Clinical outcome of follicular lymphoma patients receiving chemo-immunotherapy in the PRIMA study is not affected by FCGR3A and FCGR2A polymorphisms


1Centre Léon Bérard, Lyon, France
2Université Claude Bernard, UMR CNRS5239, Lyon, France
3Département d’Hématologie, Centre Hospitalier Universitaire, Montpellier UMR-CNRS5235, Montpellier, France
4Peter MacCallum Cancer Centre, & University of Melbourne, Australia
5CHU Henri Mondor, Créteil, France
6Ghent University, Ghent, Belgium
7Institut Bergonié and Université Victor Segalen Bordeaux 2, Bordeaux, France
8CHU de Nancy, Nancy, France
9Hôpital Saint-Louis, AP-HP, Paris, France
10Institut Paoli Calmettes, Marseille, France
11UCL, Mont-Godinne, Yvoir, Belgium
12CHU de Dijon, Dijon, France
13Frankston Hospital, Frankston, Australia
14CHU de Reims, Reims, France
15Centre Henri Becquerel and UMR INSERM U918, Rouen, France
Institut Gustave Roussy, Villejuif, France

Hospices Civils de Lyon, Pierre-Bénite, France

Corresponding author:
Gilles Salles, MD, PhD
Service d’Hématologie, Centre Hospitalier Lyon-Sud
165, chemin du Grand Revoyet
69495 Pierre-Bénite Cedex
Tel: +33 4 78 86 43 02; fax: +33 4 78 86 43 55
email: gilles.salles@lyon-chu.fr

Running head: FCGR3A and FCGR2A in follicular lymphoma
This work was supported in part by a grant from the Institut National du Cancer (INCa, Paris, France) French Ministry of Health.
Presented in part at the 11th international conference on malignant lymphoma (ICML), Lugano, Switzerland, June 15-18 2011.
ABSTRACT

In patients with follicular lymphoma treated with single-agent rituximab, single nucleotide polymorphisms (SNP) in the *FCGR3A* gene are known to influence response and progression-free survival (PFS). The prognostic role of *FCGR3A* and *FCGR2A* polymorphisms in patients with follicular lymphoma treated with rituximab and chemotherapy combination remains controversial and has not been evaluated in the context of rituximab maintenance. *FCGR3A* and *FCGR2A* SNPs were evaluated in respectively 460 and 455 patients treated in the PRIMA study to investigate whether these were associated with response rate and patient outcome after rituximab chemotherapy induction and 2-year rituximab maintenance. In this representative patient cohort, complete and unconfirmed complete responses after rituximab chemotherapy were observed in 65%, 67%, 66% (*P*=0.86) and 60%, 72%, 66% (*P*=0.21) of *FCGR3A* VV, VF, FF and *FCGR2A* HH, HR, RR carriers, respectively. After two years of rituximab maintenance (or observation), response rates did not differ between the different genotypes. PFS measured from either treatment initiation or randomization to observation or maintenance was not influenced by these polymorphisms. These data indicate that *FCGR3A* and *FCGR2A* polymorphisms do not influence response rate and outcome when rituximab is combined with chemotherapy or used as maintenance treatment. The PRIMA study is registered with clinicaltrials.gov (NCT00140582).
INTRODUCTION

The prognosis of patients with follicular lymphoma (FL) remains very variable and the natural history of this lymphoma is typified by multiple episodes of relapse.\(^1\) Despite a relatively long overall survival (OS), advanced stage FL generally remains an incurable disease. To more accurate predict a patient’s prognosis, a variety of scores based on presenting clinical and simple biological characteristics, such as the follicular lymphoma international prognostic index (FLIPI) score, have been proposed.\(^2\) The decision to initiate treatment is usually determined by the presence of a clinically significant tumor mass, hematopoietic compromise or symptomatic disease.\(^3\)

Recent biological data have suggested that the host–tumor interaction may influence disease behavior, and assessment of specific cellular components within the tumor microenvironment may provide additional prognostic information.\(^4\) The outcome of patients could also be influenced by inherent host immunologic factors. For example, recent studies have shown that the prognosis of patients with FL was associated with germline polymorphisms in immune-response elements, such as the cytokine genes \(IL1RN\), \(IL2\), \(IL8\) and \(IL12\).\(^5\) The prognosis of patients with FL has dramatically improved with the introduction of anti-CD20 monoclonal antibodies (mAbs).\(^6-9\) The therapeutic activities of these mAbs may also be affected by patient biological characteristics such as polymorphisms in Fcγ receptors (FC\(\gamma\)R) genes.\(^10\) The affinity of the Fc portion of anti-CD20 mAb on FC\(\gamma\)Rs is modulated by specific single nucleotide polymorphisms (SNPs) in \(FCGR\) genes. For instance, the SNP rs396991
leads to the amino acid substitution of a valine (V) for a phenylalanine (F), the FCγRIIIA 158V having a higher affinity of human immunoglobulin G1 (hu-Ig G1) than FCγRIIIA F allele. Similarly, the polymorphism (rs1801274) in FCGR2A gene lead to FcγRIIA with either a histidine (H) or a phenylalanine (R) at amino acid position 131, where the FCγRIIA 131H variant binds more strongly to hu-Ig G1. The possible clinical consequence is a difference in the quality and the duration of response to anti-CD20 mAbs. Some clinical studies have shown that patients homozygous for FCGR3A 158VV have an improved response to treatment with single-agent rituximab. However, the effect of this polymorphism on longer-term outcome as well as its consequences in the context of immunochemotherapy are more controversial. The biological and clinical relevance of others SNPs such as FCGR2A in patients with FL also need to be clarified.

