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ORIGINAL REPORT

Polymorphisms in FcγRIIIA (CD16) Receptor Expression Are Associated With Clinical Response to Rituximab in Waldenström's Macroglobulinemia

Steven P. Treon, Mark Hansen, Andrew R. Branagan, Sigitas Verselis, Christos Emmanouilides, Eva Kimby, Stanley R. Frankel, Nikolaos Touroutoglou, Barry Turnbull, Kenneth C. Anderson, David G. Maloney, and Edward A. Fox

From the Bing Program for Waldenström's Macroglobulinemia, Dana-Farber Cancer Institute, Harvard Medical School, Boston; CareStat, Newton, MA; UCLA Medical Center, Los Angeles, CA; Greenebaum Cancer Center, University of Maryland, Baltimore, MD; Nevada Cancer Center, Las Vegas, NV; Fred Hutchinson Cancer Research Center, Seattle WA; and Karolinska University Hospital, Stockholm, Sweden.

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to Steven P. Treon, MD, MA, PhD, Bing Program for Waldenström's Macroglobulinemia, Dana-Farber Cancer Institute, LG102, 44 Binney St, Boston, MA 02115; e-mail: steven_treon@dfci.harvard.edu.

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ABSTRACT

Purpose
Rituximab is an important therapeutic for Waldenström's macroglobulinemia (WM). Polymorphisms in FcγRIIIA (CD16) receptor expression modulate human immunoglobulin G1 binding and antibody-dependent cell-mediated cytotoxicity, and may therefore influence responses to rituximab.

Patients and Methods
Sequence analysis of the entire coding region of FcγRIIIA was undertaken in 58 patients with WM whose outcomes after rituximab were known.

Results
Variations in five codons of FcγRIIIA were identified. Two were commonly observed (FcγRIIIA-48 and FcγRIIIA-158) and predicted for amino acid polymorphisms at FcγRIIIA-48: leucine/leucine (L/L), leucine/arginine (L/R), and leucine/histidine (L/H). Polymorphisms at FcγRIIIA-158 were phenylalanine/phenylalanine (F/F), phenylalanine/valine (F/V), and valine/valine (V/V). A clear linkage between these polymorphisms was detected and all patients with FcγRIIIA-158F/F were always FcγRIIIA-48L/L, and patients with either FcγRIIIA-L/R or -L/H always expressed at least one valine at FcγRIIIA-158 ($P \leq .001$). The response trend was higher for patients with FcγRIIIA-48L/H (38.5%) versus -48L/R (25.0%) and LL (22.0%), and was significantly higher for patients with FcγRIIIA-158V/V (40.0%) and -V/F (35%) versus -158F/F (9.0%; $P = .030$). Responses for patients with FcγRIIIA-48L/L were higher when at least one valine was present at FcγRIIIA-158 ($P = .057$), thereby supporting a primary role for FcγRIIIA-158 polymorphisms in predicting rituximab responses. With a median follow-up of 13 months, no significant differences in the median time to progression and progression-free survival were observed when patients were grouped according to their FcγRIIIA-48 and -158 polymorphisms.

Conclusion
The results of these studies therefore support a predictive role for FcγRIIIA-158 polymorphisms and responses to rituximab in WM.

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INTRODUCTION

Monoclonal antibodies have been used successfully to treat patients with B-cell malignancies, including Waldenström's macroglobulinemia (WM). Most of these efforts to date have focused on the use of rituximab, a chimeric human immunoglobulin G1 (IgG1) monoclonal antibody, which targets CD20, an antigen that is widely expressed in WM.[1] With the use of standard-dose rituximab therapy (ie, four infusions at 375 mg/m^2/wk), responses (defined as ≥ 50% decline in serum IgM) have been observed

in 20% to 30% of patients.[2-6] Higher response rates (40% to 50%) have been reported with the use of extended rituximab therapy (ie, eight infusions at 375 mg/m^2/wk delivered at weeks 1 to 4 and 12 to 16).[7,8] Studies combining rituximab with chemotherapy or with immunomodulators have also shown encouraging findings.[9-12] In view of the above studies, the Consensus Panel III of the Second International Workshop charged with making treatment recommendations for WM recommended the use of rituximab in primary as well as salvage therapy of WM.[13]

