Published in final edited form as:

Leuk Lymphoma. 2009 May ; 50(5): 723–727. doi:10.1080/10428190902829441.

Genetic polymorphism of the inhibitory IgG Fc receptor FcγRIIb is not associated with clinical outcome in patients with follicular lymphoma treated with rituximab

WEN-KAI WENG¹ and RONALD LEVY²

¹ Division of Blood and Marrow Transplantation, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

² Division of Medical Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

Abstract

Polymorphisms of activating FcγRIIIa (CD16) and FcγRIIa (CD32a) have been found to predict rituximab response, probably because of the relative efficiency of different FcγR variants in performing antibody-dependent cellular cytotoxicity. The inhibitory FcγRIIb (CD32b) has an opposing effect on effector cells. Here, we examined whether an FcγRIIb 232 isoleucine (I)/threonine (T) polymorphism predicts rituximab response in 101 patients with follicular lymphoma. Eighty-four patients were 232 I/I, 15 were 232 I/T and two were 232 T/T. The response rate was similar among the three groups. The 2-year progression free survival (PFS) and median time to progression (TTP) were not different between I/I and I/T groups. The TTP was not determined in T/T group because of small number of patients. The FcγRIIIa 158 V/V and FcγRIIa 131 H/H genotypes continued to emerge as independent predictors for higher response rate and longer TTP. This study is the first to determine whether inhibitory FcγRIIb play a role in rituximab's anti-tumor effect in humans.

Keywords

Follicular lymphoma; rituximab; FcγR; polymorphism; antibody-based immunotherapy; lymphoma and Hodgkin disease; immunotherapy

Introduction

Although the anti-CD20 mAb, rituximab, is effective in treating B-cell NHL, the exact mechanism of its anti-tumor effect is still under investigation. The role for antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity has been suggested [1–4]. Whether ADCC or complement-mediated process is the main mechanism is still controversial. Supporting the role of ADCC, polymorphisms of activating IgG Fc receptor (FcγR) have been found to predict the rituximab response in patients with follicular lymphoma [5,6]. In ADCC, rituximab binds to CD20 and then bridges the natural killer (NK) cells and macrophages via the FcγR on these effector cells. The cells then become activated and kill the antibody-coated tumors [7,8]. The effectiveness of ADCC largely depends on how well the effector cells are activated after the engagement of FcγR. Three

classes of FcγR are found on effector cells that regulate their activation: FcγRIIa (CD32a), FcγRIIIa (CD16) activate and FcγRIIb (CD32b) inhibits activation. FcγRIIIa is expressed on both NK cells and macrophages, whereas FcγRIIa and FcγRIIb are found only on macrophages. Polymorphisms in the extra-cellular domains of FcγRIIIa and FcγRIIa have been identified to affect their ability to interact with the Fc of immunoglobulin. For the FcγRIIIa, receptor of valine allele at position 158 (158 V) binds human IgG1 better than the one of 158 phenylalanine (158 F) allele [9]. In the case of FcγRIIa, receptor of histidine allele at position 131 (131 H) binds the Fc of rituximab better than the one of 131 arginine (131 R) allele (Weng and Levy, unpublished observation). This increased binding also translates to enhanced activation of effector cells and better ADCC [10,11] that may lead to clinical efficacy of rituximab therapy. In contrast to FcγRIIIa and FcγRIIa, an inhibitory FcγRIIb inhibits ADCC when being co-engaged by antibody [12]. FcγRIIb is expressed by both macrophages and B lymphocytes. Recently, a 232 I/T polymorphism at the transmembrane domain of FcγRIIb was identified that may affect the inhibitory property of this receptor [13,14]. Therefore, it is possible that this polymorphism may influence the effector cells' ability to perform ADCC and their anti-tumor effect. In the present study, we tested whether the FcγRIIb 232 I/T polymorphism may correlate with rituximab response.