The PRIMA study recently showed that patients with high tumor burden FL which responded to immunochemotherapy have improved progression-free survival (PFS) after two years of rituximab maintenance. Pre-specified ancillary biological objectives of the PRIMA study included clarification, in the context of a prospective trial, the role of intrinsic FCGR polymorphisms in patients with FL treated with rituximab and chemotherapy (R-CT) and to determine their prognostic impact for patients treated by rituximab maintenance after R-CT. Our genetic studies focused on FCGR3A and FCGR2A polymorphisms in respectively 460 and 455 patients treated in the PRIMA study.
PATIENTS AND METHODS

Study population
Peripheral-blood DNA samples were prospectively obtained from 460 patients with FL included in the open-label, international, multicentre randomized PRIMA study which enrolled a total of 1,217 patients with untreated high tumor burden FL. Only patients with confirmed grade 1, 2 or 3a FL were included in the present biological study. The FLIPI scores were assessed before treatment. Patients were firstly treated by one of the 3 protocol-specified standard immunochemo therapy regimens (induction phase). The three rituximab combinations used in the PRIMA study were CVP (cyclophosphamide 750 mg/m² on day 1, vincristine 1.4 mg/m² [capped at 2 mg] on day 1, prednisone 40 mg/m² on days 1–5, repeated every 3 weeks for 8 cycles), CHOP (cyclophosphamide 750 mg/m² on day 1, vincristine 1.4 mg/m² [capped at 2 mg] on day 1, doxorubicin 50 mg/m² on day 1, prednisone 100 mg on days 1–5, repeated every 3 weeks for 6 cycles), and FCM (fludarabine 25 mg/m² on days 1–3, cyclophosphamide 200 mg/m² on days 1–3, mitoxantrone 6 mg/m² on day 1, repeated every 4 weeks for 6 cycles). Rituximab (375 mg/m² at each infusion) was administered on day 1 of each chemotherapy course. Two additional rituximab infusions were administered in patients treated with CHOP (every 3 weeks after the last cycle) and FCM (2 weeks after the first and the fourth cycles) to ensure equivalent exposure to the antibody during induction for all patients. Response to this induction therapy was assessed 2 to 4 weeks after the last treatment course. After this induction phase, patients who obtained a complete response (CR), an unconfirmed CR (CRu) and a partial response (PR) were randomized in a 1:1 ratio to observation or rituximab maintenance (12 infusions of 375 mg/m² at 8 week
intervals). Patients were evaluated clinically every 8 weeks during the 2-year maintenance phase and by CT scan every 6 months. Patients with bone marrow involvement at diagnosis underwent bone marrow evaluation at the end of the maintenance phase. Thereafter, a clinical evaluation and a CT scan were performed respectively every 3 and 6 months for a period of 3 years. This study was conducted in accordance with the Declaration of Helsinki. All patients signed a consent form for participation in specific studies of germline polymorphisms approved by the ethics committees of Lyon University Hospitals. The PRIMA study is registered with clinicaltrials.gov (NCT00140582).

**Laboratory analysis**

DNA was extracted from peripheral blood mononuclear leukocytes before any treatment. The two SNPs in *FCGR3A* and *FCGR2A* were named according to the SNP500 Cancer database (http://snp500cancer.nci.nih.gov) and identified according to the ID numbers of the NCBI dbSNP database, rs396991 and rs1801274, respectively. The SNP in *FCGR3A* was analyzed using specific fluorescent dye-labeled (FAM and VIC) MGB probes (Applied Biosystems, Foster City, California, USA). Real-time PCR analysis was performed on an ABI Prism 7000 Sequence Detection System (Applied Biosystems) using a total volume of 25 μl with 2μL of DNA (5ng/μl). The forward and reverse primer pairs and MGB probes were obtained from the SNP500Cancer database. PCR conditions were as follows: 50°C for 2 minutes, 95°C for 10 minutes, and 35 cycles at 92°C for 15 seconds and 67°C for 60 seconds. *FCGR2A* genotyping varied slightly, as a complete commercially available assay (Applied Biosystems, Carlsbad, CA, USA) containing primers, probes and TaqMan® Genotyping Master Mix was used. The amplification program for *FCGR2A* consisted
of 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. All analyses were performed in duplicate.