With the increased use of rituximab in the treatment of WM, there has been growing interest in understanding what patient-related differences might account for the heterogeneous responses observed in WM, particularly because CD20 is widely expressed in WM, and surviving rituximab-coated tumor cells may be found in WM patients many months after antibody therapy.[1,14] Increasing evidence has pointed to both quantitative and qualitative differences in natural killer (NK) cell function to explain rituximab clinical activity. Higher circulating NK cell levels and responses to rituximab have been reported in patients with low-grade non-Hodgkin's lymphoma (NHL), suggesting that antibody-dependent cell-mediated cytotoxicity (ADCC) enacted by NK cells may be a primary mechanism by which rituximab functions.[15,16] Moreover, as the recent studies by Cartron et al[17] suggest, responses to rituximab may depend on polymorphisms present in the FcγRIIIA receptor, a receptor mainly expressed on NK cells.[18-20]

Polymorphisms in positions 48 and 158 of the FcγRIIIA receptor expression have been reported to influence human IgG1 binding and ADCC.[19-22] Polymorphisms at position 158 result in either valine (V) or phenylalanine (F) expression, the former of which is associated with increased depletion of peripheral-blood B-cells[23] and response to rituximab in patients with follicular NHL,[17,24] but not chronic lymphocytic leukemia. [25] At position 48, polymorphisms of the FcγRIIIA receptor result in expression of either leucine (L), arginine (R), or histidine (H), the first of which is linked to FcγRIIIA-158F polymorphisms, and the latter two of which is linked to FcγRIIIA-158V polymorphisms.[21,22] However, the binding of IgG1 to FcγRIIIA appeared to occur independent of position 48 polymorphisms in this study.[21]

The contribution of FcγRIIIA receptor polymorphisms at position 158, as well as at other positions, in response to rituximab has not been reported for WM. WM may be a particularly relevant disease model for gaining understanding of the role of FcγRIIIA receptor polymorphisms to rituximab response. Unlike other lymphomas, WM is uncommonly associated with adenopathy (< 20% of patient cases) and is centered in the bone marrow, where monoclonal antibodies are more readily able to bind and saturate tumor cells. This eliminates the penetration of antibody into bulky disease as a variable for response to rituximab. In this study, we performed sequencing for the entire DNA coding region of FcγRIIIA in 58 patients with WM who received rituximab, and report the association of the two most common polymorphism sites (FcγRIIIA-48 and -158) with clinical responses.

PATIENTS AND METHODS

Patient Characteristics and Therapy

Patients with an established clinicopathologic diagnosis of WM who received single-agent rituximab therapy were eligible for this study. The study was approved by the institutional review board of the Dana-Farber Cancer Institute (Boston, MA), and informed consent was obtained from all patients. All patients received rituximab at 375 mg/m^2/wk. The number of weekly infusions received by patients was as follows: four (n = 34), eight (n = 19), six (n = 2), two (n = 2), and three (n = 1).

Response Assessment

Complete response (CR) was defined as having resolution of all symptoms, normalization of serum IgM levels with complete disappearance of IgM paraprotein by immunofixation, and resolution of any adenopathy or splenomegaly. Patients achieving a partial response (PR) were defined as achieving a ≥ 50% reduction in serum IgM levels. No patients achieved a CR; hence, the overall response rate reflected only PR patients. The median time to disease progression (TTP) and progression-free survival were determined for all patients and for responders in each polymorphism group, respectively.

