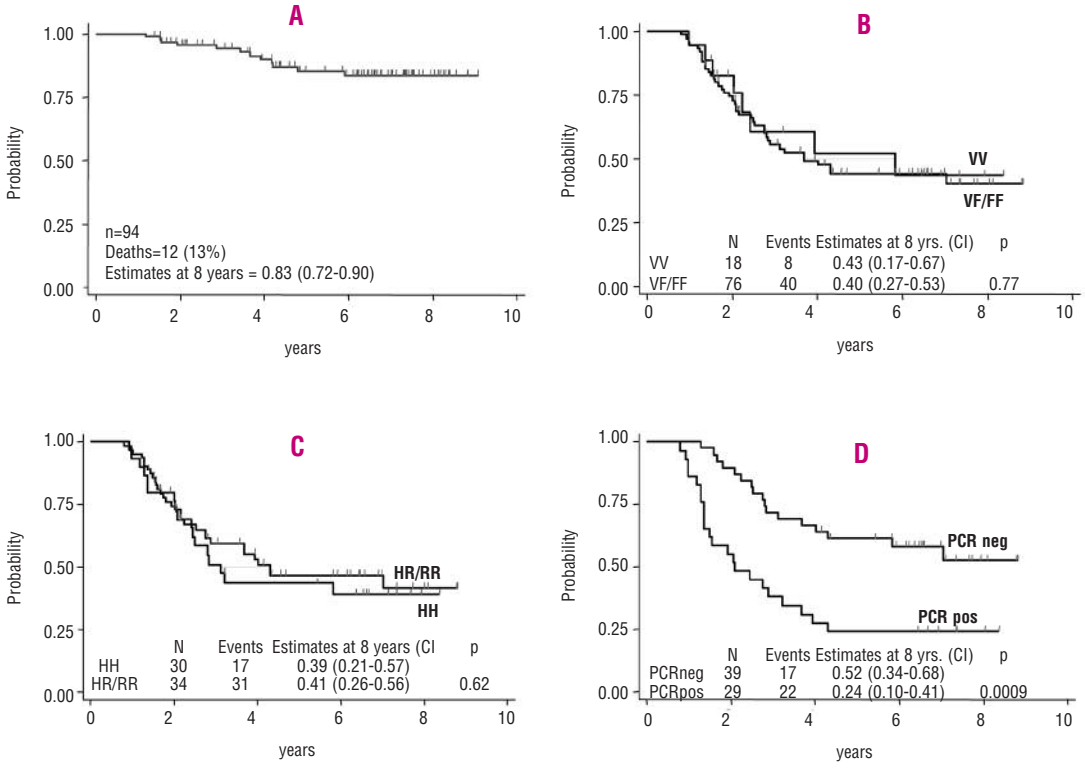

Figure 1. Clinical outcome of 94 FL-NHL patients treated with sequential CHOP and Rituximab. OS of study patients (Panel A). EFS according to: FcγRIIIA-VV genotype (Panel B), FcγRIIA-HH genotype (Panel C) or the molecular evidence of BCL2/IgH$^+$ cells detected in the BM 1 year after the end of treatment with Rituximab (Panel D). OS was calculated from the beginning of CHOP chemotherapy to the date of death or date of last follow-up. The EFS was calculated from the start of CHOP chemotherapy to the date of the first event (relapse, disease progression, treatment failure as defined by the need for any additional anti lymphoma treatment or death for any cause) or last follow-up.

