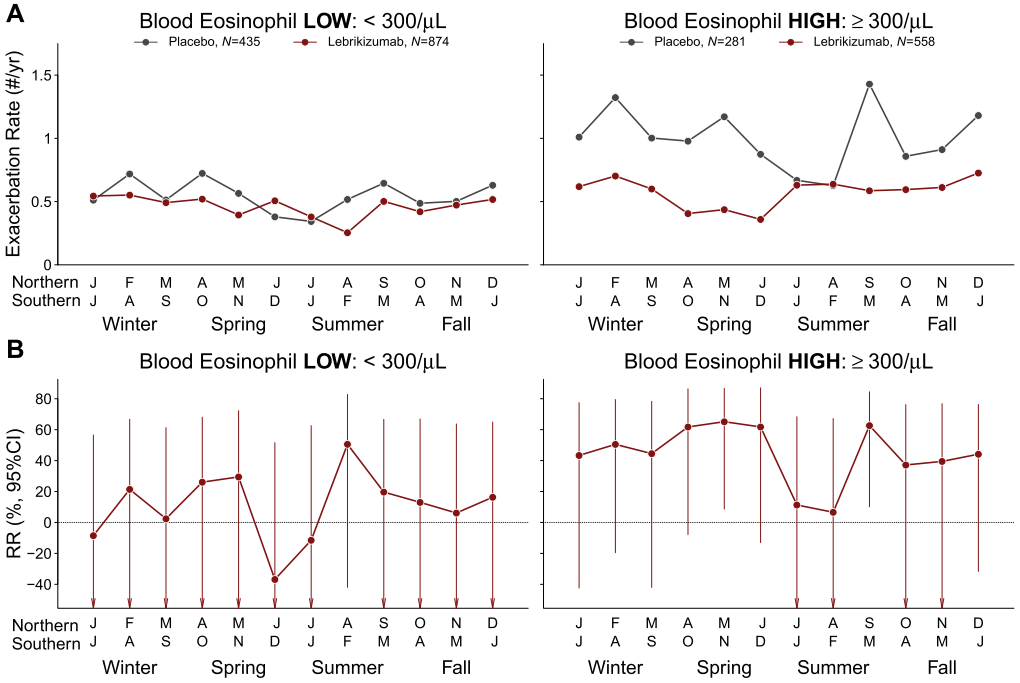

FIG 2. Seasonal analysis of exacerbations in subjects defined by baseline blood eosinophil counts. Unadjusted exacerbation rates **(A)** and treatment effect **(B)** expressed as percent exacerbation rate reduction (RR) are plotted as a function of normalized hemisphere month (northern hemisphere reference). Analyses for subjects with baseline blood eosinophils less than 300/μL or 300/μL or more are plotted on left and right panels, respectively. The months for corresponding hemispheric season are annotated in plot margins. CI segments terminated by arrowheads indicate censored values; CI extends past plot limit.

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REFERENCES

1. Johnston NW, Sears MR. Asthma exacerbations, 1: epidemiology. Thorax 2006;61:722-8.
2. Monto AS. The seasonality of rhinovirus infections and its implications for clinical recognition. Clin Ther 2002;24:1987-97.
3. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. Nature 2008;454:445-54.
4. Holtzman MJ. Asthma as a chronic disease of the innate and adaptive immune systems responding to viruses and allergens. J Clin Invest 2012;122:2741-8.
5. Mansson A, Cardell LO. Role of atopic status in Toll-like receptor (TLR)7- and TLR9-mediated activation of human eosinophils. J Leukoc Biol 2009;85:719-27.
6. Fajt ML, Wenzel SE. Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. J Allergy Clin Immunol 2015;135:299-310; quiz 311.
7. Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH, et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. N Engl J Med 2011;364:1005-15.
8. Teach SJ, Gill MA, Togias A, Sorkness CA, Arbes SJ Jr, Calatroni A, et al. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. J Allergy Clin Immunol 2015;136:1476-85.
9. Hanania NA, Korenblat P, Chapman KR, Bateman ED, Kopecky P, Paggiaro P, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. Lancet Respir Med 2016;4:781-96.

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Association of a *TNFSF13B* (BAFF) regulatory region single nucleotide polymorphism with response to rituximab in antineutrophil cytoplasmic antibody–associated vasculitis

To the Editor:

Rituximab is effective at inducing and maintaining remission in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV).[1,2] The wide interpatient variability in the duration of B-cell depletion and time to relapse as well as the significant relapse risk after treatment, costs, and adverse event rates necessitate improved patient stratification.[3]

Several biomarkers have been explored[4,5] in diseases treated with rituximab; however, all require validation before they can enter clinical practice; among them, a role for candidate single nucleotide polymorphisms (SNPs) has been proposed.[6] However, no SNP has so far shown a reliable association with response to treatment and no such study has been performed in AAV. We tested a panel of candidate SNPs to investigate their potential association with response to rituximab in 2 large cohorts of patients with AAV.