Analysis of FcγRIIIA Polymorphisms

DNA was extracted from peripheral-blood leukocytes using a kit (Qiagen, Valencia, CA). Sequences for the primers used in these studies are listed in Table 1. Exons 1 (primers 1sF and 1nR) and 2 (primers 2sF and 2nR) were each amplified as single polymerase chain reaction (PCR) amplicons; exons 3 and 4 were each amplified in two overlapping amplicons (primer 3nF with 3sR and primer 3sF with 3nR for exon 3; primer 4nF with 4sR and primer 4sF with 4nR for exon 4); and the coding region plus some of the 3′

Table 1. Primer Sequences Used in the Analysis of FcγRIIIA Polymorphisms

Primer Name	Sequence (5′ > 3′)
1sF	TGTAAAACGACGGCCAGTGGCTGGGGAAAGGCTGTTTACTT
1nR	CAGGAAACAGCTATGACCCTGAACCCAAGGCATCTCAAA
2sF	TGTAAAACGACGGCCAGTGCAAGAGGCATGAACAGTGGAG
2nR	CAGGAAACAGCTATGACCCTGCTAACCCCACATCAGCATTT
3nF	TGTAAAACGACGGCCAGTCACCAAGCATGGGTTTGCAAT
3sF	TGTAAAACGACGGCCAGTAATGGTACAGGGTGCTCGAGAA
3nR	CAGGAAACAGCTATGACCAGTGGGACCACACATCATCTCAT
3sR	CAGGAAACAGCTATGACCGCACCTGTACTCTCCACTGTCGTC
4nF	TGTAAAACGACGGCCAGTTGCAGGGTTGACTCCCAATCT
4sF	TGTAAAACGACGGCCAGTGTCACATATTTACAGAATGGCAAAGG
4nR	CAGGAAACAGCTATGACCCCAACTCAACTTCCCAGTGTGATT
4sR	CAGGAAACAGCTATGACCGCAGAAGTAGGAGCCGCTGTCT
5nF	TGTAAAACGACGGCCAGTGGTGAGCTGTCCTCTGCTCAGATA
5sR	CAGGAAACAGCTATGACCGAAATGTTCAGAGATGCTGCTGCT

untranslated region of exon 5 were amplified in a single amplicon using seminested primers (primer 4sF with 5sR for the first round, followed by primer 5nF with 5sR for the second round). The components of the 10-μL PCR reaction were 20 mmol/L Tris-HCl (pH8.4); 50 mmol/L KCl; 1.5 mmol/L $MgCl_2$; 0.1 mmol/L in each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and thymidine triphosphate; 0.1 μmol/L of each primer; 5 ng/μL of genomic DNA (or a 1:100 dilution of the first round exon 5 PCR for the second round reaction); and 0.05 U/μL Taq polymerase (Taq Platinum, GIBCO BRL, Gaithersburg, MD). The thermocycling conditions were 94°C for 4 minutes, followed by 11 cycles, each with a denaturing step at 94°C for 30 seconds and an extension step at 72°C for 20 seconds, and with a 20-second annealing step that decreased 1°C/cycle, beginning at 60°C in the first cycle and decreasing to 50°C in the 11th cycle; the 11th cycle was then repeated 25 times. A 6-minute incubation at 72°C followed by a 4°C soak completed the program. Each strand of each PCR product was sequenced using dye primer chemistry (Applied Biosystems Inc, Foster City, CA). The first predicted amino acid of the extracellular domain 1 was designated as amino acid 1, and the first nucleotide position of the start codon was designated as nucleotide position 1. Others have designated the first amino acid of the signal sequence as the first amino acid.[20]

Statistical Analysis

Comparison of study parameters was performed using two-tailed Student's *t* tests for continuous variables and two-tailed Fisher's exact tests for variables categoric in nature. TTP analyses were restricted to responding patients and were assessed using Kaplan-Meier methodology with log-rank statistics for inferential comparisons. All analyses were performed using SAS (version 8; SAS Institute, Cary, NC) software and *P* values $\leq .05$ were deemed to be statistically significant.

RESULTS

FcγRIIIA Sequencing and Polymorphisms

The clinical and laboratory features of the 58 WM patients evaluated in this study are summarized in Table 2. Sequence analysis of the entire coding region of FcγRIIIA was accomplished for all 58 patients and revealed variations in five codons (Fig 1), two of which were commonly observed (FcγRIIIA-48 and FcγRIIIA-158) and have been described previously.[21,22] Two distinct nucleotide changes at position 197 within the third exon (T→G, resulting in arginine; T→A, resulting in histidine) were detected, which predicted for the following amino acid polymorphisms at FcγRIIIA-48: leucine/leucine (L/L), leucine/arginine (L/R), and leucine/histidine (L/H). The frequencies of these FcγRIIIA-48 polymorphisms for the 58 patients were as follows: L/L, 70.7%; L/H, 22.4%; and L/R, 6.9%.