**Statistical analysis**

Clinical characteristics, prognostic parameters, response to induction treatment and outcome were compared between the 460 patients available for FCGR genotyping and all patients included in the PRIMA study to ensure that our study sample was representative of the general patient population. Response to induction phase was assessed according to Cheson criteria.\(^{18}\) Patients could be classified as having a CR, a CRu, a PR or no response with stable or progressive disease (SD and PD). Response was also evaluated at the end of the 2-year rituximab maintenance phase using similar response criteria. PFS and OS defined by international criteria could be evaluated from the date of registration before induction phase or from the time of randomization to rituximab maintenance or observation.\(^{18}\) The correlation between FCGR genotypes and initial characteristics, response to treatment and outcome were assessed. A Chi-square test was used to examine associations between genotypes and patient characteristics, treatment response and treatment toxicities. Time-to-event parameters were estimated by the Kaplan-Meier product limit method and compared by log-rank test.
RESULTS

Clinical Results

The clinical characteristics of the 460 patients with FL of the FCGR cohort in this ancillary study were comparable to those of the whole PRIMA cohort (1193 patients with complete data who received induction treatment), except for the more frequent presence of B-symptoms in patients of the PRIMA study (33% vs. 27%, \(P=0.02\)) (Supplemental Table 1). A majority of the 460 analyzed patients received induction treatment with R-CHOP (n=374, 81%). Other patients received R-CVP (n=84, 18%) or R-FCM (n=2, <1%). A slightly different treatment distribution was reported in the PRIMA study: R-CHOP n=881, 74%; R-CVP n=268, 22%; R-FCM n=44, 4%.

Evaluation of response after induction therapy in the 460 analyzed patients of the FCGR study showed that 159 patients (35%) were in CR, 147 in CRu (32%), 128 in PR (28%), 21 (5%) had SD or PD, but 5 patients (1%) could not be evaluated. Among the 1019 patients that were randomized,\textsuperscript{17} after induction treatment, 398 had been analyzed for FCGR study, 196 patients in the observation arm and 202 into the maintenance arm, one patient in the maintenance arm died immediately after the randomization. We evaluated the primary endpoint (PFS in randomized patients) of the PRIMA study in this subpopulation. With a median follow-up of 36 months, the 3-year PFS rate was 79.2% (95% confidence interval [CI] 72.6–84.4%) in the rituximab maintenance arm and 56.2% (95% CI 48.8–63.0%) in the observation arm (\(P<0.0001\)), thereby confirming the results of the PRIMA study (Supplemental Figure 1).
Genotype frequencies of FCGR3A and FCGR2A alleles

FCGR3A was tested in all 460 patients; the frequency of the VV, VF and FF alleles was 15%, 47% and 38%, respectively. FCGR2A was tested in 450 patients; the distribution of the HH, HR and RR alleles was 27%, 50% and 23%, respectively. These polymorphism distributions were consistent with Hardy-Weinberg equilibrium.

As the PRIMA study is an international project, 78 genomic DNA samples were obtained from Australian and 382 from European (France and Belgium) patients. The allele distribution was not different among patients from different continents. In accordance with French law, no mention of race or ethnicity was made. Comparison of patient characteristics according to FCGR3A and FCGR2A allele status showed no difference between disease characteristics and each SNP. Similarly, the distribution of alleles for these two SNPs was similar between patients treated by R-CHOP, R-CVP and R-FCM (Table 1).

Response to induction therapy according to FCGR3A and FCGR2A alleles

Among the 460 patients genotyped, respectively 455 and 450 patients with FCGR3A and FCGR2A SNPs were assessable for response after induction therapy. The quality of response after immunochemotherapy was not influenced by the FCGR3A and FCGR2A allele status (Table 2). For FCGR3A polymorphism, CR/CRu was observed in 65% in of patients with the VV allele, in 67% with the VF allele and 66% with the FF allele. For FCGR2A polymorphism, CR/CRu was observed in 60% patients with the HH allele, in 72% with the HR allele and 66% with the RR allele. Results were similar when analyses were restricted to patients treated by R-CHOP induction therapy: 67%, 69% and 71% of patients with VV, VF, FF FCGR3A alleles,
and 64%, 72% and 69% of patients with HH, HR, RR FCGR2A alleles, respectively (Table 2).

