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Attainment of complete/very good partial response following rituximab-based therapy is an important determinant to progression-free survival, and is impacted by polymorphisms in *FCGR3A* in Waldenstrom macroglobulinaemia

Steven P. Treon,[1,2] Guang Yang,[1,2] Christine Hanzis,[1] Leukothea Ioakimidis,[1] Sigitas J. Verselis,[3] Edward A. Fox,[2,3] Lian Xu,[1] Zachary R. Hunter,[1] Hsiuyi Tseng,[1] Robert J. Manning,[1] Christopher J. Patterson,[1] Patricia Sheehy[1] and Barry Turnbull[4]

[1]Bing Center for Waldenstrom's Macroglobulinaemia, Dana Farber Cancer Institute, [2]Harvard Medical School, [3]Molecular Diagnostics Center, Dana Farber Cancer Institute, and [4]BioBridges, Boston MA, USA

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Correspondence: Steven P. Treon M.D., M.A., Ph.D., Bing Center for Waldenström's Macroglobulinaemia, Dana Farber Cancer Institute, M547, 44 Binney Street, Boston MA 02115 USA.
E-mail: steven_treon@dfci.harvard.edu

Summary

The incorporation of rituximab into various regimens has improved depth of response in Waldenstrom macroglobulinaemia (WM), though the impact of achieving better responses remains to be determined. We examined response depth on progression-free survival (PFS) in 159 rituximab-naïve WM patients who received rituximab-based therapy. The median follow-up was 33·5 months, and categorical responses were as follows: complete response (CR, 8·8%); very good partial response (VGPR, 13·2%); partial response (50%); minor response (18·9%); Non-Responders (8·8%). Sequencing for polymorphic variants of *FCGR2A*, *FCGR2B*, and *FCGR3A* was performed, and impact on response depth determined. Achievement of better categorical responses was incrementally associated with improved PFS ($P < 0{\cdot}0001$). No separation was observed between CR and VGPR, and attainment of at least a VGPR was associated with improved time-to-progression. Neither age, serum IgM, haematocrit, platelet count, serum $\beta_2$microglobulin, WM International Prognostic Scoring System score, and treatment group predicted for CR/VGPR. Polymorphisms at *FCGR3A*-48 and -158 were associated with improved categorical responses, particularly attainment of CR/VGPR ($P \leq 0{\cdot}03$). The attainment of CR/VGPR was associated with significantly longer PFS in rituximab-naïve WM patients undergoing rituximab-based therapy, and was predicted by polymorphisms in *FCGR3A*.

Keywords: Waldenstrom macroglobulinemia, complete response, very good partial response, FCGR3A, rituximab.

With the incorporation of rituximab into the upfront therapy of Waldenström macroglobulinaemia (WM), the attainment of higher response rates, as well as very good partial response (VGPR) and/or complete response (CR) rates has improved (Dimopoulos *et al*, 2009; Treon, 2009). The attainment of better categorical responses, particularly a VGPR or better has been associated with improved progression-free (PFS) and/or overall survival (OS) in patients with multiple myeloma (Lahuerta *et al*, 2008; Wang *et al*, 2010; Moreau *et al*, 2011), though its influence in WM remains to be delineated as also are determinants for the achievement of better categorical responses.

One important determinant for the activity of rituximab, an IgG_1 class molecule, is the ability to bind to Fcγ receptors (FcγR) on effector cells, which are specific for IgG_1 class antibodies. The binding of FcγR with the antibody Fc domain triggers tumour cell killing through natural killer (NK) cell antibody-dependent cell-mediated cytotoxicity (ADCC) and macrophage-mediated phagocytosis (Manches *et al*, 2003). There are three distinct FcγR classes (FcγRI, FcγRII, and FcγRIII), which are encoded by eight genes in close proximity to one another at chromosome 1q21-23 (Grundy *et al*, 1989; de Wit *et al*, 1993). Each FcγR class expresses isotypes that function as either activating (FcγRI, FcγRIIa, FcγRIIc and FcγRIIIa) or inhibitory receptors (FcγRIIb and FcγRIIIb) (Ravetch & Bolland, 2001).