Diallelic single-nucleotide changes in position 526 (T→G) were also detected by sequencing, which predicted for the following amino acid polymorphisms at FcγRIIIA-158: phenylalanine/phenylalanine (F/F), phenylalanine/valine (F/V), and valine/valine (V/V). The frequencies for these FcγRIIIA-158 polymorphisms in the 58 patients were as follows: F/F, 37.9%; F/V, 44.8%; and V/V, 17.2%. A clear linkage between the FcγRIIIA-48 and FcγRIIIA-158 polymorphisms was detected. All patients with the FcγRIIIA-158F/F polymorphism were FcγRIIIA-48L/L, whereas patients with either the FcγRIIIA-L/R or -L/H polymorphisms expressed at least one valine at FcγRIIIA-158

Table 2. Summary of Features for All Patients Evaluated in This Study

No. of Patients	58
Age, years	
Median	64
Range	39-86
Sex, %	
Male	57
Female	43
No. of prior treatments	
0	23
1	20
2	10
3	5
Serum IgM, mg/dL	
Median	3,220
Range	252-10,200
Hct, %	
Median	32.9
Range	20-45.6
PLT, mm^3	
Median	188
Range	17-478
No. of rituximab infusions	
2	2
3	1
4	34
6	2
8	19
Response to rituximab	
CR	0
% CR	0.0
PR	15
% PR	25.9

Abbreviations: IgM, immunoglobulin M; Hct, hematocrit; PLT, platelets; CR, complete response; PR, partial response.

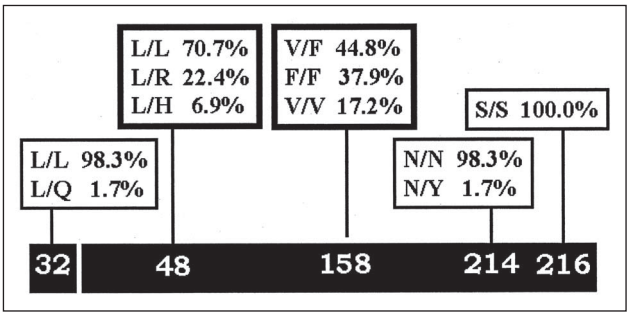

Fig 1. Predicted amino acid polymorphisms in the FcγRIIIA receptor for 58 patients with Waldenström's macroglobulinemia. Sites and percentage of patients displaying each predicted amino acid polymorphism are shown. L, leucine; R, arginine; H, histidine; Q, glutamine; V, valine; F, phenylalanine; S, serine; N, asparagine; Y, tyrosine.

($P = .059$). Variations at nucleotide positions 32, 216, and 694 were also detected. Six of the 58 patients (10.3%) demonstrated a single nucleotide change at position 216 (G→A), which did not result in an amino acid change (serine→serine). A single nucleotide change (T→A) at position 32 (in the signal peptide) was detected in one patient, which predicted for an amino acid change from leucine to glutamine (Q) resulting in a change in expression from L/L→L/Q, whereas in another patient, an (A→T) change at nucleotide position 694 predicted for a change from asparagine (N) to tyrosine (Y) and a change in expression from N/N→N/Y at amino acid 214.

Patient Characteristics and Polymorphisms

Given that variations in the signal peptide and at amino acid 214 were found in only two of the 58 patients, and given that no amino acid change was predicted at position 54 from the single nucleotide substitution at that locus, no additional analysis was undertaken for these variations in FcγRIIIA genomic expression. Analysis of age, male-to-female ratio, prior number of treatments, pretherapy serum IgM levels, hematocrit, and platelet count, as well as the number of rituximab infusions received for patients with any of the FcγRIIIA-48 or FcγRIIIA-158 polymorphisms, did not demonstrate any significant differences (Tables 3 and 4).