Response at the end of 2-year rituximab maintenance according to FCGR3A and FCGR2A alleles

Treatment maintenance provided an advantage for response in patients with all FCGR3A and FCGR2A genotypes (Table 2). As observed at the end of induction therapy, no difference in response rates was observed at 2 years from randomization in either the observation or the rituximab maintenance arms according to FCGR3A or FCGR2A genotypes. To better examine the effect of FCGR polymorphisms during rituximab maintenance and determine whether the FCGR3A or FCGR2A genotype might influence response improvement, we focused our analysis on patients who attained PR after induction and achieved CR/CRu after rituximab maintenance. For FCGR3A, among the 57 patients in PR after induction therapy and secondly randomized to the maintenance arm, 30 (57%) were in CR/CRu after 2 years of rituximab treatment, 15 (26%) remained in PR, 8 (14%) progressed and 4 (7%) were not evaluated. The proportion of patients whose response status converted from PR to CR/CRu was not statistically different between the VV (n=4, 40%), VF (n=14, 52%) and FF (n=12, 60%) alleles ($P=.58$). For FCGR2A, among the 56 patients in PR after induction, 30 (54%) obtained a CR/CRu after maintenance treatment, 15 (27%) remained in PR, 7 (13%) progressed and 4 (7%) were not evaluated. The genotyping of patients whose response status converted from PR to CR/CRu showed no difference in allele distribution: HH (n=11, 61%), HR (n=14, 50%), RR (n=5, 50%) ($P=.84$).
PFS and OS according to FCGR3A and FCGR2A alleles

With a median follow-up of 44 months from the time of registration in the PRIMA study, the 3-year PFS rates for patients with FCGR3A VV, VF and FF were 69.9% (95% CI 57.2–79.4%), 69.1% (95% CI 62.3–74.9%) and 67.3% (95% CI 59.8–73.7%), respectively (P=0.68) (Figure 1A). These rates were 67.1% (95% CI 57.8–74.8%), 65.9% (95% CI 59.1–71.7%) and 75.8% (95% CI 66.0–83.1%), respectively, for HH, HR and RR carriers of FCGR2A SNP (P=0.46) (Figure 1B). No difference in median OS from registration was seen between the three allele groups in FCGR3A and FCGR2A SNPs (data not shown). Similar results were obtained when the analysis was restricted to patients treated with R-CHOP (data not shown).

A comparison of PFS from randomization with a median follow-up of 36 months was then performed between patients with FCGR3A VV, VF and FF alleles. PFS rates for patients with VV, VF and FF alleles in FCGR3A were 66.0% (95% CI 44.8–80.7%), 53.0% (95% CI 42.2–62.6%) and 56.8% (95% CI 44.7–67.3%), respectively, (P=0.48) (Figure 2 A1) in the observation arm, and 79.8% (95% CI 60.4–90.4%), 78.6% (95% CI 68.3–85.9%) and 79.6% (95% CI 67.9–87.4%), respectively, in the rituximab maintenance arm (P=0.46) (Figure 2 A2). The 3-year PFS rate of VV carriers was thus slightly superior to that of non-VV carriers in the observation arm, but this difference was not significant (66% vs. 54.6% P=0.31) and the curves were overlapping in the rituximab maintenance arm (79.8% vs. 79% P=0.70).

The 3-year PFS rates according to FCGR2A HH, HR and RR alleles were 54.4% (95% CI 39.3–67.2%), 52.7% (95% CI 42.1–62.3%) and 62.5% (95% CI 46.7–74.9%), respectively, in the observation arm (P=0.26) (Figure 2 B1) and 80.9% (95% CI 67.3–89.3%), 77.7% (95% CI 67.7–84.9%) and 83.9% (95% CI 67.6–92.4%), respectively, in the rituximab maintenance arm (P=0.15).
respectively, in the rituximab maintenance arm \((P=0.79)\) (Figure 2 B2). These rates were not different between HH alleles and non-H alleles in the observation \((54.4\% \text{ vs. } 55.7\% \ P=0.47)\) and the rituximab arms \((80.9\% \text{ vs. } 79.4\% \ P=0.64)\).

**Influence of FCGR3A and FCGR2A alleles according to tumor bulk and FLIPI risk groups**

As previous reports showed that response to rituximab could be influenced by the presence of bulky disease,\(^{14}\) we analyzed whether FCGR SNPs had a differential effect for patients according to tumor bulk. We performed sub-analyses in patients with or without bulky tumors (> or < 7 cm) as prospectively collected (this parameter was one of the inclusion criteria) and in the different prognostic groups of the FLIPI \((\leq 1, 2, 3-5 \text{ risk factors})\).\(^{2}\) The quality of response after the induction phase, PFS from registration and from randomization for patients allocated to rituximab maintenance were not influenced by FCGR3A or FCGR2A genotypes in the different subgroups analyzed (data not shown).

