

## Fc $\gamma$ RIIIa and Fc $\gamma$ RIIa polymorphisms do not predict response to rituximab in B-cell chronic lymphocytic leukemia

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**In follicular lymphoma (FL), genomic polymorphisms corresponding to the expression of valine (V) or phenylalanine (F) at amino acid 158 of Fc $\gamma$ RIIIa alter the binding affinity of immunoglobulin G1 (IgG1) to the receptor and have been associated with varied responses to rituximab. We examined Fc $\gamma$ RIIIa polymorphisms of 30 CLL patients with the phenotypes V/V (n = 6), V/F (n = 12), and F/F (n = 12)**

**treated with thrice-weekly rituximab (375 mg/m<sup>2</sup>) for 4 weeks to correlate polymorphism type with infusion toxicity and response. Infusion toxicity (grade 3 or greater or hypoxia/hypotension requiring transient cessation of therapy) was observed equally among the groups (V/V, 50%; V/F, 33%; F/F, 41.6%; P = .78). The response to rituximab was also similar among the different polymorphism pheno-**

**types (V/V, 33%; V/F, 41.6%; F/F, 50%). These data suggest that Fc $\gamma$ RIIIa polymorphisms are not predictive of response in CLL and that, unlike the case with FL, mechanisms of tumor clearance other than antibody-dependent cellular cytotoxicity may be more important. (Blood. 2004; 103:1472-1474)**

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### Introduction

Rituximab is a chimeric monoclonal antibody directed against CD20, an antigen found most B-cell malignancies, including non-Hodgkin follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL). As a single agent, rituximab induces objective responses in more than 50% of FL and CLL patients with minimal toxicity because of its B-cell selectivity.<sup>1-4</sup> These favorable results have led to considerable interest in combining rituximab with other agents in FL and CLL. However, understanding how rituximab mediates its *in vivo* biologic effects in these diseases is likely to enhance the effective implementation of combination therapy.

Multiple mechanisms have been proposed for the activity of rituximab, including antibody-dependent cellular cytotoxicity (ADCC),<sup>5,6</sup> complement-dependent cytotoxicity (CDC),<sup>6,7</sup> and a direct proapoptotic effect.<sup>8,9</sup> Although F(ab')<sub>2</sub> rituximab homodimers were shown to be effective in inducing apoptosis of B-cell lymphoma cell lines *in vitro*,<sup>10</sup> other works have recently established that ADCC is important as a predominant mechanism of lymphoma cell clearance and that Fc $\gamma$  receptors (Fc $\gamma$ R) are critical for the *in vivo* actions of rituximab in non-Hodgkin lymphoma (NHL).<sup>11</sup> In a xenograft model of human lymphoma, knocking out the Fc $\gamma$ R loci in mice showed a complete abrogation of response to rituximab.<sup>11</sup> In contrast, knocking out the inhibitory Fc $\gamma$ RIIB in mice resulted in enhanced response to rituximab in the same xenograft model.<sup>11</sup> In addition, the recent demonstration in NHL patients that the response to rituximab is dependent on specific Fc $\gamma$ RIIIa polymorphisms supports the importance of ADCC in the *in vivo* actions of rituximab.<sup>12</sup> The activating Fc $\gamma$ R on natural killer (NK) cells and monocytes (Fc $\gamma$ RIIIa) and on

monocytes (Fc $\gamma$ RIIa) mediates ADCC. Genomic polymorphism corresponding to phenotype expression of valine (V) or phenylalanine (F) at amino acid 158 on the Fc $\gamma$ RIIIa greatly influences the affinity of IgG1 to the Fc $\gamma$  receptor. Studies have demonstrated the stronger binding of antibody to homozygous Fc $\gamma$ RIIIa-158V NK cells than to homozygous Fc $\gamma$ RIIIa-158P or heterozygous NK cells.<sup>13,14</sup> Similarly, polymorphism related to the expression of histidine (H) or arginine (R) at amino acid 131 in Fc $\gamma$ RIIa affects the binding affinity of IgG1.<sup>15</sup> Forty-nine patients with previously untreated FL, patients homozygous for Fc $\gamma$ RIIIa-158V (V/V), had a significantly higher (100%) clinical response rate than those with heterozygous (V/F) or homozygous phenylalanine (F/F) genotype (67%).<sup>12</sup> Furthermore, polymerase chain reaction (PCR) detection of *bcl-2* rearranged cells was significantly lower in the homozygous V/V group. In contrast, the Fc $\gamma$ RIIa-131 polymorphism was not associated with response to rituximab.