Patient Responses and Polymorphisms

FcγRIIIA-48 polymorphisms and rituximab response. The overall response rate for patients treated with rituximab, based on their predicted amino acid polymorphisms in FcγRIIIA-48, were as follows: nine of 41 (22.0%) for FcγRIIIA-48L/L; one of four (25%) for FcγRIIIA L/R; and five of 13 (38.5%) for FcγRIIIA L/H (Table 5). Although an increased overall response rate was observed among patients with the predicted FcγRIIIA-48L/H versus FcγRIIIA-48L/L and -L/R polymorphisms, these differences did not reach statistical significance.

FcγRIIIA-158 polymorphisms and rituximab response. An increased response rate was observed among patients with the predicted FcγRIIIA-158V/V (four of 10; 40.0%) or -158 V/F (nine of 26; 35.0%) polymorphisms versus those patients with the FcγRIIIA-158F/F (two of 22; 9.0%) polymorphism (V/V *v* F/F, $P = .059$; V/F *v* F/F, $P = .045$; V/V and V/F *v* F/F, $P = .03$; Table 6).

Table 3. Summary of Patient Features According to Their FcγRIIIA-48 Polymorphism

Characteristic	FcγRIIIA-48L/L	FcγRIIIA-48L/H	FcγRIIIA-48L/R
No. of Patients	41	13	4
%	70.7	22.4	6.9
Age, years			
Median	63	68	61
Range	39-86	51-86	53-65
Sex, %			
Male	56	69	25
Female	44	31	75
No. of prior treatments			
0	15	5	3
1	17	2	1
2	6	4	0
3	3	2	0
Serum IgM, mg/dL			
Median	3,000	4,200	5,300
Range	583-8,970	285-10,200	252-7,530
Hct, %			
Median	33.9	30.4	33.8
Range	20-45.6	28-41	27.1-43.4
PLT, mm^3			
Median	179	211	242
Range	43-478	17-472	225-283
No. of rituximab infusions			
2	1	0	1
3	1	0	0
4	23	8	3
6	1	1	0
8	15	4	0

NOTE. P = not significant for comparison of all variables among the three polymorphism groups.
Abbreviations: L/L, leucine/leucine; L/H, leucine/histidine; L/R, leucine/arginine; IgM, immunoglobulin M; Hct, hematocrit; PLT, platelets.

Table 4. Summary of Patient Characteristics According to Their FcγRIIIA-158 Polymorphism

Characteristic	FcγRIIIA-158F/F	FcγRIIIA-158V/F	FcγRIIIA-158V/V
No. of Patients	22	26	10
%	37.9	44.8	17.2
Age, years			
Median	63	63	67
Range	39-86	45-86	49-80
Sex			
Male	64	50	60
Female	36	50	40
No. of prior treatments			
0	10	9	4
1	7	11	2
2	4	3	3
3	1	3	1
Serum IgM, mg/dL			
Median	3,050	2,980	3,900
Range	583-8,970	252-7,780	1,300-10,200
Hct, %			
Median	32.6	32.6	35.6
Range	20-45.6	23-43.4	20-41
PLT, mm^3			
Median	156	221	216
Range	43-478	17-405	136-472
No. of rituximab infusions			
2	0	0	2
3	0	0	1
4	11	17	6
6	1	1	0
8	10	8	1

NOTE. P = not significant for comparison of all variables among the three polymorphism groups.
Abbreviations: F/F, phenylalanine/phenylalanine; V/F, valine/phenylalanine; V/V, valine/valine; IgM, immunoglobulin M; Hct, hematocrit; PLT, platelets.

