VOLUME 28 · NUMBER 13 · MAY 1 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

BCL-2/IgH Polymerase Chain Reaction Status at the End of Induction Treatment Is Not Predictive for Progression-Free Survival in Relapsed/Resistant Follicular Lymphoma: Results of a Prospective Randomized EORTC 20981 Phase III Intergroup Study

Marinus H.J. van Oers, Evelyn Tönnissen, Martine Van Glabbeke, Livia Giurgea, Joop H. Jansen, Richard Klasa, Robert E. Marcus, Max Wolf, Eva Kimby, Andrej Vranovsky, Harald Holte, Anton Hagenbeek, and Bert A. van der Reijden

Academic Medical Center, Amsterdam; Radboud University Nijmegen Medical Centre, Nijmegen; University Medical Center, Utrecht; the Netherlands; European Organisation for Research and Treatment of Cancer, Brussels, Belgium; British Columbia Cancer Agency, Vancouver, Canada; Addenbrook Hospital, London, United Kingdom; Peter MacCallum Cancer Center, Melbourne, Australia; Karolinska Institutet, Stockholm, Sweden; National Institute of Cancer, Bratislava, Slovak Republic; and Radium Hospital, Oslo, Norway.

Submitted July 23, 2009; accepted December 17, 2009; published online ahead of print at www.jco.org on April 5, 2010.

Supported by F. Hoffmann- La Roche Pharmaceuticals.

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

Clinical Trials repository link available on JCO.org.

Corresponding author: Marinus H.J. van Oers, MD, PhD, Department of Hematology F4-224, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands; e-mail: M.H.vanOers@AMC.UVA.NL.

0732-183X/10/2813-2246/$20.00

DOI: 10.1200/JCO.2009.25.0852

ABSTRACT

Purpose

The prognostic value of residual BCL2/immunoglobulin heavy chain (*BCL2/IgH*) –positive cells in peripheral blood (PB) or bone marrow (BM) after induction treatment in follicular lymphoma (FL) is still controversial. In a prospective randomized phase III intergroup trial of 465 patients with relapsed/resistant follicular lymphoma (FL), we showed that addition of rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisone induction results in increased overall and complete response rates, and that rituximab maintenance strongly improves median progression-free survival (PFS) as well as overall survival. Here, we studied whether *BCL2/IgH* major break point levels in PB/BM correlated with response rates/quality for the induction phase and PFS for the maintenance phase.

Patients and Methods

Samples were obtained before and after induction therapy and at the end of the 2 years maintenance/observation period. *BCL2/IgH* major break point–positive cells were quantified by genomic quantitative polymerase chain reaction in 792 samples from 238 patients.

Results

Pretreatment *BCL2/IgH* levels had no significant prognostic value for overall response or complete remission rates after induction treatment, but pretreatment positive BM results had an adverse prognostic value for PFS from first randomization ($P = .023$). Importantly, *BCL2/IgH* levels at the end of induction treatment had no prognostic value for PFS from second randomization. The highly significant improved PFS by rituximab maintenance was observed in both *BCL2/IgH* PB/BM–positive and –negative groups.

Conclusion

Postinduction *BCL2/IgH* major break point status in BM/PB is not useful for decisions on subsequent therapy for patients with relapsed/resistant FL.

J Clin Oncol 28:2246-2252. © 2010 by American Society of Clinical Oncology

INTRODUCTION

In up to 80% of patients, follicular lymphoma (FL) is characterized by the presence of the t (14;18) translocation, resulting in *BCL2*/immunoglobulin heavy chain (*BCL2/IgH*) gene fusion. In the majority of the patients, complete remissions (CR) or partial remissions (PR) can be obtained with chemotherapy, but relapse rates are very high. After relapse, both the response rate and duration after subsequent salvage treatment regimens steadily decrease.[1,2] The chimeric anti-CD20 monoclonal antibody rituximab has improved treatment outcome to such an extent that the combination of rituximab and chemotherapy is now the standard induction treatment for both previously untreated and relapsed FL.[3-7]

Although some studies have shown that patients achieving CR on induction have a better prognosis, other studies failed to show improved survival with treatment regimens producing higher CR rates (reviewed in[8]). These discrepancies might partially be due to different levels of minimal residual disease (MRD) in patients in clinical CR. Thus, the value of molecular monitoring of MRD in FL by quantitative *BCL2/IgH* polymerase chain reaction (PCR) has been addressed in several studies. Conflicting results

have been reported, both as to whether pretreatment *BCL2/IgH* levels are predictive for treatment response,[9-11] and as to the prognostic value of *BCL2/IgH* levels after either standard[11-16] or myeloablative chemotherapy.[17-22] Important factors contributing to these controversies are differences in numbers (generally small) and clinical characteristics of patients studied, treatments applied, time of sampling, material analyzed (blood *v* bone marrow), and PCR assays used.[8]