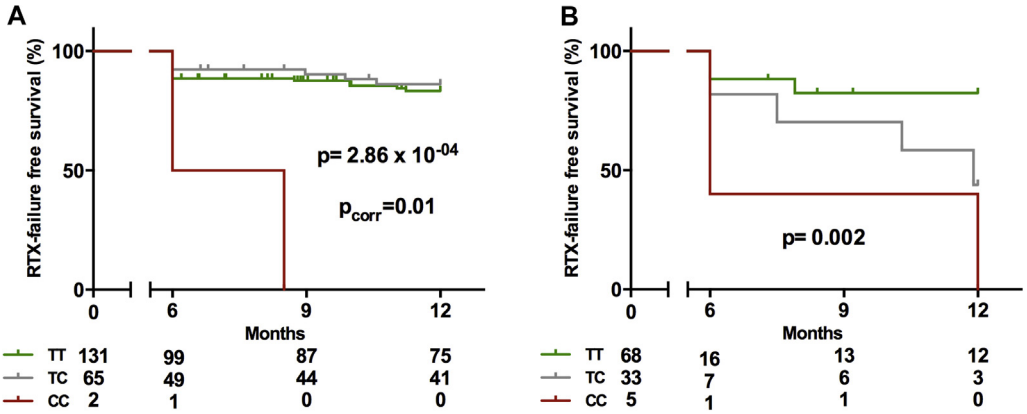

FIG 1. Rituximab (RTX) failure-free survival in an exploratory cohort of 213 patients **(A)** and in a replication cohort of 109 patients **(B)** stratified according to the genotypes of the SNP rs3759467 of the gene *TNFSF13B*.

TABLE I. Association results for the SNP rs3759467 after fixed-effects weighted meta-analysis for the 2 main outcomes assessed in our study assuming a recessive model

Outcome assessed	Primary cohort		Replication cohort		Meta-analysis	
	OR-HR	*P* value	OR-HR	*P* value	OR-HR	*P* value
Rituximab failure risk at 6 mo	9.1	.06489	8.6	.008996	8.8	.0065
Time to rituximab failure	12.4	7×10^{-04}	5.39	.0024	7.3	8.5×10^{-06}

Rituximab failure risk at 6 months has been explored using a recessive model of the Cochrane-Armitage test. Time to rituximab failure has been explored using a recessive model of Cox-proportion hazards regression model. OR and HR have been reported where appropriate.
HR, Hazard ratio; *OR*, odds ratio.

We included patients with granulomatosis with polyangiitis (GPA, Wegener's) and microscopic polyangiitis (MPA) who received rituximab mainly for relapsing or refractory disease. We aimed at identifying associations between the tested SNPs and the rate of rituximab failure at 6 months and time to rituximab failure (relapse). For further details, see the Methods section in this article's Online Repository at www.jacionline.org.

We enrolled 213 patients in the primary and 109 in the replication cohort (see Table E1 in this article's Online Repository at www.jacionline.org). Across the primary cohort, a mean of 0.88 ± 0.19 SNPs per sample could not be called and no SNP had a *P* value of less than .05 for departure from Hardy-Weinberg Equilibrium (HWE) (see Table E2 in this article's Online Repository at www.jacionline.org).

In the primary cohort, the *TNFSF13B* SNP rs3759467 was associated with time to rituximab failure ($P = 2.86 \times 10^{-04}$, $P_{corr} = .01$) (Fig 1, *A*; see Table E3 in this article's Online Repository at www.jacionline.org). We genotyped this SNP in the replication cohort (test for deviation from HWE $P = .7627$; rate of missing calls, 3%) where the association with time to rituximab failure was confirmed ($P = .002$) (Fig 1, *B*).

Because the results suggested a recessive effect for the SNP rs3759467, we used recessive models for the analysis in the replication cohort and reanalysis of the primary cohort. Meta-analyses of the 2 cohorts confirmed an association between the *TNFSF13B* SNP rs3759467 and the 2 end points ($P = .0065$ and 8.5×10^{-06}, respectively) (Table I).

We then compared the main clinical characteristics of the carriers of the CC genotype with the carriers of the TC and TT genotypes (see Table E4 in this article's Online Repository at www.jacionline.org) and found a higher rate of detectable peripheral B cells 6 months after rituximab in carriers of the CC genotype (50% vs 14%; $P = .0146$) as well as a smaller reduction in IgM levels (1.5 [1.4-10.92] and 1 [1-1.33]; $P = .01539$).

The haplotype analyses of the 5′ regulatory region of the *TNFSF13B* gene based on the genotyped SNPs confirmed an association of the risk of rituximab failure at 6 months for the haplotype including the risk allele of the SNP rs3759467 (see Table E5 in this article's Online Repository at www.jacionline.org).

In view of the small number of MPO-ANCA–positive patients and the fact that all the minor homozygous carriers for the rs3759467 SNP were PR3-ANCA positive, we reanalyzed the primary cohort according to ANCA specificity (see Table E6 in this article's Online Repository at www.jacionline.org); the association with the B-cell activating factor (BAFF) SNP rs3759467 was limited to the PR3-ANCA subgroup (see Tables E6 and E7 in this article's Online Repository at www.jacionline.org).