Analysis of combined FcγRIIIA-48 and -158 polymorphisms and rituximab response. Given that patients with the FcγRIIIA-158F/F polymorphism always expressed FcγRIIIA-48L/L, and those patients with either the FcγRIIIA-L/R or -L/H polymorphisms always expressed at least one valine at FcγRIIIA-158, we next analyzed the relationship of FcγRIIIA-48 and -158 polymorphisms and response to rituximab. The response rates combining both polymorphisms are summarized in Table 7. Among patients possessing the FcγRIIIA-48L/L polymorphism, an increased response rate (36.8 *v* 9.0%; P = .057) was observed in patients predicted to be carrying at least one valine (V/V or V/F) versus those predicted to be expressing F/F at FcγRIIIA-158. Because all patients possessing the FcγRIIIA-48L/R or H polymorphism expressed at least one valine amino acid at FcγRIIIA-158, no associations for

Table 5. Summary of WM Patient Responses to Rituximab According to Their FcγRIIIA-48 Polymorphism

Polymorphism	No. of Patients	Response Rate	%
FcγRIIIA-48L/L	41	9	22.0
FcγRIIIA-48L/R	4	1	25.0
FcγRIIIA-48L/H	13	5	38.5
FcγRIIIA-48L/R or H	17	6	35.3

NOTE. P = not significant for all response comparisons.
Abbreviations: WM, Waldenström's macroglobulinemia; L/L, leucine/leucine; L/R, leucine/arginine; L/H, leucine/histidine; H, histidine.

Table 6. Summary of WM Patient Responses to Rituximab According to Their FcγRIIIA-158 Polymorphism

Polymorphism	No. of Patients	Response Rate	%
FcγRIIIA-158F/F	22	2	9.0
FcγRIIIA-158V/F	26	9	35.0*
FcγRIIIA-158V/V	10	4	40.0†
FcγRIIIA-158V/F or V	36	13	36.0‡

Abbreviations: WM, Waldenström's macroglobulinemia; F/F, phenylalanine/phenylalanine; V/F, valine/phenylalanine; V/V, valine/valine; V, valine.
*P = .045 for comparison of response rate to patients with FcγRIIIA-158F/F polymorphism.
†P = .059 for comparison of response rate to patients with FcγRIIIA-158F/F polymorphism.
‡P = .030 for comparison of response rate to patients with FcγRIIIA-158F/F polymorphism.

Table 7. Summary of WM Patient Responses to Rituximab According to the Combined FcγRIIIA-48 and -158 Polymorphisms

FcγRIIIA-48 Polymorphism	FcγRIIIA-158 Polymorphism	No. of Patients	Response Rate	%
-48L/L	-158F/F	22	2	9.0
	-158V/-	19	7	36.8*
-48L/R	-158V/-	4	1	25.0
-48L/H	-158V/-	13	5	38.5†
-48L/R or H	-158V/-	17	6	35.3‡

NOTE. P = not significant for all comparisons except those listed below.
Abbreviations: WM, Waldenström's macroglobulinemia; L/L, leucine/leucine; F/F, phenylalanine/phenylalanine; V, valine; L/R, leucine/arginine; L/H, leucine/histidine; H, histidine.
*P = .057 for comparison of responses to patients with FcγRIIIA-48L/L and -158F/F polymorphisms.
†P = .075 for comparison of responses to patients with FcγRIIIA-48L/L and -158F/F polymorphisms.
‡P = .059 for comparison of responses to patients with FcγRIIIA-48L/L and -158F/F polymorphisms.

response activity with the -158 F/F polymorphism could be undertaken. However, the response rate for patients with the FcγRIIIA-48L/R or H polymorphisms was increased when compared with that of patients with the FcγRIIIA-48L/L and -158F/F polymorphism (35.3% *v* 9.0%; P = .059). This effect was slightly more pronounced for patients with the FcγRIIIA-48L/H polymorphism (38.5% *v* 9.0%; P = .075). No significant difference in response rate (35.3% *v* 36.8%) was found among patients who possessed FcγRIIIA-48L/R or H, and those with the FcγRIIIA-48 polymorphism who expressed at least one valine at position 158.

With a median follow-up of 13 months, the median TTP for all patients when grouped according to their FcγRIIIA-48 and -158 polymorphisms was not significantly different (Table 8). Similarly, no significant difference in the median progression-free survival was observed when responding patients were grouped by their FcγRIIIA-48 and -158 polymorphisms (Table 8).