(95% vs. 88%, p=not significant, ns) and for patients with FcγRIIA-HH or FcγRIIA HR/RR (87% vs. 91%, *p*=ns). Furthermore, the rate of complete remission was similar irrespective of the different FcγRs variants: 78% for FcγRIIIA-VV, 70% for FcγRIIIA-VF/FF (*p*=ns), 67% for FcγRIIA-HH, and 73% for FcγRIIA HR/RR (*p*=ns). Finally, no correlation was found between the FcγRs genotypes and the achievement of molecular response (*data not shown*). Univariate and multivariate analysis at diagnosis showed positive predictive factors of clinical response after chemo-immunotherapy were the lack of bulky disease and normal LDH values. By contrast, the clinical stage, the involvement of extra-nodal sites (beyond BM), age, sex, B symptoms, BM infiltration, or the polymorphism of FcÁRIIIA–VV or the FcγRIIA-RR were not statistically significant (Table 1). With a median follow-up of 5.8 years (range 1.18-9.04) the OS of these patients is 83% at 8 years (Figure 1A). This provides further evidence that the addition of Rituximab contributes to an increase in OS in FL patients.[12-14] The EFS of patients with FcγRIIIA-VV was 43% compared to 40% in patients bearing the combined FcγRIIIA-VF/FF (*p*=ns) (Figure 1B). Similarly, the EFS of patients with FcγRIIA-HH was 39% compared to 41% for patients with the FcγRIIA-RR/HR (*p*=ns) (Figure 1C). By contrast, we confirm that the achievement of a durable BM clearance of BCL2/IgH$^+$ cells detected by PCR analysis is associated with a better clinical outcome. Indeed, the long-term EFS of patients who proved PCR negative 1 year after the end of CHOP and Rituximab treatment remains significantly better compared to that of patients who never converted or rapidly lost a molecular response (52% vs. 24%, *p*=0.0009) (Figure 1D). The study showed FcγRs variants did not influence prognosis. This may have been due to several factors. It may be related to the different clinical setting in which Rituximab was employed compared to previous studies: a positive correlation between FcγRIIIA and FcγRIIA polymorphisms had been previously reported when Rituximab was used as single agent, both in untreated FL patients[6] or as a second or further line of treatment.[7] Our results are in keeping with those reported by Boettcher and colleagues[9] in a group of 75 FL patients treated with R-CHOP. In contrast to our results and those of Boettscher's, Kim and co-workers reported a positive correlation between V/V carriers and response in DLBCL treated with R-CHOP.[8] Apart from the different histology, these different results could perhaps be explained, at least in part, by the high proportion of V/V phenotype in the Asiatic population. In addition, the high remission rate obtained in our study may make it difficult to demonstrate differences in remission rate between V/V and F carrier patients, particularly if a large

part of the therapeutic efficacy is due to chemotherapy. However, in contrast with this hypothesis, we previously showed that both CHOP and Rituximab can achieve a similar 2 log decrease of BCL2/IgH+ cells detectable in the bone marrow.[15] As far as the FcγRIIA is concerned, the lack of influence of the H/R polymorphism at position 131 is not surprising given the lack of effect of this amino acid on human IgG1 binding to this receptor.[16] Thus, while FcγRs variants are likely to play an important role when Rituximab is used alone, our results support the hypothesis that ADCC, and/or phagocytosis mediated by FcγRIIIA and FcγRIIA, may not be the most relevant mechanisms of action when this chimeric antibody is used in combination with chemotherapy. On the other hand, CHOP chemotherapy may also enhance ADCC/phagocytosis via other FcγRs[17] thus decreasing differences previously attributable to FcγRIII polymorphisms. Indeed, different chemotherapy regimens do not reduce the number or function of circulating NK cells.[18]

To summarize, although FcγRIIIA-158VV and FcγRIIA-131HH genotypes are predictors of better clinical and molecular response in FL patients treated with Rituximab alone, this association seems to be lost when Rituximab is used in combination with or after chemotherapy.

Authors' Contributions

EC, GAP, DT and SS performed the molecular analysis; AnR and AP followed the patients and provided blood and marrow samples; EO performed statistical analysis, JG and RF critically revised the manuscript; EC and AR planned the project, supervised the work and wrote the manuscript.

Conflicts of interest

The authors reported no potential conflicts of interest.

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