Here we report on the results of *BCL2/IgH* PCR analysis performed in the setting of a large, prospective, randomized, phase III, intergroup trial evaluating rituximab in remission induction and maintenance treatment of 465 patients with relapsed/resistant FL. This study showed that addition of rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) induction results in increased overall and CR rates, and that rituximab maintenance strongly improves median progression-free survival and overall survival.[23]

Questions addressed in the present molecular side study were: do preinduction *BCL2/IgH* levels correlate with quality of response and progression-free survival? Do postinduction *BCL2/IgH* levels correlate with further progression-free survival (PFS)? Are *BCL2/IgH* levels predictive of the benefit of rituximab?

PATIENTS AND METHODS

Patients

This randomized, phase III study (European Organisation for Research and Treatment of Cancer [EORTC] 20981) was conducted at 130 centers in Canada, Australia/New Zealand, Europe, and South Africa. Major eligibility criteria were: older than 18 years, CD20-positive grade 1 to 3 FL, stage III/IV at initial diagnosis, and relapse after or resistant to a maximum of two nonanthracycline-containing chemotherapy regimens.[23] Written informed consent was obtained according to the local rules. The study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines.

Study Design and Treatment

Both study design and treatment have been described in detail.[23] In brief, eligible patients were randomly assigned to remission induction with either six cycles of standard CHOP once every 3 weeks, or CHOP plus rituximab (375 mg/m^2 intravenously at day 1 of each cycle of CHOP). Those with stable disease or progression after three cycles of CHOP with or without rituximab, went off study. Patients in CR/PR after six cycles underwent a second random assignment to either observation or maintenance treatment with rituximab (375 mg/m^2 intravenously every 3 months, until relapse or for a maximum period of 2 years).

Molecular Study

Peripheral blood (PB) and bone marrow (BM) samples were obtained before the start and at the end of the induction therapy (in responding patients) and at the end of the 2 years maintenance/observation period. Samples were directly frozen at −20°C to −80°C on withdrawal. For central quantitative PCR analysis, samples were shipped on dry ice to the Central Hematology Laboratory, Radboud University Medical Center, Nijmegen, the Netherlands. All samples were analyzed in duplicate in a blinded manner without any prior knowledge of *BCL2/IgH* and clinical status. Genomic DNA was isolated using a high salt lysis protocol. Briefly, samples were thawed at 37°C, diluted in TSE (Tris, HCl, NaCl buffer) and subsequently incubated at 60°C for 1 hour in the presence of proteinase K and sodium dodecyl sulfate (SDS), followed by a further incubation o/n at 56°C. Samples were thoroughly mixed with a 6M NaCl solution followed by a centrifugation step at 2,500 rpm. DNA was precipitated by addition of 96% ethanol to the supernatant. DNA was dissolved in TE (Tris/EDTA) buffer. The percentage of *BCL2/IgH* major break point region (MBR) –positive cells was quantified by quantitative PCR using 500 ng genomic DNA. PCRs were performed using 300 nmol/L each primer, 160 nmol/L probe, 0.625 U AmpliTaq Gold, 1× Taqman A buffer (Applied Biosystems, Foster City, CA), 5 mmol/L $MgCl_2$ in a total volume of 25 μL according to the manufacturer's instructions (Applied Biosystems). Samples were heated for 10 minutes at 95°C and amplified for 45 cycles of 30 seconds at 95°C and 1.5 minutes at 60°C (ABI Prism 7700 Sequence detecter; Applied Biosystems). The 5′-3′ sequences of used primers and probes (TET labeled) were GTT TGA CCT TTA GAG AGT TGC TTT ACG (BCL2-2983), ACC TGA GGA GAC GGT GAC C (JHCON), and ACA GAC CCA CCC AGA GCC C (MBR TET), respectively. In all experiments, both a log dilution series consisting of genomic DNA isolated from the DOHH2 cell line diluted in DNA isolated from three *BCL2/IgH*-negative Epstein-Barr virus transformed cell lines and a no template control were included. The primer-probe combination reproducibly detected the equivalent one DOHH2 cell in 10,000 negative cells. The reference gene albumin was quantified in duplicate on each sample to normalize for PCR and DNA input variations. 5′-3′ sequences of used primers and probes (VIC labeled) were TGA AAC ATA CGT TCC CAA AGA GTT T (ALB-F), CTC TCC TTC TCA GAA AGT GTG CAT AT (ALB-R), TGC TGA AAC ATT CAC CTT CCA TGC AGA (ALB VIC), respectively. Only samples yielding Ct values of 20.7 ± 2.0 at a threshold of 0.05 for albumin were included. For each sample, the percentage of *BCL2/IgH*-positive cells was measured in duplicate with Taqman software using the dilution series. Normalization was performed by dividing the percentage of *BCL2/IgH*-positive cells with the correction factor $2^{(20.7-CtS)}$ in which CtS represents the obtained albumin Ct for the individual samples using a threshold of 0.05. The value of 20.7 was the average Ct at a cycle threshold of 0.05 derived from 100 samples using 500 ng DNA. *BCL2/IgH* values were recorded on data forms and sent to the EORTC in Brussels, Belgium, for statistical analysis. Samples with no amplification signal were scored negative.