It is possible, however, that the relative importance of ADCC as a mechanism for the activity of rituximab may differ in B-cell malignancies other than NHL. CLL differs significantly from FL from an immunophenotypic<sup>15,16</sup> and a genomic<sup>17</sup> standpoint, with most tumor cells having dim CD20 antigen expression. Studies have demonstrated that CLL cells are often resistant to complement-mediated cytotoxicity<sup>6,18</sup> and that, in responding patients receiving rituximab,<sup>19</sup> caspase-dependent apoptosis may be the predominant mechanism by which tumor cell elimination occurs. Although the effector-to-tumor cell ratio and the T<sub>H</sub>2 cytokine profile in CLL do not favor effective ADCC, the importance of Fc $\gamma$ RIIIa has not been explored in CLL. Herein, we examine the importance of Fc $\gamma$ RIIIa

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**Table 1. Clinical features of CLL patients by FcγRIIIa polymorphism type**

	FcγR V/V, n = 6	FcγR V/F, n = 12	FcγR F/F, n = 12	P
Median age, y (range)	69 (54-79)	56 (50-74)	64 (54-79)	.14
No. male (%)	4 (66)	7 (58)	12 (100)	.04
Median WBC count, ×10 <sup>9</sup> /L (range)	59.1 (8.1-175.3)	64.7 (2.5-152.2)	72.6 (4.1-173.4)	.80
No. stage III/IV (%)	5 (83)	10 (83)	6 (50)	.32
Mean prior regimens (range)	3 (1-6)	2 (0-6)	2 (0-4)	.39
No. prior nucleoside analog treatments (%)	5 (83)	5 (42)	7 (58)	.24
No. with lymphadenopathy (%)	5 (83)	11 (92)	12 (100)	.39
No. with infusion toxicity (%)	3 (50)	4 (33)	5 (42)	.78
No. partial responses	2	5	6	.70
(95%) CI	33 (4-78)	42 (15-72)	50 (21-78)	> .2

WBC indicates white blood cell.

and FcγRIIIa polymorphisms to response and toxicity observed with rituximab treatment in CLL.

## Patients, materials, and methods

### Patient samples and cell processing

Patients were enrolled in this previously reported, institutional review board (Walter Reed Army Medical Center, Johns Hopkins Oncology Center, and The Ohio State University)–approved, multicenter trial.<sup>4</sup> All patients gave written, informed consent before participation. Patients were required to have histologically documented CLL as defined by the modified National Cancer Institute (NCI) criteria<sup>20</sup> or to have small lymphocytic lymphoma as defined by the International Working Formulation classification.<sup>21</sup> Treatment included stepped-up administration of thrice-weekly rituximab for 4 consecutive weeks, as previously described.<sup>4</sup> Response to rituximab was judged 2 months after therapy according to the modified National Cancer Institute (NCI) criteria.<sup>20</sup> Infusion toxicity was defined by the presence of hypoxemia, hypotension, or dyspnea, which required transient discontinuation of the infusion and supportive intervention. Cells were obtained before rituximab treatment, and mononuclear cells were isolated from blood using density-gradient centrifugation (Ficoll-Hypaque Plus; Pharmacia Biotech, Piscataway, NJ). Cells were then viably cryopreserved in 10% dimethyl sulfoxide (DMSO), 40% fetal calf serum, and 50% RPMI medium.

### Analysis of FcγRIIIa and FcγRIIIa polymorphisms

Pretreatment samples for analyses of V/F 158 FcγRIIIa and FcγRIIIa 131 H/R polymorphisms were available for 30 (90%) of 33 patients enrolled in this trial. DNA was extracted using the QIAamp kit according to the manufacturer’s instructions (Qiagen, Valencia, CA). Assessment of the of FcγRIIIa and FcγRIIIa polymorphisms was performed as previously described.<sup>12</sup> All samples were analyzed in duplicate, with identical results.

### Statistical analysis

Pretreatment clinical features, observed toxicity, and response to therapy among the polymorphism groups were compared using  $\chi^2$  analysis for categorical data and the nonparametric Kruskal-Wallis test for continuous data. A *P* value of .05 was considered statistically significant.