Materials and methods

Patient population

This study included 101 patients with follicular lymphoma, who were treated at Stanford Medical Center between 1993 and 2005 with rituximab as a single agent. They were selected in this study because of the availability of their tissue samples and their known clinical response to rituximab. The patient characteristics were summarised in Supplemental Table I. Twenty patients had received rituximab as first-line therapy and 81 patients were treated for relapsed disease. All 101 patients received rituximab for the first time. Ninety five patients had four weekly and five patients had eight weekly infusions of 375 mg/m², and one had four weekly infusions of 250 mg/m². No maintenance rituximab was given after initial 4–8 weeks of infusion. The median follow-up after rituximab therapy was 3.43 years for the entire group. Clinical responses were determined between 1 and 3 months after last rituximab infusion and every 3 months thereafter according to the Cheson Criteria retrospectively by reviewing patient charts and medical records [15]. Maximal responses were observed at 1–3 months in all but four patients, who had partial responses at 1–3 months and showed further tumor shrinkage at later time points. This study was conducted according to an institutional review board-approved protocol and informed consent was obtained from all patients.

Analysis of FcγR polymorphism

Genomic DNA was prepared from tumor cells or PBMC using a QIAGEN DNA extraction kit or prepared from the serum as described [16]. Genotyping of FcγR polymorphisms was performed using the TaqMan technology on an ABI Prism 7900HT Sequence Detector System (Applied Biosystems, Foster City, CA). In brief, FcγRIIIa, FcγRIIa and FcγRIIb-specific primer pairs flanking the polymorphic sites were used for amplification of genomic DNA in presence of probes specific to different alleles. Probes specific to FcγRIIIa 158 V, FcγRIIa 131 H and FcγRIIb 232 T alleles were labelled with VIC and probes specific to FcγRIIIa 158 F, FcγRIIa 131 R and FcγRIIb 232 I alleles were labelled with FAM. Each sample was set up as duplicate. The final determination of FcγR genotypes was performed using Allelic Discrimination protocol in SDS software provided by Applied Biosystems.

Statistical analysis

The clinical responses were compared using two-tailed Fisher exact test (PRISM for Macintosh, GraphPad Software, San Diego, CA). A logistic regression analysis including age ($\geq$ or <60 years), Stage (III vs. IV), presence of bulky disease, number of extranodal sites ($\geq$ or <2), prior bone marrow transplantation, Fc γ RIIa, Fc γ RIIb and Fc γ RIIIa genotype was used to identify independent prognostic variables influencing the clinical responses (StatView 5.0.1, SAS, Cary, NC). Using Cox Proportional Hazard Model, a multi-variant analysis was performed to identify independent prognostic variables influencing the PFS (StatView 5.0.1, SAS, Cary, NC).

Results

Clinical response to rituximab therapy and Fc γ RIIb 232 I/T polymorphism

ADCC is the leading candidate for rituximab's anti-tumor action *in vivo*. The effectiveness of ADCC largely depends on the activation of effector cells after engagement of their Fc γ R with antibody-coated target cells. Patients with high-affinity genotypes of the activating Fc γ RIIIa 158 V/V or Fc γ RIIa 131 H/H had higher rituximab response rate [5,6]. On the other hand, the inhibitory Fc γ R has been shown to negatively affect the rituximab's efficacy in a mouse model [17]. Recently, 232 I/T polymorphism of Fc γ RIIb was found to affect its inhibitory ability in B cells [13,14,18] and probably effector cell function during ADCC. Here, we tested whether 232 I/T polymorphism predicts the rituximab response in a group of 101 patients with follicular lymphoma, which included the 87 patients described in our previous report [6], of which longer follow-up is now available. In this new sample set, 84 (83%) were homozygous 232 I/I, 15 (15%) were heterozygous 232 I/T and 2 (2%) were homozygous 232 T/T. The numbers of patients having the heterozygous I/T and homozygous T/T genotype were relatively low (17 patients).