Polymorphisms within the *FCGR2A*, *FCGR2B* and *FCGR3A* genes can affect binding to the Fc portion of IgG immuno-

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globulins (de Haas *et al*, 1996; Koene *et al*, 1997). Polymorphisms at amino acid positions 48 and 158 on *FCGR3A*, and at 131 on *FCGR2A* have also been reported to impact clinical outcome to rituximab-based therapy in patients with indolent non-Hodgkin lymphoma (NHL), WM, as well as B-cell depletion in patients with systemic lupus erythematosus (Cartron *et al*, 2002; Anolik *et al*, 2003; Weng & Levy, 2003; Treon *et al*, 2005a; Paiva *et al*, 2008; Persky *et al*, 2009; Pierz *et al*, 2010). At *FCGR3A*-48, the expression of Leucine (L), Arginine (R) or Histidine (H) results in tri-allelic polymorphisms. Individuals who express L/R or L/H *versus* L/L at *FCGR3A*-48 demonstrate more avid IgG_1 binding (Koene *et al*, 1997). The expression of L/R or L/H at FcγRIIIa-48 is also associated with higher response rates to rituximab monotherapy *versus* L/L in WM patients (Treon *et al*, 2005a). At *FCGR3A*-158, the expression of valine (V) either in a homozygous (V/V) or heterozygous manner with phenylalanine (V/F), results in more avid IgG_1 binding *versus* phenylalanine alone (F/F) (Koene *et al*, 1997; Wu *et al*, 1997). Higher response rates to rituximab monotherapy have also been observed among patients with indolent NHL, including WM, who express at least one valine at *FCGR3A*-158 (Cartron *et al*, 2002; Anolik *et al*, 2003; Weng & Levy, 2003; Treon *et al*, 2005a; Paiva *et al*, 2008; Persky *et al*, 2009; Pierz *et al*, 2010).

The *FCGR2A* gene also displays two common polymorphisms at amino acid positions 27 and 131. At *FCGR2A*-27, glutamine (Q) and/or trypthophan (W) can be expressed, though do not appear to effect IgG_1 binding, while the impact of polymorphisms at this site on rituximab response remains to be clarified (Warmerdam *et al*, 1990). At *FCGR2A* -131, the expression of arginine (R) and histidine (H) generates variants with high (H/H), intermediate (H/R) and low (R/R) affinity for IgG_2, but not IgG_1 binding (Warmerdam *et al*, 1991; Parren *et al*, 1992). Despite the lack of impact on IgG_1 binding, higher response rates to rituximab have been reported in patients with follicular lymphoma expressing H/H at *FCGR2A* -131 (Weng & Levy, 2009).

The gene encoding FcγRIIb, an inhibitory FcγR, also displays a polymorphism at amino acid position 187 which can result in expression of either isoleucine (I) or tryptophan (T). Expression of T at *FCGR2B*-187 is associated with augmented inhibitory signalling in response to IgG_1 antibodies, though in one study in follicular NHL patients, polymorphisms at *FCGR2B*-187 did not predict for clinical response to rituximab monotherapy (Weng & Levy, 2009).

While the impact of FcγR polymorphisms has been investigated in WM patients receiving rituximab monotherapy, their impact on predicting outcome, including depth of response, has not been investigated in patients receiving combined rituximab therapy. We therefore examined the impact of categorical responses on PFS on 159 WM patients, who were rituximab naïve and who received a rituximab-based regimen. We also assessed the impact of previously validated prognostic determinants including advanced age, serum IgM, haematocrit, serum $\beta_2$microglobulin (B_2M), the WM International Prognostic Staging System (IPSS) score, previous treatment status, treatment, and polymorphisms in *FCGR2A*, *FCGR2B* and *FCGR3A* on categorical response attainment (Dimopoulos *et al*, 2005; Dhodapkar *et al*, 2009; Morel *et al*, 2009).