**Outcome according to FCGR3A and FCGR2A alleles stratified by lymphocyte counts**

As it was previously reported that a lymphocyte count at diagnosis \(\geq 1 \text{ G/L} \) influenced response and event-free survival in rituximab-treated patients,\(^{14}\) we hypothesize that FCGR polymorphism could play a distinct role in patients with low or high lymphocyte counts. We then analyzed the potential influence of FCGR3A and FCGR2A genotypes on response and outcome in those subgroups. At baseline, lymphocyte counts were available in 406 of the genotyped patients. In those patients, the median lymphocyte count was 1.26 G/L, with 259 patients presenting with a lymphocyte count.
count ≥1 G/L and 147 <1G/L, respectively. Responses to induction therapy or at the end of rituximab maintenance (in the 192 patients randomized to this treatment arm) were not influenced by \textit{FCGR3A} genotypes in high and low lymphocyte count groups. Similar analyses were performed for \textit{FCGR2A} SNP, and no difference in term of response was observed in the two lymphocyte count groups. The PFS from registration for \textit{FCGR3A} was not different between the 3 genotypes in the low lymphocyte count group: the 3-year PFS rate was 70.6\%, 68.6\%, 67.1\% for VV, VF and FF carriers, respectively (\(P=0.47\)). No difference was either observed in high lymphocyte count group with a 3-year PFS rate of 73.9\%, 69.8\%, 67.6\% for VV, VF and FF carriers, respectively (\(P=0.78\)). Similar results were obtained according to the \textit{FCGR2A} polymorphism. Evaluation of PFS from randomization in the rituximab arm also showed no difference between the different genotypes of \textit{FCGR3A} and \textit{FCGR2A} in patients with low or high lymphocyte count evaluated at time of randomization (not shown).

\textbf{Effect of \textit{FCGR} genotypes on hematological toxicities during treatment}

A previous study has shown that \textit{FCGR3A} polymorphisms could be associated with the degree of neutropenia manifest in the context of rituximab treatment after autologous transplantation.\textsuperscript{19} We therefore explored the relationship between neutropenia and \textit{FCGR} SNPs during induction treatment and rituximab maintenance. During induction treatment, 347 and 341 patients presented at least one episode of grade 2-4 neutropenia in the \textit{FCGR3A} and \textit{FCGR2A} genotyped cohorts, respectively. No difference in the frequencies of neutropenia was observed between \textit{FCGR3A} VV (n=48, 71\%)\), VF (n=168, 78\%) and FF (n=131, 74\%) patients (\(P=.38\)) and between \textit{FCGR2A} HH (n=88, 72 \%), HR (n=173, 77\%), RR (n=80, 78\%) patients (\(P=.27\)).
When the analysis was restricted to grade 3/4 neutropenia during induction treatment, for *FCGR3A* SNP, 34 VV (50%), and 252 F carriers (FV or FF) patients (66%) respectively presented at least one episode of grade 3 neutropenia ($P=0.02$). However, this difference was not observed for the *FCGR2A* SNP. During rituximab maintenance, 7 (10%), 12 (6%), 10 (6%) of patients with *FCGR3A* VV, VF and FF genotypes had at least one episode of grade 2-4 neutropenia ($P=0.34$). Similarly, no difference was observed for *FCGR2A*: 11 HH (9%), 13 HR (6%) and 4 RR (4%) of patients presented respectively at least one grade 2-4 neutropenia ($P=0.13$). Again, no difference were observed when the analyses were restricted to grade 3/4 neutropenia.

**DISCUSSION**

In this large prospective sub-study of the PRIMA trial, we found no influence of *FCGR3A* or *FCGR2A* polymorphisms on the response or outcome of patients with high tumor burden FL receiving a rituximab chemotherapy combination and rituximab maintenance. These observations were similar regardless of the tumor bulk or FLIPI category, and were unaffected by the peripheral blood lymphocyte count. In contrast to the endpoints related to rituximab efficacy, we observed that *FCGR3A* polymorphisms were associated with the rate of grade 3-4 neutropenia during the combination of rituximab and chemotherapy. Given the low number of patients developing this adverse event, we cannot exclude that this observation may be a chance association of limited clinical relevance. However, in accordance with previous reports, this result suggests that immune mechanisms mediated by NK cells may play a role in rituximab induced neutropenia.$^{19}$
The rationale to investigate these polymorphisms was based on previously published \textit{in vitro} and \textit{in vivo} studies. It is well established that the affinity of Fc region of hu-Ig G1 with FcγRIIIA is influenced by some SNPs in \textit{FCGR3A}, for example the presence of a valine (V) conferring a higher affinity than the presence of phenylalanine (F) at codon 158.\textsuperscript{20} \textit{In vitro}, with therapeutic mAbs, these data were confirmed, with rituximab more efficiently binding and activating NK cells in VV carriers compared to FF carriers.\textsuperscript{21,22} However, when analyzing the efficacy of B-cells lysis by NK cells obtained from healthy donors, it was found that differences in this lytic activity according to \textit{FCGR3A} genotypes were only observed at low (< 0.01 µg/mL) rituximab concentrations.\textsuperscript{22} Serum concentrations of rituximab obtained \textit{in vivo}, after infusions given every 3 weeks or every 2 months, usually substantially exceed these levels,\textsuperscript{23-26} although the concentration of antibody at tumor sites remain unknown. More recently, it was reported that some peripheral blood parameters reflecting NK cells activation assessed 4 hours after an initial infusion of rituximab were lower in FF than in FV/VV patients.\textsuperscript{27} However, the significance of these findings with repeated rituximab infusions, or in terms of clinical response remains unknown. Considering the other mechanisms of action of rituximab (apoptosis, complement dependent cytotoxicity, phagocytosis) that could also eventually account for its therapeutic activity, a definite relationship between NK cells activation and the efficacy of the antibody \textit{in vivo} is still lacking.