In the MPO-ANCA subgroup, a different association emerged in the primary cohort with the SNP rs6822844 in the *IL2-IL21* area (rituximab failure risk at 6 months and time to rituximab failure, $P = 4.2 \times 10^{-04} - P_{corr} = .03$ and $P = 1.9 \times 10^{-04} - P_{corr} = .0068$, respectively) (Table E6). However, this was not replicated (rituximab failure risk at 6 months and time to rituximab failure, $P = .153$ and .172, respectively) in the context of a small sample size (19 patients), although there was a trend to an increased risk of treatment failure at 6 months for the carriers of the T allele (see Table E8 in this article's Online Repository at www.jacionline.org).

We have identified an SNP (rs3759467) in the 5′ regulatory region of the gene *TNFSF13B* (BAFF) able to predict response to rituximab in 2 cohorts of patients with AAV. Interestingly, the carriers of the unfavorable genotype showed in addition to a poor response to rituximab, a higher proportion of detectable B cells 6 months after infusion and a smaller reduction in IgM levels.

A BAFF level increase after rituximab treatment has been described and a central role for this cytokine in AAV pathogenesis

has also been proposed.[7] The 5′ regulatory region of the *BAFF* gene includes several SNPs that may have a modulatory effect; the -TTTT- haplotype of this region has been associated with BAFF levels[8] and response to rituximab in rheumatoid arthritis.[6] In our study, a trend toward better response in carriers of this haplotype was also observed.

The role of the SNP rs3759467 will need to be clarified; this is part of a TAAT- binding site; the lack of a heterozygous effect may suggest that the losing of both sites is required to obtain the phenotype causing poor response. It seems likely that this SNP modulates B-cell survival and/or activity; this might be a consequence of higher baseline BAFF levels or greater BAFF increases after a B-cell–depleting event.

Interestingly, our findings were restricted to the subgroup of patients with positive PR3-ANCA probably due to power limitations in the MPO-ANCA subgroup where the reanalyses identified a different association in the *IL2-IL21* area, as already described in systemic lupus erythematosus.[9]

Our study has limitations: a sample size relatively small for a genetic study and the retrospective nature of data collection may be weaknesses; however, at the time of this writing, it would not have been possible to enroll a comparable prospective cohort. We also have to acknowledge the closeness of the *P* value for the departure from HWE for the SNP rs3759467 in the primary cohort; however, the replication of the data in the context of a *P* value for the departure from HWE far from significance ($P = .7627$) is reassuring on the reliability of the overall finding.

In conclusion, we have identified a *TNFSF13B* (BAFF) SNP (rs3759467) associated with response to rituximab in 2 independent cohorts of patients with AAV. This SNP may be useful in identifying patients likely to respond poorly to rituximab and for whom alternative treatments should be considered. Further studies are required to confirm this finding and to clarify its mechanism of action.

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REFERENCES

1. Jones RB, Tervaert JW, Hauser T, Luqmani R, Morgan MD, Peh CA, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. N Engl J Med 2010;363:211-20.
2. Guillevin L, Pagnoux C, Karras A, Khouatra C, Aumaître O, Cohen P, et al. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. N Engl J Med 2014;371:1771-80.
3. Roberts DM, Jones RB, Smith RM, Alberici F, Kumaratne DS, Burns S, et al. Rituximab-associated hypogammaglobulinemia: incidence, predictors and outcomes in patients with multi-system autoimmune disease. J Autoimmun 2015;57:60-5.
4. Nasrallah M, Pouliot Y, Hartmann B, Dunn P, Thomson E, Wiser J, et al. Reanalysis of the Rituximab in ANCA-Associated Vasculitis trial identifies granulocyte subsets as a novel early marker of successful treatment. Arthritis Res Ther 2015;17:262.
5. Leandro MJ, Cambridge G, Ehrenstein MR, Edwards JC. Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. Arthritis Rheum 2006;54:613-20.
6. Fabris M, Quartuccio L, Vital E, Pontarini E, Salvin S, Fabro C, et al. The TTTT B lymphocyte stimulator promoter haplotype is associated with good response to rituximab therapy in seropositive rheumatoid arthritis resistant to tumor necrosis factor blockers. Arthritis Rheum 2013;65:88-97.
7. Bader L, Koldingsnes W, Nossent J. B-lymphocyte activating factor levels are increased in patients with Wegener's granulomatosis and inversely correlated with ANCA titer. Clin Rheumatol 2010;29:1031-5.
8. Nossent JC, Lester S, Zahra D, Mackay CR, Rischmueller M. Polymorphism in the 5' regulatory region of the B-lymphocyte activating factor gene is associated with the Ro/La autoantibody response and serum BAFF levels in primary Sjogren's syndrome. Rheumatology 2008;47:1311-6.

9. Marquez A, Davila-Fajardo CL, Robledo G, Rubio JL, de Ramón Garrido E, García-Hernández FJ, et al. IL2/IL21 region polymorphism influences response to rituximab in systemic lupus erythematosus patients. Mol Biol Rep 2013;40:4851-6.