Patients and methods

Patients with the consensus panel diagnosis of WM, who were rituximab-naïve, and who received a rituximab-containing regimen were eligible for this analysis. The study was approved by the Dana Farber Cancer Institute/Harvard Cancer Center Institutional Review Board. Response determinations were made using modified consensus criteria, and response rates determined on an intent-to-treat basis (Kimby *et al*, 2006; Treon *et al*, 2009a,b). A CR was defined as having resolution of all symptoms, normalization of serum IgM levels with complete disappearance of IgM paraprotein by immunofixation, a bone marrow biopsy demonstrating no evidence of disease, and resolution of any adenopathy or splenomegaly. A VGPR, partial response (PR), and a minor response (MR) were defined as achieving a ≥90%, 50–89%, and 25–49% reduction in serum IgM levels, respectively. Patients with no response (NR) were defined as having <25% change in serum IgM levels. Progressive disease occurred with ≥25% increase in serum IgM level from the lowest attained response value or progression of clinically significant disease-related symptom(s). PFS was calculated from the start of therapy using the Kaplan–Meier method. The primary endpoint of this study was impact of best-attained categorical response achievement on PFS. The impact of advanced age (≥65 years), serum B_2M level, serum IgM level, haematocrit, WM IPSS Score, previous treatment status, treatment, and polymorphisms at *FCGR2A*-27, -131, FCGR2B-187, and FCGR3A-48, -158 were also assessed for their impact on categorical response attainment.

Genotyping analysis of FCGR2A, FCGR2B and FCGR3A polymorphisms

The *FCGR* gene polymorphisms were determined by allele-specific polymerase chain reaction (PCR) and directed sequencing of genomic DNA as previously described (Treon *et al*, 2005a; Hatjiharissi *et al*, 2007). Genomic DNA derived from peripheral blood was available for 75 patients and was extracted using a DNA isolation kit following the manufacturer's instructions (Qiagen, Valencia, CA, USA).

Statistical analysis

Comparison of pre- and post-treatment parameters was performed using a two-tailed student's *t*-test on Microsoft Excel™ software. Non-parametric testing of pre- and post-treatment variables was performed by Chi Square testing (Vassar Stats). Kaplan–Meier curves for PFS were plotted and compared with the use of the log-rank test. Prognostic factors

for PFS were determined by means of the Cox proportional hazards model using univariate analysis. A *P*-value ≤0·05 was deemed to be significant for all comparisons. Either *P*-value or indication of non-significance (NS) is denoted in text and figures.

Results

Baseline patient characteristics

Baseline patient characteristics were as follows: median age 62 (range 32–86) years; serum IgM 36·7 (range 4·58–124) g/l; haematocrit 31·7% (range 14·5–50·5%); platelet count 235 (range 24–597) $\times 10^9$/l; B_2M 2·9 (range 1·0–13·7) g/l, and serum $B_2M \geq 3{\cdot}0$ g/l (n = 82; 52%). WM IPSS score was I (n = 64; 40%); II (n = 57; 36%); and III (n = 38; 24%). One hundred and seven patients (67·3%) were previously untreated. All patients were on a rituximab-containing regimen with either cyclophosphamide (Cy-R; n = 58; 35%); fludarabine (Flu-R; n = 43; 27%); immunomodulatory agent (Imid-R; n = 35; 22%); or bortezomib (Bo-R; n = 23; 15%) as previously reported (Treon *et al*, 2008a,b, 2009a,b; Ioakimidis *et al*, 2009). The median follow-up for all patients was 33·5 months, and the major and overall response rates were 72% and 91% respectively, with categorical responses as follows: CR (n = 14; 8·8%); VGPR (n = 21; 13·2%); PR (n = 79; 50%); MR (n = 30; 18·9%); NR (n = 14; 8·8%). The median number of rituximab infusions for all patients was 6 (range 3–8), and was not significantly different between patients stratified by treatment group or categorical response attainment. The median PFS by treatment groups were as follows: Cy-R, 34 months; Flu-R, 48·0 months; Imid-R, 39·1 months; and Bo-R, >35·9 months, and did not differ by log rank analysis (P = 0·207).

Impact of categorical response attainment on progression-free survival

Achievement of better categorical response was incrementally associated with improved PFS ($P < 0{\cdot}0001$ by log rank analysis), though no separation of curves was observed with the current follow-up between CR and VGPR (Fig 1A). The estimated median time-to-progression (TTP) for patients with CR and VGPR was >90 and >75 months, respectively. For patients with PR and MR, the median TTP was 42·8 and 30·8 months, respectively. For non-responders, the median TTP was 10·6 months. Among responders, the attainment of at least a VGPR was associated with improved TTP (Fig 1B; P = 0·04). Insufficient events (four deaths) did not permit for an assessment of categorical response impact on overall survival at this time.