Based on this biological rationale, many retrospective studies in patients treated with therapeutic IgG1 mAbs analyzed the correlation between \textit{FCGR} polymorphisms and treatment response or outcome.\textsuperscript{12,13,28-30} Considering specifically FL, eight studies
have previously examined these correlations.\textsuperscript{12,13,15,31-35} The first demonstration of the variability in the quality of response to rituximab treatment according to \textit{FCGR3A} polymorphism was derived from analysis of a cohort of 49 patients with low tumor burden FL receiving 4 infusions of rituximab as first-line treatment.\textsuperscript{12} Significantly higher clinical and molecular response rates were demonstrated in VV carriers, and a possible improvement in PFS was suggested. In contrast, no influence of the \textit{FCGR2A} polymorphism was found in this study.\textsuperscript{12} A second cohort of 87 patients receiving single agent rituximab was retrospectively analyzed for the effect of \textit{FCGR3A} and \textit{FCGR2A} polymorphisms and reported their influence on both response rates and PFS.\textsuperscript{13} In the context of a prolonged rituximab treatment schedule (4 weekly infusions followed by 4 infusions administered every 2 months), again used as single agent, response and event-free survival were improved mainly for patients with \textit{FCGR3A} VV genotypes.\textsuperscript{14,33} This study of 171 patients also established that \textit{FCGR3A} status was an independent prognostic factor for event-free survival.\textsuperscript{14} These latter studies were retrospective and included both untreated and previously treated patients. Preliminary results obtained in the prospective study assessing rituximab single agent followed by two years of rituximab maintenance in patients with low-tumor burden FL were recently reported.\textsuperscript{32} In this study, \textit{FCGR3A}, \textit{FCGR2A} and \textit{FCGR2B} SNPs were not found to influence the outcome of patients receiving rituximab maintenance.\textsuperscript{32} When considering rituximab plus chemotherapy combinations, three prior retrospective reports (including 75 to 102 patients) failed to demonstrate any impact of \textit{FCGR2A} and \textit{FCGR3A} status on prognosis.\textsuperscript{15,31,35} However, discordant results were reported from a prospective clinical trial including 76 patients treated with chemotherapy in combination with either rituximab or I-131 tositumomab, where \textit{FCGR3A} alleles were associated with OS.\textsuperscript{33} Two additional
studies should be mentioned in this context. First, the outcome of patients with FL treated with chemotherapy alone or under watchful waiting does not appear to be affected by *FCGR3A* or *FCGR2A* polymorphisms,\(^ {33,36}\) clearly indicating that the effects of FcγR variability on outcome are restricted to patients receiving mAb therapy. Secondly, the identification of *FCGR2A* polymorphism impact may potentially only reflect the linkage disequilibrium between the *FCGR3A* rs396991 and the *FCGR2A* rs1801274 SNPs rather than a true biological mechanism related to the affinity of the FcγRIIA allotypes.\(^ {37,38}\)

The current results represent the largest cohort prospectively analyzed, and clarify this debate. The characteristics and outcome of the 460 patients who provided consent for genotyping did not meaningfully differ from those of the whole PRIMA study. The relative proportion of *FCGR3A* and *FCGR2A* SNP genotypes, mainly from Europe and Australia, was in accordance with other studies and with the Hardy-Weinberg equilibrium. As the PRIMA study design included two steps, we were able to analyze the effects of *FCGR* SNPs both after rituximab-chemotherapy induction and also for those patients with responsive disease who received rituximab maintenance. In accordance with other reports from smaller retrospective studies,\(^ {15,31,35}\) our data indicate that *FCGR3A* and *FCGR2A* polymorphisms have no impact on response rate and PFS for patients treated with rituximab chemotherapy combinations. Therefore, the effects of FcγR polymorphisms on the therapeutic activity of rituximab are only observed when this antibody is used as single agent. One possible explanation is that chemotherapy agents may alter immune mechanisms such as ADCC in those patients. However, this does not preclude the therapeutic activity of the mAbs when associated with chemotherapy.\(^ {39}\) These results
are also in line with those observed in the context of other B-cell malignancies, such as diffuse large B-cell lymphoma or chronic lymphocytic leukemia. More surprising in this regard was the lack of correlation between FCGR genotypes and response after maintenance or PFS in those patients that received 2 years of rituximab maintenance. Again, the effects of previous chemotherapy may partly account for these observations, by altering the ADCC effector functions. Our analysis of lymphocyte count utilized levels at registration and at time of randomization and is limited by the lack of evaluation of lymphocyte subsets, such as NK lymphocytes for instance. Moreover, lymphocyte subsets are not the sole immune effectors of anti-CD20 mAbs, the role of macrophages also being important. Another hypothesis is that the schedule of rituximab administration (one infusion of 375 mg/m² every 2 months for 24 months) abolishes the variability of the therapeutic effects related to FCGR polymorphisms. With this schedule, sustained levels of rituximab are likely to be achieved between each infusion, probably leading to a saturation of FCγR binding. It is also conceivable that the efficacy of rituximab according to FCGR polymorphisms may be observed in patients with low tumor burden, not represented in the current study; however, we did not observed any influence of FCGR genotypes in patients without tumor bulk.