The 232 I/I, I/T and T/T groups were not different in terms of patient characteristics (Supplemental Table I). The response rate in three groups was similar at 1–3, 6, 9 and 12 months after rituximab therapy (64% vs. 67% vs. 50% at 1–3 months, $p=0.898$; 52% vs. 62% vs. 50% at 6 months, $p=0.805$; 41% vs. 54% vs. 50% at 9 months, $p=0.615$; 35% vs. 46% vs. 0% at 12 months, $p=0.425$). The PFS at 2 years was 20% for patients with 232 I/I and 43% for I/T using the Kaplan-Meier estimation with median TTP of 6.9 and 9.2 months for the two groups, respectively [Figure 1(a)]. The PFS and TTP were not determined in T/T group because of small number ($N=2$). Additionally, the Fc γ RIIb 232 I/T polymorphism showed no impact on the response rate in a multivariate analysis by logistic regression analysis (Table I), or on PFS using Cox Proportional Hazard Model (Table II).

Fc γ RIIIa 158 V/V and Fc γ RIIa 131 H/H genotype still associate with better rituximab response

In this extended patient population with longer follow-up, Fc γ RIIIa 158 V/V and Fc γ RIIa 131 H/H once again emerged as the only two independent positive predictors for higher response rate and longer PFS after rituximab therapy in multivariate analyses [Tables I and II, Figures 1(b) and 1(c)]. More than two extranodal sites was identified as a negative predictive factors for PFS (Table II).

Discussion

The inhibitory Fc γ R has been shown to be involved in the anti-tumor effect of antibodies. In one such example, the anti-tumor effect of rituximab significantly increased in mice deficient in the inhibitory Fc γ R [17], suggesting that inhibitory Fc γ R may negatively affect the function of the effector cells. Recently, a newly identified Fc γ RIIb 232 I/T

polymorphism has been shown to affect the downstream signalling pathway through B cell receptors (BCR) in B cells [14,18]. In this case, BCR-mediated calcium mobilization was attenuated by the 232 I allele more efficiently than by the 232 T allele. This may be because of a relative deficiency in recruiting phosphatase into the signalling complex and failure of the Fc γ RIIb of 232 T allele to migrate to lipid rafts after BCR ligation [18]. It is possible that Fc γ RIIb 232 I/T polymorphism may affect the function of effector cells, such as macrophages, during the process of ADCC and, thereby affect the efficacy of rituximab. However, in the present study, we found no association between Fc γ RIIb 232 I/T polymorphism and clinical response to rituximab [Tables I and II, Figure 1(a)]. This observation has several important implications: First, in contrast to B cells, the 232 I/T polymorphism may have a lesser effect on the inhibitory signal on effector cells. Although it is possible that Fc γ RIIb-bearing macrophages do not play a major role in the anti-tumor effect of rituximab, the independent predictive value of Fc γ RIIa 131 H/R polymorphism suggests otherwise, as macrophages but not NK cells express the Fc γ RIIa. Because Fc γ RIIb is also expressed on lymphoma B cells, whether the Fc γ RIIb 232 I/T polymorphism has opposing effect on effector cells and target lymphoma cells is unclear. In this regard, a recent publication failed to identify a correlation between Fc γ RIIb expression on lymphoma cells and the clinical response to combination therapy with rituximab and cyclophosphamide/doxorubicin/vincristine/prednisone (CHOP) chemotherapy in patients with diffuse large B cell lymphoma [19]. However, the influence of Fc γ RIIb expression on treatment response in patients with follicular lymphoma is unknown. Secondly, the low number ($n=17$) of patients having T allele makes it difficult to detect a difference in clinical outcome between different genotype groups. On the other hand, only 15 patients have Fc γ RIIa V/V genotype and they had significantly better clinical outcome. It argues that the Fc γ RIIb 232 I/T polymorphism either have no impact on clinical outcome or the impact was too small to detect with limited number of patients in this study.