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Longitudinal variation of serum periostin levels in adults with stable asthma

To the Editor:

Periostin is a matricellular protein upregulated by IL-4 and IL-13 and released by airway epithelial cells. Its levels in peripheral blood are associated with eosinophilic airway inflammation in patients with poorly controlled asthma despite maximal inhaled corticosteroid (ICS) therapy.[1] High levels of serum periostin in asthmatic patients are associated with higher rates of severe exacerbations and greater FEV_1 decrease with time.[2] There is also evidence that high serum periostin levels can predict responsiveness to mAb therapy directed against IL-4 receptor subunit alpha,[3] IL-13,[4] and IgE.[5]

To better understand the profile of periostin as a biomarker in asthmatic patients, we have recently defined the reference ranges of periostin in a population of adults with asthma[6] and a population of adults without asthma or chronic obstructive pulmonary disease.[7] These studies suggest that periostin is not a measure that can usefully discriminate between patients with asthma across a range of severities or from a population without asthma or chronic obstructive pulmonary disease. A characteristic that would need to be established if periostin is to be used as a biomarker in asthmatic patients is its short-term variability in patients with stable asthma. The objective of this study was to determine the variation in serum periostin levels in adults with stable asthma receiving long-term ICS and long-acting β-agonist (LABA) therapy. We hypothesized that there would be little intraparticipant variation in periostin levels in the absence of an asthma exacerbation.

This prospective cohort study recruited 60 adults aged between 18 and 75 years. All participants had a doctor's diagnosis of asthma and were receiving stable treatment regimens of an ICS and LABA. Ethical approval was given by the New Zealand Health and Disability Ethics Committee (13/NTB/185). The trial was prospectively registered with the Australia New Zealand Trial Registry (ACTRN126140000235606). Informed written consent was obtained for all participants. Participants attended the Medical Research Institute of New Zealand (MRINZ) outpatient facility for 11 visits over 8 weeks. There were daily visits for the first 5 days, followed by a sixth visit at day 10, weekly visits for 4 weeks, and a final visit 3 weeks later (for full methodology and study plan, see Table E1 in this article's Online Repository at www.jacionline.org). At baseline, participants answered 3 health questionnaires to determine asthma severity, quality of life, and health status; performed basic spirometry and had fraction of exhaled nitric oxide (FENO) measured; and had venous blood drawn for analysis of full blood counts, serum IgE levels, and serum periostin levels. Venous blood for the measurement of serum periostin was collected at all 11 visits. The Asthma Control Questionnaire (ACQ), scoring asthma severity in the week before data collection, was administered at both baseline and every visit from visits 6 to 11. Spirometry was performed with a MasterScreen Pneumo device (MasterScreen Version 2.0; CareFusion, Hoechberg, Germany). FENO values were measured with a nitric oxide monitor (NIOX MINO; Aerocrine AB, Solna, Sweden), according to American Thoracic Society (ATS) guidelines.[8] Serum periostin levels were determined by using the clinical trial version of the Elecsys periostin immunoassay (Roche Diagnostics, Penzbery, Germany). This assay is an automated electrochemiluminescence immunoassay based on the sandwich principle using the same antibodies reported previously.[1]

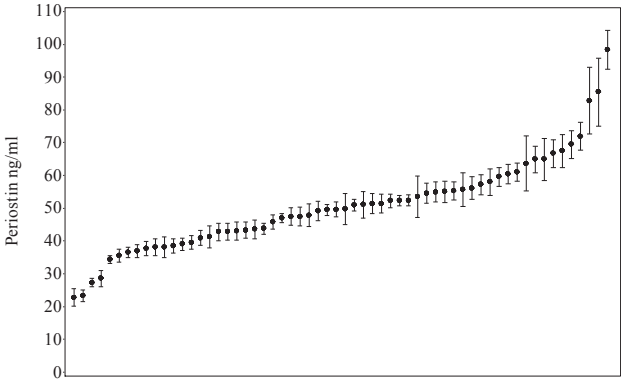

FIG 1. Individual participant mean periostin levels ±1 SD ranked by mean periostin level.

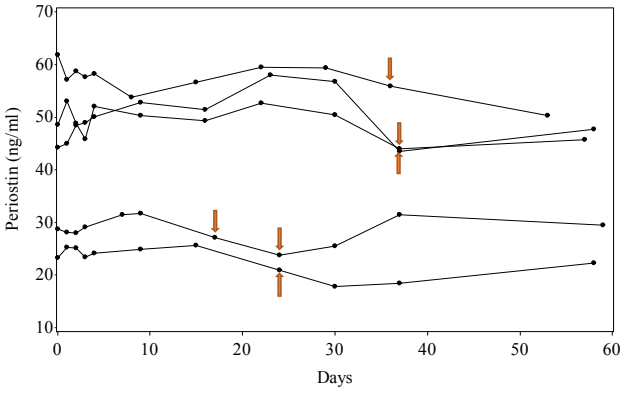

FIG 2. Joined line plot by time of periostin measurement in the 5 participants with a severe exacerbation requiring prednisone use. The *arrow* marks the periostin measurement taken after the severe exacerbation requiring prednisone.