Statistical Analysis

The primary end point for induction was response to treatment; PFS from first random assignment was a secondary end point. The primary end point for the maintenance phase was PFS (defined as interval between the date of second random assignment and date of first relapse, progression, or death). The prognostic factor analyses (comparison of outcome between *BCL2/IgH*-positive and *BCL2/IgH*-negative patients) used the Mantel-Haenszel test for trend on four ordered response categories (CR/PR/NC/PD, excluding not assessable patients), and the log-rank test for PFS.

Kaplan-Meier curves were calculated to graphically show the differences between the treatment arms. Predictive factor analysis (ie, comparison of the relative advantage of rituximab between *BCL2/IgH*-positive and *BCL2/IgH*-negative patients) used multivariate models with three variables: the randomized treatment, the *BCL2/IgH* status (positive *v* negative), and an interaction term between those two factors. Logistic models were used for response (CR + PR *v* NC + PD, excluding nonassessable cases) and Cox models for PFS. All *P* values were two sided.

RESULTS

High Correlation Between PB and BM BCL2/IgH *Major Break Point Levels*

A total of 792 samples were obtained from 238 patients. Figures 1A and 1B show the total number of samples as well as the number of *BCL2/IgH* major break point–positive samples obtained in relation to the time point of sampling and the total number of patients on study. As shown the samples were evenly distributed among the treatment arms, both for induction and maintenance (Appendix Table A1, online only). Pretreatment 49% (67 of 137) of the patients had a *BCL2/IgH*-positive BM and 43% (78 of 183) were *BCL2/IgH* positive in the PB.

The *BCL2/IgH*-positive and -negative patients were well balanced as to sex, performance status, time since initial diagnosis, Ann Arbor stage, bulky disease, B symptoms, and histologic BM involvement. However, the patients with *BCL2/IgH*-positive BM tended to be older ($P = .044$, test for trend), whereas the patients with *BCL2/IgH*-positive