Thirdly, given the close proximity of these Fc γ R genes on Chromosome 1, the possibility of linkage disequilibrium between Fc γ RIIa and Fc γ RIIb has been raised to explain the predictive value of Fc γ RIIa 131 H/R polymorphism. Recent studies have identified a linkage disequilibrium between Fc γ RIIa V allele and Fc γ RIIb H allele in Caucasian population [20,21]. This linkage disequilibrium has been suggested to be the reason for the association between Fc γ RIIa H/H genotype and rituximab response. However, the lack of association between Fc γ RIIb polymorphism and rituximab efficacy helps to confirm the specificity of the predictive value of the two activating Fc γ R polymorphisms. Indeed, in multivariate analyses, Fc γ RIIa 158 V/V and Fc γ RIIa 131 H/H emerged as two independent predictors for higher response rate and longer TTP (Tables I and II). Consistent with our finding in rituximab therapy, the predictive value of these two Fc γ R polymorphisms was also observed in other therapeutic antibodies including anti-HER2/Neu antibody [22–24]. We have also found that high affinity Fc γ RIIa 158 V/V genotype was associated with better clinical outcome in patients receiving active idiotype vaccination [25]. In this case, we postulated that having a more effective Fc receptor bearing effector cells enhanced the action of anti-tumor antibodies induced by vaccination.

Finally, the lack of association between Fc γ RIIb 232 I/T polymorphism and rituximab efficacy does not rule out a role for inhibitory Fc γ RIIb in rituximab's anti-tumor effect as the animal model has suggested [17]. It is possible that the expression level of Fc γ RIIb may have a profound effect on rituximab's clinical efficacy. A direct measurement of the Fc γ RIIb level on the surface of effector cells of individual patients will help to address this possibility. However, the un-availability of effector cell specimen prevent this kind of analysis in this study.

Although the Fc γ RIIb 232 I/T polymorphism has been shown to affect the signalling property of this inhibitory receptor and to associate with auto-immune diseases [26], we found no association between this polymorphism and response to rituximab therapy in patients with follicular lymphoma. However, the true contribution of inhibitory Fc γ RIIb in rituximab's clinical efficacy is still unclear. Clinical trials using the next generation of anti-tumor antibodies with increased affinity to activating Fc γ RIIIa and Fc γ RIIa and decreased affinity to inhibitory Fc γ RIIb will help answer this question.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work is supported by grants CA34233 and CA33399 from the U.S. Public Health Service-National Institutes of Health (NIH). W.-K. W. is recipient of a NIH-NCI K08 Award CA111827. R. L. is an American Cancer Society Clinical Research Professor.

References

1. Anderson DR, Grillo-Lopez A, Varns C, Chambers KS, Hanna N. Targeted anti-cancer therapy using rituximab, a chimeric anti-CD20 antibody (IDEC-C2B8) in the treatment of non-Hodgkin's B-cell lymphoma. *Biochem Soc Trans* 1997;25:705–708. [PubMed: 9191187]
2. Golay J, Zaffaroni L, Vaccari T, Lazzari M, Borleri G-M, Bernasconi S, et al. Biological response of B lymphoma cells to anti-CD20 monoclonal antibody rituximab *in vitro*: CD55 and CD59 regulate complement-mediated cell lysis. *Blood* 2000;95:3900–3908. [PubMed: 10845926]
3. Weng W-K, Levy R. Expression of complement inhibitors CD46, CD55, and CD59 on tumor cells does not predict clinical outcome after rituximab treatment in follicular non-Hodgkin lymphoma. *Blood* 2001;98:1352–1357. [PubMed: 11520782]
4. Manches O, Lui G, Chaperot L, Gressin R, Molens J-P, Jacob M-C, et al. *In vitro* mechanisms of action of rituximab on primary non-Hodgkin lymphomas. *Blood* 2003;101:949–954. [PubMed: 12393572]
5. Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor Fc γ R IIIa gene. *Blood* 2002;99:754–758. [PubMed: 11806974]
6. Weng W-K, Levy R. Two immunoglobulin G Fc receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol* 2003;21:3940–3947. [PubMed: 12975461]
7. Fanger MW, Shen L, Graziano RF, Guyre PM. Cytotoxicity mediated by human Fc receptors for IgG. *Trend Immunol* 1989;10:92–99.
8. Graziano RF, Ranger MW. Fc γ RI and Fc γ RII on monocytes and granulocytes are cytotoxic trigger molecules for tumor cells. *J Immunol* 1987;139:3536–3541. [PubMed: 2960735]
9. Koene HR, Kleijer M, Algra J, Roos D, von dem Borne A, de Haas M. Fc γ RIIIa-158 V/F polymorphism influences the binding of IgG by natural killer cell Fc γ RIIIa, independently of the Fc γ RIIIa-48 L/R/H phenotype. *Blood* 1997;90:1109–1114. [PubMed: 9242542]
10. Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, et al. High resolution mapping of the binding site on human IgG1 for Fc γ RI, Fc γ RII, Fc γ RIII, and Fc γ Rn and design of IgG1 variants with improved binding to the Fc γ R. *J Biol Chem* 2001;276:6591–6604. [PubMed: 11096108]
11. Vance BA, Huizinga TWJ, Wardwell K, Guyre PM. Binding of monomeric human IgG defines an expression polymorphism of Fc γ RIII on large granular lymphocyte/natural killer cells. *J Immunol* 1993;151:6429–6439. [PubMed: 8245476]
12. Nimmerjahn F, Ravetch JV. Fc γ receptors: old friends and new family members. *Immunity* 2006;24:19–28. [PubMed: 16413920]