Predictive factors for attainment of CR/VGPR in rituximab naïve WM patients receiving rituximab-based therapy

We next examined the impact of previously established prognostic criteria on categorical response attainment. Neither age, serum IgM, haematocrit, platelet count, serum B_2M, WM IPSS score, previous treatment status or treatment group predicted for CR/VGPR attainment (P = NS). Since in previous studies, we showed that polymorphisms in *FCGR3A*-158 predicted outcome to single agent rituximab therapy, we also examined the impact of *FCGR3A*-158, as well as other important polymorphisms implicated in modulating IgG antibody binding and activation: *FCGR3A*-48, *FCGR2A*-27, *FCGR2A*-131, and *FCGR2B*-187. These studies were performed in 75/159 (47·2%) patients for whom genomic DNA was available for genotyping, and were representative across categorical response categories, i.e. CR/VGPR (18/35; 51·4%); PR (37/79; 46·8%); MR (11/30; 36·6%); NR (9/14; 64·2%); P = NS. The distribution of this patient subset across response categories showed no differences in baseline age, serum IgM, haematocrit, platelet count, serum B_2M, WM IPSS score, previous treatment status or treatment group (P = NS). Polymorphisms at *FCGR3A*-158 and *FCGR3A*-48 were associated with attainment of CR/VGPR. Fourteen of 18 (77·8%) patients attaining CR/VGPR expressed at least one valine (V/-) at *FCGR3A*-158 in comparison to 17/57 (29·8%) patients who were either non-responders or who attained a less than VGPR

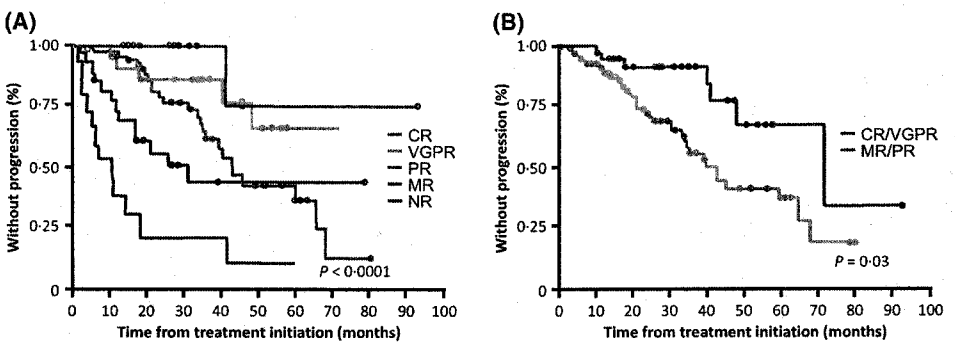

Fig 1. Impact of categorical response attainment on progression-free survival (A) and time-to-progression (B). CR, complete response; VGPR, very good partial response; PR, partial response; MR, major response; NR, no response; SD, stable disease.

response ($P = 0{\cdot}008$). At *FCGR3A*-48, 8/18 (44·4%) of patients who attained CR/VGPR expressed either LH or LR, in comparison to 9/57 (15·5%) of patients who were either non-responders or who attained a less than VGPR response ($P = 0{\cdot}03$). In contrast, polymorphisms at *FCGR2A*-27, *FCGR2A*-131, and *FCGR2B*-187 were not associated with CR/VGPR attainment.

Discussion

The incorporation of rituximab into various regimens has improved the depth of response in WM, though the impact of achieving better responses remains to be determined. The outcome of this study supports that attainment of at least a VGPR is associated with significantly longer PFS in rituximab-naïve WM patients treated a rituximab-containing regimen. This study, which examined a broader array of available combination rituximab therapies for WM patients, affirms the findings from two of our previous studies (Treon *et al*, 2009a,b) that encompassed patients treated with either bortezomib- or fludarabine-based rituximab therapy. In these studies, improved PFS was also observed in patients achieving VGPR or better (Treon *et al*, 2009a,b). As part of these efforts, we also examined the impact of previously defined prognostic factors on categorical response attainment. Similar to the findings recently reported by Laszlo *et al* (2010) among predominantly rituximab naïve patients who received subcutaneous cladribine and rituximab, the WM IPSS score did not predict for categorical response attainment. In addition, serum IgM and B_2M levels, which were reported as a determinants of response in some, but not other rituximab treatment-based studies in WM patients, did not predict for CR/VGPR in this larger study population (Dimopoulos *et al*, 2004, 2008; Treon *et al*, 2005b; Dhodapkar *et al*, 2009; Morel *et al*, 2009).

