

## FC $\gamma$ RIIIa and FC $\gamma$ RIIa polymorphisms are not associated with response to rituximab and CHOP in patients with diffuse large B-cell lymphoma

**We investigated the association of FC $\gamma$ RIIIa and FC $\gamma$ RIIa polymorphisms and response to R-CHOP in 58 patients with diffuse large B-cell lymphoma (DLBCL). FC $\gamma$ RIIIa and FC $\gamma$ RIIa polymorphisms did not influence response, event-free or overall survival. These results suggest that ADCC via FC $\gamma$ RIIIa and FC $\gamma$ RIIa may not be the major mechanism of activity of the R-CHOP combination in DLBCL.**

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Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid neoplasm accounting for approximately 30-40% of all non-Hodgkin lymphomas (NHLs). Recently, the combination of rituximab (R) and CHOP (cyclophosphamide, doxorubicin, vincristin, prednisone) led to significant improvement in the outcomes of patients with DLBCL, establishing R-CHOP as the new standard treatment.<sup>1,2</sup> The proposed mechanisms of rituximab activity include: antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), direct induction of apoptosis and chemosensitization of tumor cells to the cytotoxic effects of chemotherapy.<sup>3,4</sup> The clinical importance of each is still a matter of debate. ADCC is mediated by effector cells that engage the Fc portion of the antibody via receptors for immunoglobulin (Fc $\gamma$ Rs). Three Fc $\gamma$ R classes (Fc $\gamma$ RI, II, III) and eight subclasses have been described with significantly different haplotype distribution between various ethnic groups.<sup>5</sup> Fc $\gamma$ RIIIa receptor is expressed on natural killer (NK) cells, macrophages, while Fc $\gamma$ RIIa is expressed on neutrophils and macrophages. Genomic polymorphism of the Fc $\gamma$ RIIIa receptor corresponding to pheno-

typic expression of valine (V) or phenylalanine (F) at position 158 influences the binding of IgG1 to this receptor.<sup>6</sup> It has been shown that NK cells with valine homozygous Fc $\gamma$ RIIIa receptors (V/V) have a higher affinity to rituximab than those with phenylalanine homozygous receptors (F/F) resulting in more effective ADCC.<sup>7</sup> Several studies investigated the influence of Fc $\gamma$ RIIIa and Fc $\gamma$ RIIa polymorphisms on response to rituximab-containing treatment in different types of lymphoma. In follicular lymphoma (FL), patients with Fc $\gamma$ RIIIa 158 V/V phenotype respond better than F carriers to rituximab monotherapy,<sup>8,9</sup> but not to R-CHOP.<sup>10,11</sup> Fc $\gamma$ RIIIa 158 V/V phenotype is associated with improved response to rituximab monotherapy in Waldenström's macroglobulinemia,<sup>12</sup> in contrast to chronic lymphocytic leukemia (CLL).<sup>13</sup> A recently published study found that Korean DLBCL patients with Fc $\gamma$ RIIIa 158 V/V phenotype respond better to R-CHOP than F carriers, although there were no differences in event-free survival (EFS) and overall survival (OS).<sup>14</sup> In one series, patients with FL homozygous for histidine (H) on position 131 of Fc $\gamma$ RIIa responded better to rituximab monotherapy than heterozygous patients or patients homozygous for arginine (R).<sup>9</sup> Others failed to confirm this association.<sup>8,10,13,14</sup>

We examined the correlation of Fc $\gamma$ RIIIa 158 V/F and Fc $\gamma$ RIIa 131 H/R polymorphisms with treatment outcomes in a group of Caucasian patients with DLBCL receiving R-CHOP. A total of 58 patients with newly diagnosed DLBCL, Ann Arbor stage II-IV or stage I with extensive extranodal disease (IE) were included in the study. Patients were treated with a minimum of 3 cycles of rituximab (375 mg/m<sup>2</sup>) and standard CHOP. Those achieving at least partial remission (PR) continued with the treatment for an additional 1-5 cycles, depending on disease stage. Patients with a solitary residual tumor mass after R-CHOP were irradiated. Patients under 60 years of age who did not respond to R-CHOP or subsequent radiotherapy were treated with second line chemotherapy regimens followed by peripheral blood stem cell transplantation (PBSCT).

**Table 1.** Pre-treatment characteristics and treatment outcome according to FC $\gamma$ RIIIa 158 V/F and FC $\gamma$ RIIa 131 H/R polymorphisms.