13. Kyogoku C, Dijstelbloem HM, Tsuchiya N, Hatta Y, Yamaguchi A, Fukazawa T, et al. Fc γ receptor gene polymorphism in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. *Arthritis Rheum* 2002;46:1242–1254. [PubMed: 12115230]
14. Li X, Wu J, Carter RH, Edberg JC, Su K, Cooper GS, et al. A novel polymorphism in the Fc γ receptor IIB (CD32B) transmembrane region alters receptor signaling. *Arthritis Rheum* 2003;48:3242–3252. [PubMed: 14613290]
15. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphoma. *J Clin Oncol* 1999;17:1244–1253. [PubMed: 10561185]
16. Kopreski MS, Benko FA, Kwee C, Leitzel KE, Eskander E, Lipton A, et al. Detection of mutant K-ras DNA in plasma or serum of patients with colorectal cancer. *Br J Cancer* 1997;76:1293–1299. [PubMed: 9374374]
17. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptor modulate *in vivo* cytotoxicity against tumor targets. *Nature Med* 2000;6:443–446. [PubMed: 10742152]
18. Kono H, Kyogoku C, Suzuki T, Tsuchiya N, Honda H, Yamamoto K, et al. Fc γ RIIB Ile232Thr transmembrane polymorphism associated with human systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. *Human Mol Gene* 2005;14:2881–2892.
19. Camilleri-Broet S, Mounier N, Delmer A, Briere J, Casasnovas O, Cassard L, et al. Fc γ RIIB expression in diffuse large B-cell lymphomas does not alter the response to CHOP + rituximab (R-CHOP). *Leukemia* 2004;18:2038–2040. [PubMed: 15470484]
20. Lejeune J, Thibault G, Ternant D, Cartron G, Watier H, Ohresser M. Evidence for linkage disequilibrium between Fc γ RIIIa-V158F and Fc γ RIIA-H131R polymorphisms in white patients, and for an Fc γ IIIa-restricted influence on the response to therapeutic antibodies. *J Clin Oncol* 2008;26:5489–5491. [PubMed: 18955438]
21. van der Pol WL, Jansen MD, Sluiter WJ, van de Sluis B, Leppers-van de Straat FGJ, Kobayashi T, et al. Evidence for non-random distribution of Fc γ receptor genotype combinations. *Immunogenetics* 2003;55:240–246. [PubMed: 12830330]
22. Miescher S, Spycher MO, Amstutz H, de Haas M, Kleijer M, Kalus UJ, et al. A single recombinant anti-RhD IgG prevents RhD immunization: association of RhD-positive red blood cell clearance rate with polymorphism in the Fc γ RIIA and Fc γ RIIIA genes. *Blood* 2004;103:4028–4035. [PubMed: 14976055]
23. Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER2/neu-positive metastatic breast cancer. *J Clin Oncol* 2008;26:1789–1796. [PubMed: 18347005]
24. Zhang W, Gordon M, Schultheis AM, Yang DY, Nagashima F, Azuma M, et al. FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor-expression metastatic colorectal cancer patients treated with single-agent cetuximab. *J Clin Oncol* 2007;25:3712–3718. [PubMed: 17704420]
25. Weng W-K, Czerwinski D, Timmerman J, Hsu FJ, Levy R. Clinical outcome of lymphoma patients after idiotype vaccination is correlated with humoral immune response and immunoglobulin G Fc receptor genotype. *J Clin Oncol* 2004;22:4717–4724. [PubMed: 15483014]
26. Chu ZT, Tsuchiya N, Kyogoku C, Ohashi J, Wian YP, Xu SB, et al. Association of Fc γ receptor IIb polymorphism with susceptibility to systemic lupus erythematosus in Chinese: a common susceptibility gene in the Asian populations. *Tissue Antigens* 2004;63:21–27. [PubMed: 14651519]