An important observation in this study was the impact of polymorphisms in *FCGR3A*-158 and *FCGR3A*-48 for predicting CR/VGPR attainment. This was particularly significant for *FCGR3A* -158, as nearly 80% of patients who attained CR/VGPR expressed at least one valine at this polymorphic locus. The expression of at least one valine at *FCGR3A*-158 was previously shown to be associated with overall and major responses to rituximab monotherapy in patients with WM (Treon *et al*, 2005a). Polymorphisms at *FCGR3A*-48 were also associated with response to rituximab monotherapy in WM patients. However, as with our previous study, linkage disequilibrium to *FCGR3A*-158 may have accounted for this finding in the present study, as 37/37 (100%) patients who exhibited F/F at *FCGR3A*-158 also expressed L/L at *FCGR3A*-48, a polymorphism associated with less IgG binding, as well as response to single agent rituximab in WM patients. In contrast, only 1/8 patients who were V/V, and 16/30 patients who were V/F at *FCGR3A*-158, exhibited L/L ($P < 0{\cdot}0001$). The demonstration of linkage disequlibrium within polymorphic loci at *FCGR3A*, as well as between polymorphic loci at *FCGR2A* has been reported by us and others, and supports a primacy for polymorphisms at *FCGR3A*-158 in predicting outcomes to IgG class antibodies, such as rituximab (Treon *et al*, 2005a; Hatjiharissi *et al*, 2007; Lejeune *et al*, 2008).

An important realization from these studies is that clinical outcome to rituximab-based therapy is, in fact, genetically determined, and that an individual patient's genetic makeup plays an important role in heralding treatment response. Testing for *FCGR3A*-158 polymorphic testing is now cleared by the United States Food and Drug Administration for clinical use, and could be considered in future studies aimed at stratifying patients whose polymorphisms predict for lower likelihood of response to rituximab-based therapy. Such strategies could include the use of novel monoclonal antibodies that are indifferent to Fc receptor polymorphisms or are capable of inducing cell killing independent of effector cells or complement activity. One such candidate is GA101, a novel anti-CD20 humanized antibody that exhibits high rates of antibody-dependent cell mediated killing of WM cells, independent of *FCGR3A*-158 polymorphisms, as well as direct induction of apoptosis (Salles *et al*, 2008; Mössner *et al*, 2010; Yang *et al*, 2010).

The results of these studies may also have important implications in the care of WM patients, and suggest that strategies aimed at improving VGPR or better categorical responses may lead to improved outcomes. Whether these outcomes extend to non-rituximab based therapies for WM remains to be delineated. Strategies aimed at improving depth of response could include novel induction agents and combinations, maintenance rituximab, autologous stem cell transplantation, and/or radioimmunotherapy (Morschhauser *et al*, 2008; Treon *et al*, 2009c; Kyriakou *et al*, 2010).

The data presented also affirm the value of documenting MR and VGPR as formal response categories in WM patients. Previous studies have shown that clinical benefit and long-term disease control do occur with MR in WM patients (Treon *et al*, 2005b; Gertz *et al*, 2009). Also, attainment of VGPR or better was associated with improved PFS in two previous smaller series (Treon *et al*, 2009a,b). The results of this much larger study affirm the findings of those earlier trials, and support the continued presence of MR, and the incorporation of VGPR in the formal response assessment of WM patients.

In summary, the attainment of CR/VGPR is associated with significantly longer PFS in rituximab-naïve WM patients undergoing rituximab-based therapy, and is predicted by polymorphisms in *FCGR3A*. Efforts aimed at improving categorical responses may lead to improved treatment outcomes in patients with WM.

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Authorship contributions

SPT designed the study. LI, CH, RJM, CJP collected data for this study. SPT and ZRH analyzed the data. SPT and PS procured and provided patient care for subjects on this study.

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