Participants were taking a median ICS dose of 500 μg of fluticasone propionate equivalent per day and had well-controlled asthma with a mean FEV_1 of 101% of predicted value, a mean ACQ score of 0.89, and a mean FENO value of 24.6 ppb (full participants' characteristics are shown in Table E2 in this article's Online Repository at www.jacionline.org). The range of periostin values was 23.2 to 106.6 ng/mL, with a mean (SD) value of 52.2 ng/mL (16.4 ng/mL) and a median value of 48.9 ng/mL. Individual participant mean periostin levels were ranked and are depicted in Fig 1 (all periostin values per visit are shown in Table E3 in this article's Online Repository at www.jacionline.org). The estimate of interparticipant variance in periostin levels based on the raw-scale measurements was 193.6, which was large

METHODS

Eligibility criteria were a clinical diagnosis of granulomatosis with polyangiitis (GPA, Wegener's) or microscopic polyangiitis (MPA) defined according to the European Medicines Agency (EMEA) algorithm[E1] and the administration of rituximab for either relapsing or refractory disease or remission induction in few patients. Patients with a clinical diagnosis of eosinophilic granulomatosis with polyangiitis (formerly known as Churg-Strauss syndrome) were not included in this study. The primary cohort included patients enrolled at European centers with expertise in vasculitis and members of the European Vasculitis Genetics Consortium and/or of the European Vasculitis Society (Table E9); the replication cohort was enrolled at the Vasculitis and Lupus Clinic, Addenbrooke's Hospital (Cambridge, United Kingdom). All patients gave written informed consent before participation. Disease severity was assessed using the Disease Extent Index (DEI)[E2]; B-cell return was defined as a B-cell count of 0.01×10^9/L or more.

Eligibility criteria

Patients affected by GPA or MPA for whom there was indication to the beginning of treatment with rituximab as follows:

- Relapsing disease: disease flare defined as a DEI score of more than 2, physician assessment of relapsing disease, and need for immunosuppression escalation.
- Refractory disease: persistent activity of disease despite treatment with intravenous and/or oral steroids and other immunosuppressive agents and physician assessment of refractory disease for at least 3 months.
- Other indications:
  - First presentation of the disease.
  - Contraindication to standard treatment (eg, recurrent infection and fertility preservation).
  - Grumbling disease: low-grade disease activity according to physician assessment that is not formally fulfilling the inclusion criteria in terms of disease activity scoring.
  - Patients unable to taper the prednisolone below 15 mg/d on their previous therapeutic regimen.
  - Need for steroid-free regimen.

End points

The end points of the study were to identify associations between the tested SNPs and the rate of rituximab failure at 6 months and time to rituximab failure (relapse) within 12 months of the first rituximab administration. We defined rituximab failure as active vasculitis requiring escalation of immunosuppressive treatment. SNPs showing an association with at least 1 of the 2 end points in the primary cohort were analyzed in the replication cohort for confirmation and then meta-analyses of the results were performed. Subgroup analyses were performed after merging the 2 cohorts.

Genotyping

Eighteen candidate SNPs were chosen according to a biological rationale or previous reports (Table E2).[E3-E8] DNA was extracted from peripheral blood using the Qiagen DNA extraction kit; genotyping was performed using TaqMan and Sequenom platforms with the exception of the *FCGR2B* SNP rs1050501, which was genotyped via a "modified" TaqMan approach as previously described.[E9]

Statistics

Statistical analysis was performed using the software R (http://www.r-project.org) and the packages coin,[E10] survival,[E11] SNPassoc,[E12] and hapassoc.[E13]

For exploratory purposes, the primary cohort has been analyzed using the Cochrane-Armitage test with log-additive model. As a result of the exploratory analysis (recessive mechanism for the SNP rs3759467), the replication cohort has been analyzed immediately using a recessive model of the same test. We have then reanalyzed the primary cohort with the same model and a meta-analysis has been performed.

The time to rituximab failure has been assessed by Kaplan-Meier survival analysis and a log-rank test has been used to compare populations. Cox-proportional hazards regression model has been used for reanalyses of the time to rituximab failure in the primary and replication cohort using a recessive model and the results have been used for meta-analyses for this end point.

Delta of reduction of IgG and IgM levels has been assessed as the ratio between the baseline value and the value at the time point of interest.

Results are expressed as value and percentage for categorical variables and median and interquartile range or mean and SEM for continuous variables when appropriate.

In view of the observation of an association in the 5′ regulatory region of the gene *TNFSF13B*, we decided to study the haplotypes of this region because 4 of the SNPs included in the study were in strong linkage disequilibrium and organized in well-renowned haplotype blocks. We used the R package hapassoc[E13] for the identification of the haplotypes and the Cochrane-Armitage test to explore association between haplotypes and the risk of treatment failure 6 months after treatment with rituximab.

Meta-analyses were performed via a fixed-effects weighted method using the Linux version of the software metal (http://csg.sph.umich.edu/abecasis/Metal/download/). Bonferroni corrections for multiple testing were performed, with corrected P (P_{corr}) values of less than .05 considered significant.

REFERENCES

E1. Watts R, Lane S, Hanslik T, Hauser T, Hellmich B, Koldingsnes W, et al. Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis nodosa for epidemiological studies. Ann Rheum Dis 2007;66:222-7.

E2. de Groot K, Gross WL, Herlyn K, Reinhold-Keller E. Development and validation of a disease extent index for Wegener's granulomatosis. Clin Nephrol 2001;55:31-8.