In conclusion, this large series of patients with FL treated in a prospective trial indicate that the outcome of rituximab-chemotherapy followed by 2-year rituximab maintenance is not influenced by FCGR3A and FCGR2A genotypes. Other genetic determinants of anti-CD20 mAbs activity may also need to be examined in the future. New anti-CD20 mAbs are currently under development to improve ADCC, complement-dependent cytotoxicity (CDC) or induction of programmed cell death.
(PCD).\textsuperscript{10} In \textit{in vitro} study on peripheral blood mononuclear cells of healthy donors, low fucose anti-CD20 IgG1 enhanced ADCC compared to rituximab and was independent of \textit{FCGR3A} SNP.\textsuperscript{45} Clinically, a phase I/II study in relapsed or refractory B-cell malignancies has shown that objective responses to a new humanized IgG1 with afucosylated Fc appears to be independent of \textit{FCGR3A} SNP.\textsuperscript{46} Our results suggest that ADCC optimization may be more relevant to explore when using anti-CD20 antibodies alone, or in combination with other immunomodulating agents\textsuperscript{47} that may enhance cellular cytotoxicity.

ACKNOWLEDGMENTS

The authors thank MD Reynaud for editorial assistance; Celine Mabon for technical assistance; the GELARC (Groupe d’Etude des Lymphomes de l’Adultes Recherche Clinique) team, and especially Anne-Laure Borrel and Delphine Germain for study management as well as Bénédicte Gelas-Dore for statistical analyses.

AUTHORSHIP

Contribution: H.G. designed the research, performed the research, analyzed the data and wrote the manuscript; G.S. designed the research, analysed the data and wrote the manuscript; H.G., G.C., J.F.S., M.H.D., F.O., P.S., A.P., P.B., R.B., A.S., J.D., O.C., J.V.C., A.D., F.J., A.V., P.D., G.S. provided study materials or patients, collected and assembled data; H.G., G.C., J.F.S., M.H.D., F.O., P.S., A.P., P.B., R.B., A.S., J.D., O.C., J.V.C., A.D., F.J., A.V., P.D., G.S. approved final manuscript.
CONFLICTS OF INTEREST DISCLOSURE

G.C., G.S. and J.F.S. have received compensation for consultancy, advisory board and talks from Roche. O.C. has received compensation for consultancy.

The remaining authors declare no competing financial interests.

REFERENCES


33. Persky DO, Dorman D, Goldman B et al. Fc gamma receptor 3a genotype predicts overall survival in follicular lymphoma patients treated on SWOG trials with