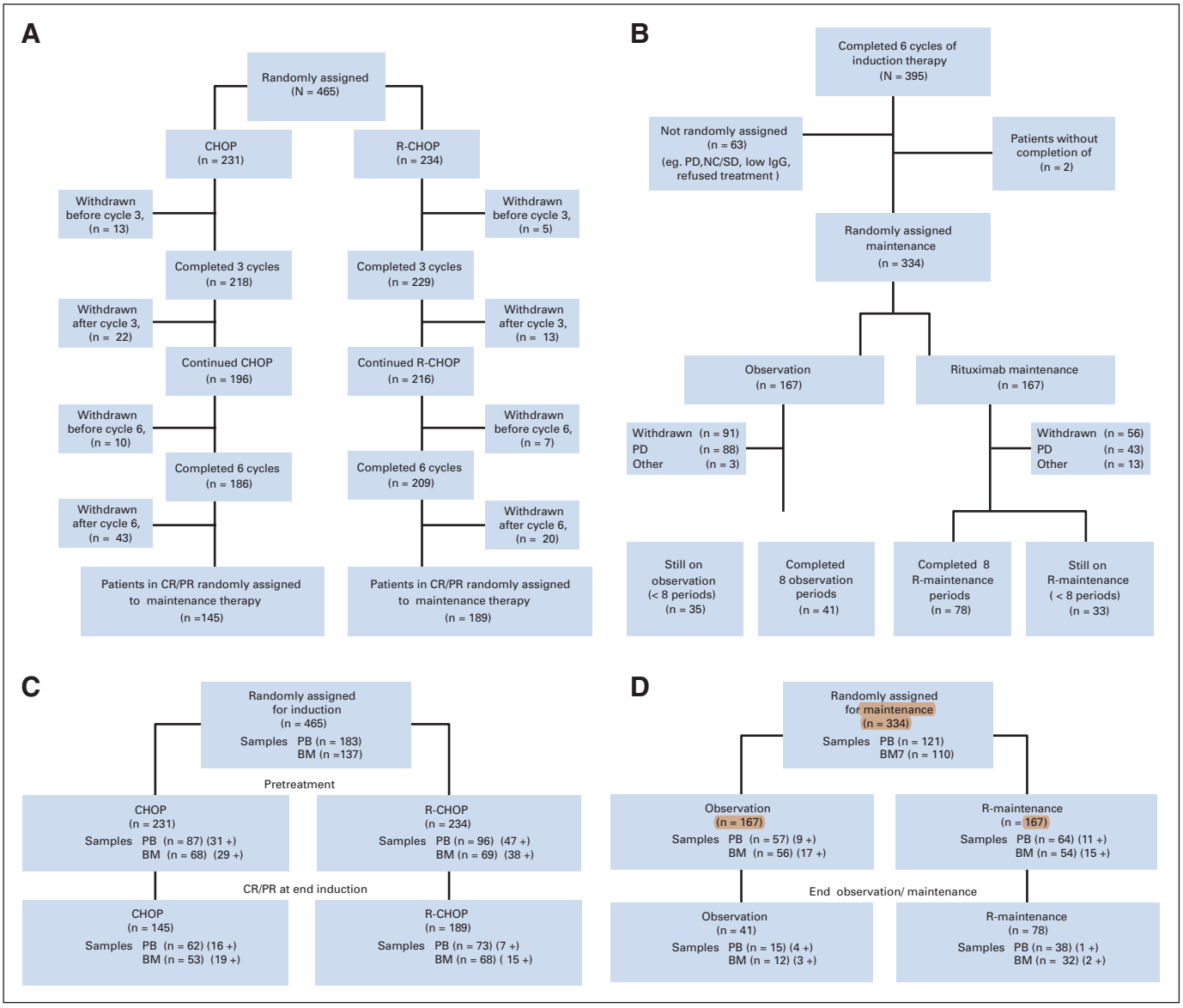

Fig 1. CONSORT diagrams for induction (A) and maintenance (B). The total number of samples as well as the number of BCL2/immunoglobulin heavy chain polymerase chain reaction–positive (+) samples obtained in relation to the time point of sampling and the total number of patients on study during the (A) induction phase and the (B) maintenance/observation phase of the study. BM, bone marrow; PB, peripheral blood; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CHOP, rituximab plus CHOP; CR, complete response; PR, partial response; Obs, observation.

PB more often had received two prior chemotherapy regimens (63% *v* 38% respectively; $P = .012$). *BCL2/IgH* positivity in BM did not correlate with histologic BM positivity.

Of the 792 samples, 261 were paired PB and BM samples: 121 at first random assignment, 102 at the end of induction treatment, and 38 at the end of the 2 years of maintenance/observation. At all three time points, we found a high correlation between *BCL2/IgH* levels in PB and BM. Using Cochran-Mantel-Haenszel statistics, the gamma coefficients were 0.96, 0.80, and 1.0 respectively (all $P < .001$).

Pretreatment BCL2/IgH *Major Break Point Levels Are Not Prognostic for Response to Induction Treatment But Do Predict PFS*

The quality of response to induction was not affected by the pretreatment *BCL2/IgH* levels in PB or BM. In patients with initially *BCL2/IgH*-positive bone marrow, ORR and CR rates were 78% (52 of 67) and 21% (14 of 67), whereas in the initially BM *BCL2/IgH*-negative patients ORR and CR were 86% (60 of 70) and 24% (17 of 70), respectively ($P = .51$). For the *BCL2/IgH* status in PB, highly similar results were obtained: ORR and CR rates in the initially *BCL2/IgH*-positive patients were 82% (64 of 78) and 22%,(17 of 78) and in initially *BCL2/IgH*-negative patients 84% (88 of 105) and 22% (23 of 105), respectively ($P = .22$; Appendix Table A2, online only). This was true for both treatment arms. Even patients with the highest *BCL2/IgH* levels ($> 10^{-3}$) did not differ in response from the *BCL2/IgH*-negative patients (data not shown). Moreover, within the group of initially *BCL2/IgH*-positive patients the response rate and quality were independent of the actual *BCL2/IgH* PCR levels.