E3. Fabris M, Quartuccio L, Vital E, Pontarini E, Salvin S, Fabro C, et al. The TTTT B lymphocyte stimulator promoter haplotype is associated with good response to rituximab therapy in seropositive rheumatoid arthritis resistant to tumor necrosis factor blockers. Arthritis Rheum 2013;65:88-97.

E4. Kim SH, Jeong IH, Hyun JW, Joung A, Jo HJ, Hwang SH, et al. Treatment outcomes with rituximab in 100 patients with neuromyelitis optica: influence of FCGR3A polymorphisms on the therapeutic response to rituximab. JAMA Neurol 2015;72:989-95.

E5. Treon SP, Hansen M, Branagan AR, Verselis S, Emmanouilides C, Kimby E, et al. Polymorphisms in FcgammaRIIIA (CD16) receptor expression are associated with clinical response to rituximab in Waldenstrom's macroglobulinemia. J Clin Oncol 2005;23:474-81.

E6. Ruyssen-Witrand A, Rouanet S, Combe B, Dougados M, Le Loët X, Sibilia J, et al. Association between -871C>T promoter polymorphism in the B-cell activating factor gene and the response to rituximab in rheumatoid arthritis patients. Rheumatology (Oxford) 2013;52:636-41.

E7. Racila E, Link BK, Weng WK, Witzig TE, Ansell S, Maurer MJ, et al. A polymorphism in the complement component C1qA correlates with prolonged response following rituximab therapy of follicular lymphoma. Clin Cancer Res 2008;14:6697-703.

E8. Robledo G, Davila-Fajardo CL, Marquez A, Ortego-Centeno N, Callejas Rubio JL, de Ramón Garrido E, et al. Association between -174 interleukin-6 gene polymorphism and biological response to rituximab in several systemic autoimmune diseases. DNA Cell Biol 2012;31:1486-91.

E9. Willcocks LC, Carr EJ, Niederer HA, Rayner TF, Williams TN, Yang W, et al. A defunctioning polymorphism in FCGR2B is associated with protection against malaria but susceptibility to systemic lupus erythematosus. Proc Natl Acad Sci U S A 2010;107:7881-5.

E10. Hothorn T, Hornik K, van de Wiel MA, Zeileis A. A Lego System for conditional inference. Am Stat 2006;60:257-63.

E11. Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model. New York: Springer; 2000.

E12. SNPassoc: SNPs-based whole genome association studies. R package version 1.9-2. 2014. Available at: http://CRAN.R-project.org/package=SNPassoc. Accessed July 15, 2014.

E13. Burkett K, McNeney B, Graham J. A note on inference of trait associations with SNP haplotypes and other attributes in generalized linear models. Hum Hered 2004;57:200-6.

TABLE E1. Patients' characteristics

Characteristic	Primary cohort (n = 213)	Replication cohort (n = 109)
Age (y)	53.3 (40.2-64.7)	57.1 (41.9-65)
Sex: male	111 (52)	48 (44)
Diagnosis		
GPA	185 (87)	94 (86)
MPA	29 (13)	15 (14)
ANCA specificity		
PR3	148 (70)	78 (72)
MPO	29 (13)	19 (17)
Negative	36 (17)	12 (11)
Prior disease duration (mo)	56.3 (12.8-113.8)	48.2 (16.5-138)
Indication for rituximab		
Relapse	108 (51)	53 (49)
Refractory disease	76 (36)	15 (14)
Other*	26 (13)	41 (38)
Rituximab dose for induction regimen		
375 mg/m^2 weekly for 4 wk	68 (32)	28 (26)
1 g 2 wk apart	143 (67)	79 (72)
Other†	2 (1)	2 (2)
No. of previous immunosuppressive agents	2 (1-3)	3 (2-3)
Cyclophosphamide	175 (82)	94 (86)
Cumulative dose (g)	10 (4-28)	10 (4-27)
Methotrexate	92 (43)	28 (26)
Azathioprine	91 (42)	76 (70)
MMF	49 (23)	60 (55)
Immunosuppression at study entry‡	1 (0-1)	1 (0-1)
Cyclophosphamide	35 (16)	15 (14)
Methotrexate	30 (14)	8 (7)
MMF	18 (9)	17 (16)
Azathioprine	14 (8)	11 (10)
IV methylprednisolone	8 (4)	17 (16)
Oral prednisolone dose	25 mg (12.5-50)	12.5 mg (10-20)
Organ involvement at study entry		
ENT	132 (62)	60 (46)
Lungs	88 (41)	21 (19)
Joints	83 (39)	26 (24)
Kidneys	82 (39)	24 (22)
Eye	59 (28)	12 (11)
Peripheral nervous system	25 (12)	9 (8)
Central nervous system	11 (5)	3 (3)
Gastrointestinal	4 (2)	0 (0)
Cardiac	4 (2)	0 (0)
DEI	5 (3.75-7)	3 (2-5)
ANCA status at study entry		
Positive by ELISA	174 (82)	73 (67)
PR3	148 (70)	59 (54)
MPO	26 (12)	14 (13)
Negative	39 (18)	36 (33)

Results are expressed as n/N (%) or median (IQR) when appropriate.
ENT, Ear, nose and throat; *IV*, intravenous; *MMF*, mycophenolate mofetil.
*Other. Includes first presentation of disease, contraindication to standard treatment, grumbling disease, patients unable to taper the prednisolone dose, need for steroid-free regimen.
†Other. Includes 500 mg 2 weeks apart (2 cases), administration of a single dose of 1 g of rituximab (1 case), administration of 3 doses of rituximab at the dose of 375 mg/m^2.
‡Excluding oral steroids, but including oral immunosuppressive agents continued for at least 3 months after the administration of the first rituximab dose.