Table 8. Summary of WM Patient TTP for All Patients and PFS for Responding Patients According to Their FcγRIIIA-48 and -158 Polymorphism Status

Polymorphism	No. of Patients	Median TTP (months)	No. of Patients	Median PFS (months)
FcγRIIIA-48L/L	42	13	9	16
FcγRIIIA-48L/R	4	14	1	4
FcγRIIIA-48L/H	13	7	5	5
FcγRIIIA-48L/R or H	17	7	6	5
FcγRIIIA-158F/F	22	12	2	7
FcγRIIIA-158V/F	26	10	9	15
FcγRIIIA-158V/V	10	12	4	12
FcγRIIIA-158V/F or V	36	11	13	15

NOTE. P = not significant for all comparisons of TTP and PFS among patients grouped by any of the FcγRIIIA-48 or -158 polymorphisms.
Abbreviations: WM, Waldenström's macroglobulinemia; TTP, time to progression; PFS, progression-free survival; L/L, leucine/leucine; L/H, leucine/histidine; L/R, leucine/arginine; H, histidine; F/F, phenylalanine/phenylalanine; V/F, valine/phenylalanine; V/V, valine/valine; V, valine.

DISCUSSION

Despite the CD20 antigen being expressed on tumor cells from nearly all patients with WM, major responses to rituximab are seen in only about half of treated patients, even with the use of extended dose schedules. Tumor-related variables including antigen loss, complement resistance antigen expression, and tumor burden have been addressed previously by us and others, and do not appear to account for the heterogeneity in response to rituximab for patients with WM.[1,7,8,14] Moreover, we previously reported finding saturating levels of rituximab on WM cells in patients who had received therapy many months earlier, suggesting that patient-related factors might also account for differential responses to rituximab in WM.[14] The possibility that patient-related differences, particularly those affecting ADCC function, might account for variable responses to rituximab in WM was also suggested by studies in related low-grade NHL patients, which correlated NK cell levels and polymorphisms in position 158 of the FcγRIIIA receptor to rituximab responses.[15-17,24]

In these studies, we sequenced all coding regions of the FcγRIIIA receptor from a series of patients with WM to first define potential polymorphisms in this receptor, and then correlated these findings to their response to rituximab. We observed single nucleotide variations in five codons of the FcγRIIIA receptor, one of which predicted for a silent amino acid change (Ser→Ser) at position 54, and four of which predicted for changes in the signal peptide, and in amino acids 48, 158, and 214. Given that variations in positions were only observed in one patient for each, we focused our analysis on the two most commonly observed polymorphisms (FcγRIIIA-48 and FcγRIIIA-158), both of which have been described previously. The genotype distributions in this study for both the FcγRIIIA-48 and FcγRIIIA-158 polymorphisms were in agreement with those previously reported.[17,22,24] Consistent with the findings of Koene et al,[21] we also observed a clear-cut linkage of the FcγRIIIA-48 and FcγRIIIA-158 genotypes. All patients with the FcγRIIIA-158F/F genotype were also FcγRIIIA-48L/L, whereas all patients with either FcγRIIIA-48L/R or -48L/H expressed at least one valine at FcγRIIIA-158.

In these studies, we observed a trend for higher overall response rates in WM patients receiving rituximab who were FcγRIIIA-48L/H versus those patients with the FcγRIIIA-48L/L or -48L/R genotype. Larger studies will be needed, however, to validate these differences statistically. The observed increases in clinical activity for rituximab in WM patients with FcγRIIIA-L/H is consistent with the