Analysis of the effect of pretreatment *BCL2/IgH* levels on PFS from first randomization showed no difference between patients

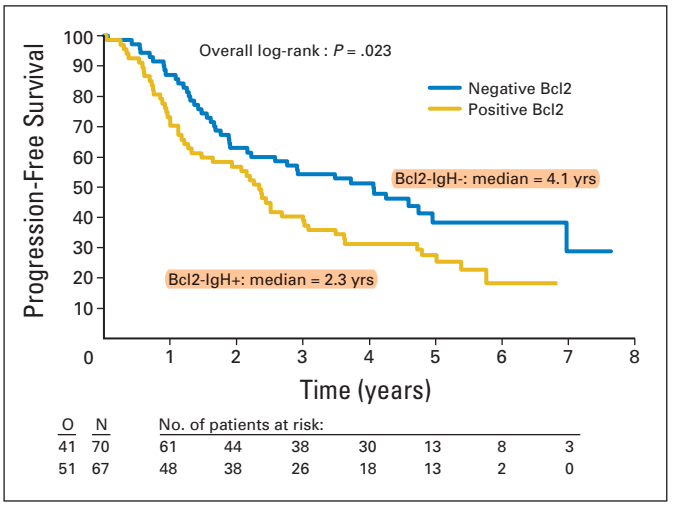

Fig 2. Progression-free survival from first randomization is significantly longer in patients with pretreatment BCL2/immunoglobulin heavy chain (*BCL2/IgH*) polymerase chain reaction (PCR) –negative bone marrow. Blue line, *BCL2/IgH* PCR negative; gold line, *BCL2/IgH* PCR positive. O, observed; N, number.

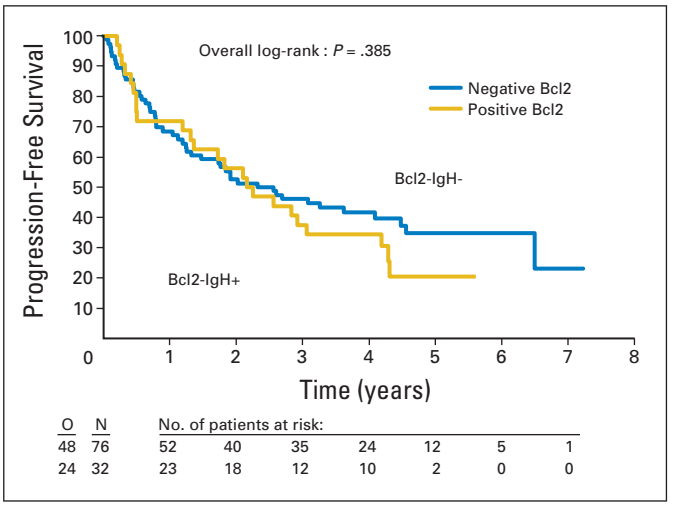

Fig 3. Progression-free survival from second randomization in relation to postinduction BCL2/immunoglobulin heavy chain (*BCL2/IgH*) polymerase chain reaction (PCR) status in the bone marrow. Blue line, *BCL2/IgH* PCR negative; gold line, *BCL2/IgH* PCR positive. O, observed; N, number.

with initially *BCL2/IgH* PCR–negative and –positive PB (2.9 *v* 2.4 years; $P = .099$). However, patients with initially *BCL2/IgH*-negative BM had a significantly longer PFS (median PFS, 4.1 years) than those with initially *BCL2/IgH*-positive BM (median PFS 2.3 years; $P = .023$; Fig 2). In a multivariate analysis including clinical prognostic factors (age, sex, performance status, number of prior treatments, time since diagnosis, stage, bulky disease, B symptoms, bone marrow involvement), the only independent prognostic factors were the type of remission induction treatment received, performance status, and the pretreatment BM *BCL2/IgH* status ($P = .018$; Table 1A).

Finally, pretreatment *BCL2/IgH* levels were not found to be predictive for the benefit of rituximab (data not shown).