TABLE E2. SNPs tested, minor allele frequency (MAF) observed, and test for deviation from HWE in the primary cohort of 213 patients with AAVs treated with rituximab

SNP	Alleles	Gene	MAF	HWE-*P*
rs396991	G/T	*FCGR3A*	0.439	0.407
rs1050501	T/C	*FCGR2B*	0.11	0.716
rs1224141	G/T	*TNFSF13B*	0.235	0.839
rs16972216	A/G	*TNFSF13B*	0.173	1.000
rs1224147	T/C	*TNFSF13B*	0.221	0.680
rs10508198	C/G	*TNFSF13B*	0.339	0.058
rs12583006	A/T	*TNFSF13B*	0.231	0.554
rs8181791	A/G	*TNFSF13B*	0.307	0.327
rs172378	A/G	*C1QA*	0.38	0.303
rs9514828	C/T	*TNFSF13B*	0.442	0.888
rs1801274	C/T	*FCGR2A*	0.495	0.407
rs1800795	C/G	*IL6*	0.359	0.881
rs6822844	G/T	*IL2-IL21*	0.127	0.542
rs3759467	T/C	*TNFSF13B*	0.172	0.057
rs28362491	DEL/ATTG	*NFKB1*	0.383	0.102
rs1800471	C/G	*TGFB1*	0.09	1.000
rs1041569	A/T	*TNFSF13B*	0.166	1.000
rs9514827	T/C	*TNFSF13B*	0.318	0.626

The test for deviation from HWE has been calculated using the R package SNPasso,[E12] and threshold for significance has been established to $P < .05$.
*MA*F, Minor allele frequency.

TABLE E3. Association of 18 candidate SNPs with the 2 main outcomes explored in the study: Rituximab failure risk at 6 months and time to rituximab failure

			Rituximab failure risk at 6 mo		Time to rituximab failure	
SNP	**Alleles**	**Gene**	***P* value**	**P_{corr}**	***P* value**	**P_{corr}**
rs396991	G/T	*FCGR3A*	.275	1	.495	1
rs1050501	T/C	*FCGR2B*	.399	1	.089	1
rs1224141	G/T	*TNFSF13B*	.006*	0.216	.268	1
rs16972216	A/G	*TNFSF13B*	.328	1	.521	1
rs1224147	T/C	*TNFSF13B*	.01*	0.36	.366	1
rs10508198	C/G	*TNFSF13B*	.295	1	.083	1
rs12583006	A/T	*TNFSF13B*	.625	1	.129	1
rs8181791	A/G	*TNFSF13B*	.489	1	.968	1
rs172378	A/G	*C1QA*	.987	1	.441	1
rs9514828	C/T	*TNFSF13B*	.854	1	.943	1
rs1801274	C/T	*FCGR2A*	.949	1	.605	1
rs1800795	C/G	*IL6*	.574	1	.735	1
rs6822844	G/T	*IL2-IL21*	.184	1	.853	1
rs3759467	A/G	*TNFSF13B*	.911	1	2.86×10^{-04}*	0.01*
rs28362491	-/ATTG	*NFKB1*	.012*	0.432	.245	1
rs1800471	C/G	*TGFB1*	.727	1	.978	1
rs1041569	A/T	*TNFSF13B*	.461	1	.88	1
rs9514827	T/C	*TNFSF13B*	.577	1	.825	1

P_{corr}, *P* corrected for multiple testing according to Bonferroni.
*Statistically significant.

TABLE E4. Main clinical characteristics of patients at time of study entry and 6 months after rituximab according to the different genotype for the SNP rs3759467 of the gene *TNFSF13B* in the overall study population (primary + replication cohorts merged)

Characteristic	Genotype TT + TC	Genotype CC	*P* value
Diagnosis (GPA)	260 of 301 (86%)	7 of 7 (100%)	.2951
Historical ANCA specificity (PR3)	209 of 294 (71%)	7 of 7 (100%)	.2453
DEI	4 (2-6)	4 (3.5-4.5)	.6925
Age (y)	54.2 (40.8-65)	54.8 (37.5-58.5)	.5965
Indication for rituximab (active flare)*	233 of 299 (78%)	6 of 7 (86%)	.6229
B-cell return at 6 mo	22 of 161 (14%)	3 of 6 (50%)	.0146†
ANCA positivity at 6 mo	137 of 244 (56%)	6 of 7 (86%)	.12
IgG delta at 6 mo‡	1.06 (0.96-1.34)	0.97 (0.76-0.97)	.09389
IgM delta at 6 mo‡	1.5 (1.04-10.92)	1 (1-1.33)	.01539†
DEI score at rituximab failure	4 (2-5)	4 (2.5-4.75)	.703

Results are expressed as n/N (%) or median (IQR) when appropriate.