	V/V	FC $\gamma$ RIIIa 158 V/F	F/F	p	H/H	FC $\gamma$ RIIa 131 H/R	R/R	p
No. (%)	16 (28)	32 (55)	10 (17)		23 (40)	27 (46)	8 (14)	
Median age, years (range)	44 (25-76)	49 (25-74)	35 (18-67)	0.29	48 (22-73)	48 (18-76)	38 (25-68)	0.34
No. male	11 (69%)	21 (66%)	5 (57%)	0.60	14 (61%)	17 (63%)	6 (75%)	0.76
PS (ECOG)								
0 or 1	9 (56%)	20 (62%)	6 (60%)	0.92	15 (65%)	15 (56%)	5 (63%)	0.78
2 - 4	7 (44%)	12 (38%)	4 (40%)		8 (35%)	12 (44%)	3 (37%)	
Stage								
IE and II	8 (50%)	14 (44%)	2 (20%)	0.27	12 (52%)	10 (37%)	2 (25%)	0.33
III and IV	8 (50%)	18 (56%)	8 (80%)		11 (48%)	17 (63%)	6 (75%)	
Age $\geq$ 60	7 (44%)	9 (28%)	1 (10%)	0.16	8 (35%)	8 (30%)	1 (13%)	0.45
Extranodal sites								
0 or 1	12 (75%)	25 (78%)	6 (60%)	0.54	20 (87%)	18 (67%)	5 (62%)	0.17
>1	4 (25%)	7 (22%)	4 (40%)		3 (13%)	9 (33%)	3 (38%)	
Elevated LDH	11 (69%)	15 (47%)	7 (70%)	0.23	11 (48%)	17 (63%)	5 (62%)	0.53
IPI								
0-2	9 (62%)	18 (57%)	5 (50%)	0.93	15 (65%)	13 (48%)	4 (50%)	0.46
3-5	7 (38%)	14 (45%)	5 (50%)		8 (35%)	14 (52%)	4 (50%)	
Bulky tumor (>10cm)	6 (38%)	6 (19%)	3 (30%)	0.36	7 (30%)	7 (26%)	2 (25%)	0.99
No. of cycles	6 (3-8)	6 (4-8)	7 (5-8)	0.12	7 (4-8)	7 (3-8)	6 (3-8)	0.19
Response								
CR	10 (62%)	26 (81%)	8 (80%)	0.36+	19 (83%)	20 (74%)	5 (62%)	0.51+
PR	2 (13%)	1 (3%)	1 (10%)		0	3 (11%)	1 (13%)	
SD, PD	4 (25%)	5 (16%)	1 (10%)		4 (17%)	4 (15%)	2 (25%)	

+CR vs. non-CR. PS: performance status; ECOG: Eastern Cooperative Oncology Group; IPI: international prognostic score; LDH: lactate dehydrogenase; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

Three younger patients with extensive disease at presentation, who achieved CR after R-CHOP, were treated with PBSCT upfront and censored for EFS analysis.

Response to treatment, EFS and OS were analyzed. For the purpose of this study, response was evaluated after three to four cycles and after the last R-CHOP cycle according to standard criteria.<sup>15</sup> The study was approved by the Ethics committee of the University Hospital Center Zagreb. All patients gave written informed consent. Assessments of the FcγRIIIa 131 H/R and FcγRIIIa 158 V/F polymorphisms were made by polymerase chain reactions followed by allele-specific restriction enzyme digestions as previously described.<sup>7</sup> Pre-treatment clinical features of patients and response to treatment according to FcγRIIIa and FcγRIIIa polymorphisms are shown in Table 1. The frequencies of V/V, V/F and F/F polymorphisms were 28%, 55%, 17%, and that of H/H, H/R and R/R polymorphisms 40%, 46% and 14% respectively. There were no significant differences regarding age, gender, performance status, LDH, extranodal involvement, international prognostic index (IPI), presence of bulky disease or number of R-CHOP cycles administered between the polymorphism groups. No association between the FcγRIIIa and FcγRIIIa polymorphism frequencies was detected ( $p=0.12$ ). Complete or unconfirmed complete remission (CR) after R-CHOP was achieved in 76% of patients (44/58). Four patients achieved PR, five had stable and five progressive disease. After a median follow up of 15 months (range 4-62), six patients (10%) relapsed and 11 (19%) patients died. Ten patients died of lymphoma progression and one from complications associated with second line treatment. The actuarial 2-year EFS rate was 64%, while 2-year OS rate was 75%. CR rates were 62%, 81%, 80% ( $p=0.36$ ) for FcγRIIIa V/V, V/F, F/F phenotypes and 83%, 74%, 62% ( $p=0.51$ ) for FcγRIIIa H/H, H/R, R/R phenotypes respectively (Table 1). FcγRIIIa and FcγRIIIa polymorphisms had no impact on EFS ( $p=0.09$  and  $p=0.50$  respectively) and OS ( $p=0.35$  and  $p=0.67$  respectively). Patients with IPI scores 0-2 had a significantly higher CR rate ( $p=0.021$ ) and better 2-year EFS rate ( $p=0.049$ ), compared to those with IPI 3-5 while differences in OS were not statistically significant.

The response to treatment with R-CHOP is comparable to results published in literature.<sup>12</sup> The finding that FcγRIIIa 158 V/F polymorphism is not associated with response to R-CHOP agrees with studies in mostly Caucasian patients with FL treated with R-CHOP<sup>10,11</sup> but differs from the recently published study by Kim and co-workers in Korean patients with DLBCL.<sup>14</sup> A possible explanation for this is that the effects of FcR polymorphisms depend on the genetic background of a given individual.<sup>5</sup> In conclusion, our study demonstrates no association of FcγRIIIa and FcγRIIIa polymorphisms and response to standard R-CHOP, suggesting that ADCC via FcγRIIIa and FcγRIIIa receptors may not be the major mechanism of the elimination of lymphoma cells in patients treated with immunochemotherapy.

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