Postinduction BCL2/IgH *Major Break Point Status Does Not Predict PFS From Second Randomization*

For patients in CR or PR after remission induction, we analyzed the effect of induction treatment on conversion from *BCL2/IgH* PCR positive to PCR negative. Conversion was much more frequent with rituximab plus CHOP than with CHOP induction: in PB 70% (28 of 40) versus 38% conversion (nine of 24; $P = .011$), and in BM 59% (19 of 32) versus 21% (five of 14; $P = .004$), respectively. Surprisingly, there was no difference between patients in clinical CR or PR as to the proportion of BM or PB PCR negativity (50% to 55%) at the end of induction treatment.

Rather unexpectedly the postinduction *BCL2/IgH* status in PB or BM did not affect PFS from second random assignment (Fig 3). More importantly, there was no difference in PFS between those patients who converted from PCR positive to PCR negative and those who remained PCR positive. This was true both for BM and PB (data not shown).

Table 1. Multivariate Analysis of Progression-Free Survival From First Randomization

Model	Estimated Hazard Ratio	*P*	
		Cox Model	Log-Rank Test
Univariate			
Bcl2-IgH in peripheral blood (binary)	1.343	.1001	.0988
Bcl2-IgH in bone marrow (binary)	1.608	.0248	.0235
Multivariate			
With peripheral blood *Bcl2-IgH* level			
Induction treatment	0.628	.0097	
Performance status	1.665	.0046	
Bcl2-IgH in peripheral blood (binary)	1.374	.0789	
With bone marrow *Bcl2-IgH* level			
Induction treatment	0.685	.0722	
Performance status	1.369	.1443	
Bcl2-IgH in bone marrow (binary)	1.654	.0180	

Abbreviation: IgH, immunoglobulin heavy chain.

Rituximab Maintenance Improves PFS Irrespective of Postinduction BCL2/IgH *Major Break Point Status*

In those patients randomly assigned for observation after remission induction, zero of 12 converted from BM *BCL2/IgH* PCR positive to negative, whereas this was the case in six of 10 of the patients on rituximab maintenance ($P = .002$). For PB, these values were zero of eight and three of six, respectively ($P = .024$). In the clinical trial, rituximab maintenance was found to improve PFS from second random assignment by almost 3 years, from 14.9 months to 51.5 months. Although the subgroups that could be analyzed were rather small, Figure 4 shows that this prolongation of PFS was independent from the postinduction *BCL2/IgH* status in BM or PB.

Finally, the few patients who were still *BCL2/IgH* PCR positive at the end of the 2 years of maintenance treatment almost all relapsed rapidly, and had a significantly shorter PFS than those who were *BCL2/IgH* PCR negative (Fig 5).

DISCUSSION

The major results of the present study are: pretreatment *BCL2/IgH* major break point levels in PB or BM do not have any prognostic value