Figure 1.

Kaplan-Meier estimates of progression free survival by FcγR polymorphism. (a) Progression free survival curves were plotted by FcγRIIb 232 I/T genotype on all 101 patients. TTP: median time to progression. (b) Progression free survival curves were plotted by FcγRIIa 131 H/R genotype. R carrier: patients with H/R and R/R. (c) Progression free survival curves were plotted by FcγRIIIa 158 V/F genotype. F carrier: patients with V/F and F/F.

Table I

Prognostic factors for clinical response: logistic regression analysis.

	1–3 months	6 months	9 months	12 months
232 I/I	0.79* (0.24–2.69) 0.712 [†]	0.50 (0.13–1.86) 0.302	0.45 (0.12–1.72) 0.245	0.81 (0.20–3.38) 0.767
158 V/V	13.47 (1.55–116.72) 0.018	11.14 (2.09–59.29) 0.005	10.94 (2.24–53.33) 0.003	19.58 (3.73–102.80) 0.001
131 H/H	3.50 (0.99–12.41) 0.052	9.32 (2.34–37.22) 0.002	8.44 (2.37–30.01) 0.001	5.62 (1.69–18.67) 0.005
Stage III vs. IV	1.29 (0.40–4.15) 0.672	1.29 (0.40–4.23) 0.669	0.66 (0.20–2.17) 0.499	1.05 (0.30–3.66) 0.941
Age ≥ 60	0.56 (0.17–1.80) 0.331	0.71 (0.21–2.40) 0.582	0.61 (0.17–2.24) 0.460	0.56 (0.14–2.28) 0.419
Prior transplant therapy	3.35 (0.58–19.22) 0.175	2.99 (0.61–14.65) 0.175	1.89 (0.38–9.42) 0.437	2.82 (0.56–14.19) 0.210
Bulky disease	0.91 (0.33–2.33) 0.842	0.88 (0.32–2.40) 0.803	0.94 (0.34–2.58) 0.899	1.91 (0.64–5.68) 0.244
≥2 extranodal sites of disease	0.66 (0.14–3.09) 0.596	0.22 (0.04–1.31) 0.096	0.21 (0.03–1.41) 0.108	0.20 (0.03–1.50) 0.116

* Odds ratio: relative odds of response to rituximab treatment, values in parentheses are 95% confidence intervals.

[†] All *p* values are two-sided and considered to be statistically significant for *p* < 0.05.

Table II

Prognostic factors for freedom from progression: cox proportional hazard model.

	Relative benefit [*]	CI	<i>p</i> [†]
232 I/I	0.76	0.39–1.47	0.409
158 V/V	2.59	1.32–5.08	0.006
131 H/H	2.68	1.46–4.92	0.001
Stage III	1.13	0.64–2.00	0.683
Age ≥ 60	0.66	0.38–1.14	0.139
Prior transplant therapy	1.66	0.78–3.59	0.193
Bulky disease	1.28	0.78–2.10	0.331
≥2 extranodal sites of disease	0.37	0.18–0.80	0.011

CI, confidence intervals.

^{*} Relative benefit: to have longer freedom from progression from last rituximab, CI: 95% confidence intervals.[†] All *p* values are two-sided and considered to be statistically significant for *p* < 0.05.