work of de Haas et al,[22] who reported increased human IgG1 binding on NK cells expressing this genotype. However, as suggested by these studies, there appears to be a direct dependence for rituximab activity in patients with FcγRIIIA-48L/L with the genotype coexpressed at FcγRIIIA-158. Patients with the FcγRIIIA-48L/L genotype expressing at least one valine at FcγRIIIA-158 demonstrated a four-fold higher response rate versus those patients coexpressing FcγRIIIA-48L/L and -158F/F. In fact, the overall response rate for patients with FcγRIIIA-48L/L expressing at least one valine at FcγRIIIA-158 (36.8%) was closer in line to that of patients with FcγRIIIA-48L/R or -48L/H (35.3%), who because of linkage always express at least one valine at FcγRIIIA-158. These observations are consistent with those reported by Koene et al,[21] who demonstrated lower cell surface binding of human IgG1 and demonstration of cytophilic IgG staining in NK cells taken from individuals who were FcγRIIIA-48L/L and -158F/F versus patients who were either FcγRIIIA-48L/L, -48L/R, or -48L/H and expressed at least one valine at FcγRIIIA-158.

Central to the findings in this study was the significant impact of polymorphisms at FcγRIIIA-158 and responses to rituximab in WM patients. A better than a four-fold higher response rate (40% *v* 9.0%) was observed in patients with FcγRIIIA-158V/V versus -158F/F polymorphism. WM patients with the FcγRIIIA-158V/F genotype, who accounted for 44.8% of the patients in this study, demonstrated response characteristics that were more closely aligned with those of patients with FcγRIIIA-158V/V versus -158F/F (Table 6). The results of these studies are consistent with previous reports demonstrating increased cell surface human IgG1 binding, cytophilic IgG1 staining, and evidence of ADCC activity by NK cells from individuals expressing FcγRIIIA-158V/V versus -158F/F. Human IgG1 binding and cytophilic IgG1 staining by NK cells from individuals that expressed FcγRIIIA-158V/F were intermediate between those observed from individuals with FcγRIIIA-158V/V and -158F/F, and were influenced by the polymorphism present at FcγRIIIA-48, as shown by Koene et al.[21] We also observed a trend for a higher overall response rate among patients expressing at least one valine at FcγRIIIA-158 and the FcγRIIIA-48L/H polymorphism in particular. Larger studies will be needed to clarify the contribution of the polymorphisms at FcγRIIIA-48 and responses in patients with the FcγRIIIA-158V/V and -158V/F genotypes.

Although these studies, coupled with those by Cartron et al[17]and Weng et al,[24] support a role for FcγRIIIA polymorphisms in predicting responses to rituximab in patients with certain lymphomas, such insight may not be applicable to all B-cell malignancies, as suggested in study of chronic lymphocytic leukemia patients receiving rituximab by Farag et al.[25] However, for certain B-cell malignancies, advance knowledge of a patient's FcγRIIIA polymorphism status may facilitate a clinician's decision to employ rituximab monotherapy versus chemotherapy alone or combined rituximab and chemotherapy. Larger studies to validate such algorithms are planned. Moreover, as suggested by the work of Shields et al,[26] monoclonal antibodies could be modified by specific amino acid substitutions to enhance FcγRIIIA binding and NK cell–mediated ADCC activity in patients with either the -158F/F or -158V/V polymorphisms, thereby potentially making such antibodies more broadly successful. Interestingly, most of these substitutions involved a less bulky amino acid, and particularly benefited interactions with FcγRIIIA-158F/F over the -158V/V receptor. Given that phenylalanine is considerably bulkier than valine, steric hindrance may prevent ideal binding of NK cells bearing the FcγRIIIA-158F/F receptor to rituximab. We have initiated molecular modeling studies examining the impact of polymorphisms at -158 and -48 on FcγRIIIA binding to rituximab that may shed additional light on the molecular mechanisms by which polymorphisms influence rituximab responses.

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Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Consultant/Advisory Role: Steven P. Treon, Biogen Idec, Genentech; David G. Maloney, Biogen Idec, Genentech. Honoraria: Steven P. Treon, Biogen Idec, Genentech; Christos Emmanouilides, Biogen Idec, Genentech; Eva Kimby, Biogen Idec, Genentech; David G. Maloney, Biogen Idec, Genentech. Research Funding: Steven P. Treon, Genentech; David G. Maloney, Genentech. For a detailed description of these categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration form and the Disclosures of Potential Conflicts of Interest section of Information for Contributors found in the front of every issue.

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