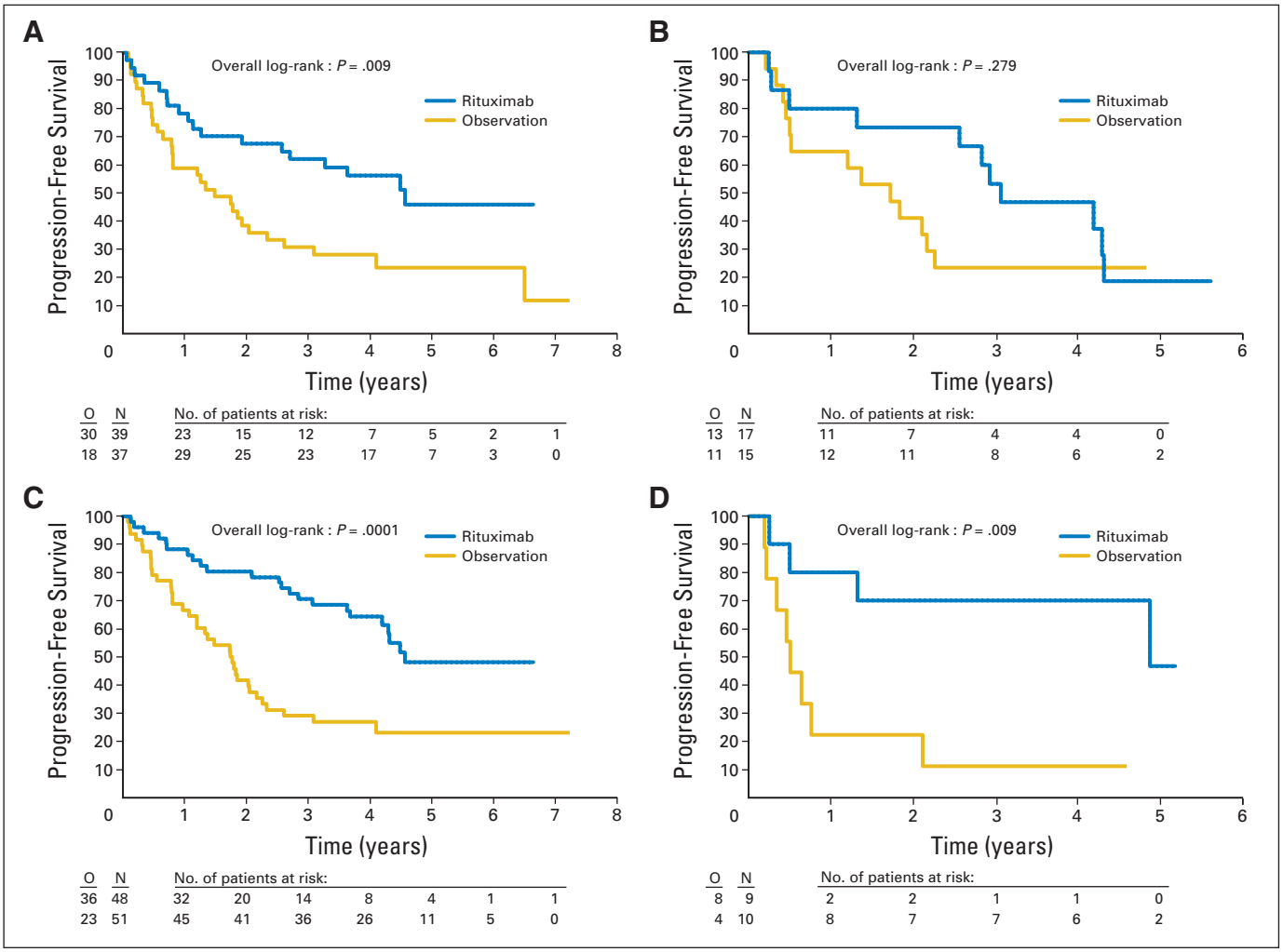

Fig 4. Rituximab maintenance treatment improves progression-free survival from second random assignment in patients with negative postinduction BCL2/immunoglobulin heavy chain (*BCL2/IgH*) polymerase chain reaction (PCR) status in (A) bone marrow and (C) peripheral blood (PB), and in patients with positive postinduction *BCL2/IgH PCR* status in (B) BM or (D) PB. Blue line, rituximab maintenance; gold line, observation; O, observed; N, number.

for overall response or CR rates after induction treatment with CHOP with or without rituximab, but a positive pretreatment *BCL2/IgH* status in BM has an adverse prognostic impact on PFS. Although rituximab plus CHOP is more effective than CHOP in inducing conversion to *BCL2/IgH* negativity in PB and BM, *BCL2/IgH* major break point PCR levels in BM and PB postinduction treatment have no prognostic value for PFS. The highly significant improvement of PFS by rituximab maintenance is independent from *BCL2/IgH* status of PB or BM at the end of induction treatment. Patients who still have a positive *BCL2/IgH* major break point PCR in PB or BM at the end of the 2 years of rituximab maintenance/observation have a significantly shorter PFS than those who are *BCL2/IgH* PCR negative.

In view of the incurability of FL, PFS is in general considered to be the most important clinical end point in FL clinical trials. The prognostic significance of pretreatment t(14;18)–positive cells in PB and BM as to this end point is controversial. Some authors found no correlation with clinical outcome,[9,25] whereas we and others found BM[11] or PB levels[10] to be predictive for PFS. Although we found an excellent correlation between the *BCL2/IgH* levels in PB and BM, others found PB levels to be lower (although the majority within 1 log difference[26]) and less predictive for relapse than BM.[12] Unfortunately all these studies are difficult to compare, because they differ not only as to the source of material analyzed (PB or BM) but also as to patient groups studied (previously untreated,[9,11] relapsed[23] or both[10]), type of treatment given, and PCR assays used. Moreover the studies were in general small. Thus, assessment of pretreatment PCR levels in PB or BM cannot be recommended.