B-cell return has been defined as B-cell count $\geq 0.01 \times 10^9$/L.

**Active flare* defined as rituximab given either for relapsing or for refractory disease.

†Statistically significant.

‡Delta has been calculated as the ratio between the value at the time of rituximab administration and the value at the time point of interest. Associations have been tested using the Mann-Whitney rank sum test.

TABLE E5. Haplotypes of the 5′ regulatory region of the gene *TNFSF13B* and their association with risk of TF 6 months after treatment with rituximab according to a log-additive and recessive models

		Rituximab failure risk at 6 mo			
		Log-additive model		Recessive model	
Haplotype	Frequency	*P* value	P_{corr}	*P* value	P_{corr}
TTAC	34%	.635	1	.58	1
CTAT	31%	.73	1	.699	1
TCAC	18%	.028*	0.28	2.6×10^{-05}*	2.6×10^{-04}*
TTTT	13%	.028*	0.28	.386	1
Pooled	4%	.475	1	.741	1

*Statistically significant.

TABLE E6. Association of 18 candidate SNPs with the 2 outcomes explored in our study (rituximab failure risk at 6 months and time to rituximab failure) according to the historical ANCA specificity

			PR3 – AAVs (n = 148)				MPO – AAVs (n = 29)			
			Rituximab failure 6/12		Time to rituximab failure		Rituximab failure 6/12		Time to rituximab failure	
SNP	**Alleles**	**Gene**	***P* value**	**P_{corr}**	***P* value**	**P_{corr}**	***P* value**	**P_{corr}**	***P* value**	**P_{corr}**
rs396991	G/T	*FCGR3A*	.150	1	.815	1	.388	1	.25	1
rs1050501	T/C	*FCGR2B*	.073	1	.401	1	.433	1	.808	1
rs1224141	G/T	*TNFSF13B*	.007	0.53	.208	1	.380	1	.672	1
rs16972216	A/G	*TNFSF13B*	.164	1	.806	1	.667	1	.705	1
rs1224147	T/C	*TNFSF13B*	.012	0.84	.253	1	.360	1	.675	1
rs10508198	C/G	*TNFSF13B*	.775	1	.206	1	.546	1	.824	1
rs12583006	A/T	*TNFSF13B*	.980	1	.162	1	.966	1	.702	1
rs8181791	A/G	*TNFSF13B*	.645	1	.829	1	.625	1	.603	1
rs172378	A/G	*C1QA*	.733	1	.481	1	.551	1	.671	1
rs9514828	C/T	*TNFSF13B*	.390	1	.533	1	.434	1	.782	1
rs1801274	C/T	*FCGR2A*	1	1	.401	1	.194	1	.562	1
rs1800795	C/G	*IL6*	.574	1	.392	1	.135	1	.287	1
rs6822844	G/T	*IL2-IL21*	.987	1	.874	1	4.2×10^{-04}*	0.03*	1.9×10^{-04}*	0.0068*
rs3759467	A/G	*TNFSF13B*	.667	1	4.8×10^{-04}*	0.017*	.452	1	.825	1
rs28362491	-/ATTG	*NFKB1*	.044	1	.263	1	.212	1	.268	1
rs1800471	C/G	*TGFB1*	.609	1	.148	1	.626	1	.363	1
rs1041569	A/T	*TNFSF13B*	.306	1	.648	1	.899	1	.668	1
rs9514827	T/C	*TNFSF13B*	.111	1	.598	1	.706	1	.534	1

Rituximab failure rates at 6 mo were compared using a Cochrane-Armitage test with log-additive model and time to rituximab failure using log-rank test.
*Statistically significant.

TABLE E7. Association results for the SNP rs3759467 in the subgroup of patients with PR3-ANCA after fixed-effects weighted meta-analysis for the 2 outcomes assessed in our study assuming a recessive model for the SNP

	Primary cohort (n = 148)		Replication cohort (n = 78)		Meta-analysis	
Outcome assessed	OR/HR	*P* value	OR/HR	*P* value	OR/HR	*P* value
Rituximab failure risk at 6 mo	8.2	.0853	9.2	.0089	8.8	.007
Time to RTX-failure	11.6	.0012	6.2	.002	8.2	8.7×10^{-06}

Rituximab failure risk at 6 months has been explored using a recessive model of the Cochrane-Armitage test. Time to rituximab failure has been explored using a recessive model of Cox-proportion hazards regression model.

TABLE E8. Genotype distribution for the SNP rs6822844 of the gene *IL2-IL21* in the subgroup of patients MPO-ANCA positive of the replication cohort

	Response at 6 mo		
Genotype rs6822844	**Positive response**	**TF**	**TF percentage**
GG	15	1	6%
GT	2	1	33%
TT	0	0	0%

The difference is not statistically significant ($P = .1722$, Cochrane-Armitage test, log-additive model) although there was a trend toward an increased risk of TF in the carriers of the T allele in small sample size (19 patients).
TF, Rituximab failure.

TABLE E9. Primary cohort recruitment according to country

Country	No. of patients
Germany	53
Italy	49
Sweden	46
Denmark	41
Czech Republic	13
Spain	11