## Results

Pretreatment features of the 30 patients separated according to FcγRIIIa polymorphism group are shown in Table 1. Six (20%) and 12 (40%) patients were homozygous for the V/V 158 FcγRIIIa and the F/F 158-FcγRIIIa polymorphisms, respectively. Although more male patients were homozygous for FcγRIIIa F/F, there was no other significant difference in the pretreatment clinical characteristics among the groups. Of the 30 patients included, 12 patients experienced infusion toxicity that required the temporary cessation of rituximab therapy and intervention. The incidence of infusion toxicity was similar (50%, 33%, and 42%; *P* = .78) among the 3 different 158-FcγRIIIa polymorphism groups. Similarly, among the 13 patients who achieved partial response to thrice-weekly rituximab therapy, there was no significant difference in response rate between the V/V, V/F, and F/F 158 FcγRIIIa polymorphism groups, with responses seen in 33% (95% confidence interval [CI], 4%-78%), 42% (95% CI, 15%-72%), and 50% (95% CI, 21%-79%), respectively (*P* = .70). Although the result is limited by the small number of cases studied within each subgroup, resulting in wide confidence intervals for the estimate of response to rituximab, the directional trend in our result was opposite what might have been expected based on the increased affinity of the V/V phenotype FcγRIIIa for the antibody. Table 2 shows the clinical baseline characteristics of the same 30

**Table 2. Clinical features of CLL patients by FcγRIIIa polymorphism type**

	FcγR H/H, n = 6	FcγR H/R, n = 17	FcγR R/R, n = 7	P
Median age, y (range)	68 (51-80)	57 (50-79)	74 (64-79)	.03
No. male (%)	5 (83)	11 (65)	7 (100)	.16
Median WBC count, ×10 <sup>9</sup> /L (range)	39.3 (3.4-154.8)	84.5 (4.8-175.3)	44.8 (2.5-173.4)	.59
No. stage III/IV (%)	3 (50)	13 (77)	6 (86)	.32
Mean prior regimens (range)	3 (1-6)	2 (0-6)	2 (0-4)	.67
No. prior nucleoside analog treatments (%)	3 (50)	10 (59)	4 (57)	.93
No. with lymphadenopathy (%)	5 (83)	16 (94)	7 (100)	.48
No. infusion toxicity (%)	2 (33)	6 (35)	4 (57)	.57
No. partial responses (95% CI)	4.67 (22-96)	5.29 (10-56)	4.57 (18-90)	.70

patients analyzed using the 131-Fc $\gamma$ RIIIa polymorphism phenotype. Similar to the results observed for the 158-Fc $\gamma$ RIIIa polymorphism, there were no significant differences in infusion toxicity or response rate among the 3 groups.

## Discussion

Our findings are in contrast to the FL results reported by Cartron et al,<sup>12</sup> which showed that the high-affinity 158-Fc $\gamma$ RIIIa V/V polymorphism was associated with the highest response rate in patients treated with rituximab.<sup>12</sup> Although the numbers of CLL patients in our series was smaller, the subset of patients with the high-affinity polymorphism had the lowest response rate. Several explanations for these findings are possible, including a different mechanism of action of rituximab in CLL compared with lymphoma or a varied patient population among the patient groups analyzed. In FL, preclinical animal studies,<sup>11</sup> the Fc $\gamma$ RIIIa polymorphism studies of Cartron et al,<sup>12</sup> and higher response rates observed in studies that combine rituximab with agents that enhance ADCC<sup>22</sup> all support ADCC as an important mechanism for the activity of rituximab. In contrast, however, our results suggest that ADCC may be a less important mechanism in CLL. The reduced expression of CD20 on the neoplastic cells of CLL compared with FL, or the presence of higher levels of soluble CD20 in CLL, may significantly reduce the efficiency of rituximab in mediating ADCC

in spite of the higher affinity of Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIa and may contribute to the lack of observed effect of Fc $\gamma$ R polymorphisms on response. In addition, it is possible that ADCC is the more important mechanism for clearance of neoplastic B cells in lymph nodes, predominantly involved in FL, compared with blood and bone marrow, which are more commonly involved in CLL. Although we have no direct data to support or refute these possibilities, a plausible alternative explanation is that rituximab acts by different mechanisms in CLL. We previously demonstrated in vivo activation of the intrinsic pathway of apoptosis in CLL patients responding to rituximab.<sup>19</sup> In addition, several cellular and genetic factors that inhibit apoptosis in CLL also diminish response to rituximab. The role of CDC is also less likely because CLL cells often dimly express CD20 and overexpress CD55 and CD59, which prevents CDC.<sup>6,18</sup> Further diminishing the major role of CDC in CLL is the lack of correlation with response to CD55 and CD59, shown in another study.<sup>23</sup> Indeed, this study, combined with the results of other studies in CLL in which cellular<sup>23</sup> and genetic<sup>24</sup> features that disrupt apoptosis are associated with diminished response, provides support for the hypothesis that signaling and apoptosis may contribute more to the therapeutic efficacy of rituximab in CLL than ADCC or CDC. These results provide justification for the emphasis on disease-specific studies to determine the mechanisms of how specific monoclonal antibody therapies work in vivo.

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