### Table 1. Correlation between FCGR3A and FCGR2A genotypes and clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>FCGR3A</th>
<th></th>
<th></th>
<th>FCGR2A</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VV</td>
<td>VF</td>
<td>FF</td>
<td>P</td>
<td>HH</td>
<td>HR</td>
</tr>
<tr>
<td>No. of patients</td>
<td>68</td>
<td>15</td>
<td>215</td>
<td>47</td>
<td>177</td>
<td>38</td>
</tr>
<tr>
<td>Europe</td>
<td>53</td>
<td>14</td>
<td>181</td>
<td>47</td>
<td>148</td>
<td>39</td>
</tr>
<tr>
<td>Australia</td>
<td>15</td>
<td>19</td>
<td>34</td>
<td>44</td>
<td>29</td>
<td>37</td>
</tr>
<tr>
<td>Male sex</td>
<td>38</td>
<td>56</td>
<td>120</td>
<td>56</td>
<td>96</td>
<td>54</td>
</tr>
<tr>
<td>Age &gt;60 years</td>
<td>26</td>
<td>38</td>
<td>90</td>
<td>42</td>
<td>63</td>
<td>36</td>
</tr>
<tr>
<td>Ann Arbor stage III/IV</td>
<td>63</td>
<td>93</td>
<td>195</td>
<td>91</td>
<td>161</td>
<td>91</td>
</tr>
<tr>
<td>ECOG PS ≥1</td>
<td>22</td>
<td>32</td>
<td>69</td>
<td>32</td>
<td>56</td>
<td>31</td>
</tr>
<tr>
<td>B symptoms present</td>
<td>16</td>
<td>24</td>
<td>60</td>
<td>28</td>
<td>47</td>
<td>27</td>
</tr>
<tr>
<td>BM involvement</td>
<td>38</td>
<td>58</td>
<td>113</td>
<td>53</td>
<td>101</td>
<td>58</td>
</tr>
<tr>
<td>Elevated LDH</td>
<td>16</td>
<td>24</td>
<td>67</td>
<td>31</td>
<td>65</td>
<td>37</td>
</tr>
<tr>
<td>Hemoglobin &lt;12 g/dL</td>
<td>18</td>
<td>26</td>
<td>39</td>
<td>18</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>β2-microglobulin ≥3 mg/L</td>
<td>18</td>
<td>28</td>
<td>52</td>
<td>26</td>
<td>49</td>
<td>30</td>
</tr>
<tr>
<td>FLIPI score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–1 risk factors</td>
<td>17</td>
<td>25</td>
<td>51</td>
<td>24</td>
<td>39</td>
<td>22</td>
</tr>
<tr>
<td>2 risk factors</td>
<td>20</td>
<td>29</td>
<td>71</td>
<td>33</td>
<td>69</td>
<td>39</td>
</tr>
<tr>
<td>3–5 risk factors</td>
<td>31</td>
<td>46</td>
<td>92</td>
<td>43</td>
<td>69</td>
<td>39</td>
</tr>
<tr>
<td>Induction regimen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-CHOP</td>
<td>54</td>
<td>79</td>
<td>175</td>
<td>81</td>
<td>145</td>
<td>82</td>
</tr>
<tr>
<td>R-CVP</td>
<td>13</td>
<td>19</td>
<td>40</td>
<td>19</td>
<td>31</td>
<td>18</td>
</tr>
<tr>
<td>R-FCM</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

ECOG indicates Eastern Cooperative Oncology Group; PS, performance status; BM, bone marrow; FLIPI, follicular lymphoma international prognostic index; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CVP, rituximab, cyclophosphamide, vincristine, and prednisone; R-FCM, rituximab, fludarabine, cyclophosphamide, and mitoxantrone; CR, complete response; CRu, unconfirmed complete response; PR, partial response; SD/PD, stable disease/progressive disease.

*The two SNPs in FCGR3A and FCGR2A were tested for Hardy-Weinberg equilibrium.*
Table 2. Clinical response according to \textit{FCGR3A} and \textit{FCGR2A} genotypes

<table>
<thead>
<tr>
<th></th>
<th>FCGR3A</th>
<th></th>
<th>FCGR2A</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VV</td>
<td>VF</td>
<td>FF</td>
<td>No.</td>
</tr>
<tr>
<td>Response to induction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>23</td>
<td>34</td>
<td>75</td>
<td>35</td>
</tr>
<tr>
<td>Cru</td>
<td>21</td>
<td>31</td>
<td>69</td>
<td>32</td>
</tr>
<tr>
<td>CR/Cru</td>
<td>44</td>
<td>65</td>
<td>144</td>
<td>67</td>
</tr>
<tr>
<td>PR</td>
<td>21</td>
<td>31</td>
<td>61</td>
<td>29</td>
</tr>
<tr>
<td>SD/PD</td>
<td>3</td>
<td>4</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>R-CHOP regimen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR/Cru</td>
<td>36</td>
<td>67</td>
<td>121</td>
<td>69</td>
</tr>
<tr>
<td>PR</td>
<td>18</td>
<td>33</td>
<td>48</td>
<td>27</td>
</tr>
<tr>
<td>SD/PD</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Response at 2-years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observation arm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR/Cru</td>
<td>15</td>
<td>58</td>
<td>37</td>
<td>43</td>
</tr>
<tr>
<td>PR</td>
<td>4</td>
<td>15</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>SD/PD</td>
<td>7</td>
<td>27</td>
<td>38</td>
<td>44</td>
</tr>
<tr>
<td>Maintenance arm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR/Cru</td>
<td>21</td>
<td>72</td>
<td>72</td>
<td>77</td>
</tr>
<tr>
<td>PR</td>
<td>4</td>
<td>14</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>SD/PD</td>
<td>4</td>
<td>14</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>

CR indicates complete response; CRu, unconfirmed complete response; PR, partial response; SD/PD, stable disease/progressive disease; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.
Figure 1. Progression-Free Survival according to FCGR3A (A) and FCGR2A (B) alleles from the time of patient registration in the PRIMA study

A. FCGR3A

B. FCGR2A
**Figure 2.** Progression-Free Survival according to *FCGR3A* (A) and *FCGR2A* (B) alleles from the time of randomization between observation and rituximab maintenance in the PRIMA study


B1. FCGR2A. Observation arm.

B2. FCGR2A. Rituximab arm.
Clinical outcome of follicular lymphoma patients receiving chemo-immunotherapy in the PRIMA study is not affected by FCGR3A and FCGR2A polymorphisms