More consensus appears to exist regarding the prognostic value of postinduction assessment of PCR levels in PB or BM. Although prolonged remissions have been described in patients persistently *BCL2/IgH* PCR positive in PB or BM,[15,16] more frequently a correlation between *BCL2/IgH* PCR negativity and prolonged PFS was demonstrated, both after treatment with conventional chemotherapy,[13,27] chemotherapy combined with rituximab,[28] chemotherapy followed by either rituximab[11] or radioimmunotherapy,[29] rituximab monotherapy[30] and, most extensively, after myeloablative regimens.[14,17-22] This correlation is often explained by different levels of eradication of MRD. However, to our opinion it is very unlikely that in FL the *BCL2/IgH* status in PB or BM can be equated to MRD sensu strictu (ie, whole-body residual disease). In contrast to the situation in leukemias

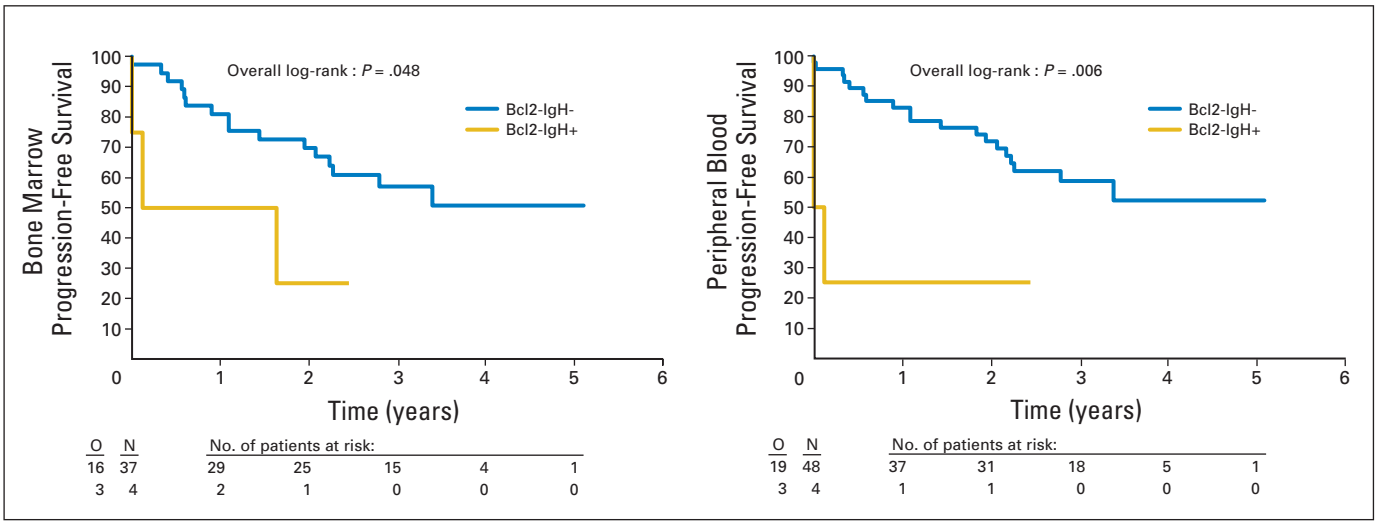

Fig 5. Progression-free survival from second random assignment in relation to BCL2/immunoglobulin heavy chain (*BCL2/IgH*) polymerase chain reaction status in bone marrow and peripheral blood at the end of rituximab maintenance treatment. O, observed; N, number.

like acute lymphoblastic leukemia and chronic myelogenous leukemia, in FL the PB and BM compartments probably are not representative for whole-body (residual) disease, because in general in FL the bulk of the disease is located in lymph nodes. A number of observations in our study support this notion. First, there was no difference in the PB or BM *BCL2/IgH* status between patients in clinical CR or PR. Secondly, our study demonstrated clear superiority of rituximab plus CHOP induction as to prolongation in PFS.[23] In this study, we showed an increased conversion to *BCL2/IgH* PCR negativity in PB and BM by rituximab plus CHOP. The most plausible inference would be to postulate a causative relationship between the two observations. However, this is not supported by our finding that the PFS from second random assignment is not related to postinduction *BCL2/IgH* status in PB and/or BM. Moreover, others have demonstrated that the PB can be cleared from MRD by one infusion of rituximab.[31] Obviously it is very unlikely that the whole-body tumor mass is zero after this single infusion. These compartmentalization effects might explain the lack of prognostic value of postinduction *BCL2/IgH* status in PB and/or BM, as observed in this study. Because the sensitivity of our real-time quantitative MBR *BCL2/IgH* major break point PCR is comparable to those used by other groups, a large proportion of false-negative or false-positive PCR test results is a very unlikely explanation for the observed lack of prognostic value of the *BCL2/IgH* PCR in PB or BM. In this study, minor break point detection was not incorporated. This might explain the relatively low percentage of pretreatment positive BM and PB samples. In contrast, this implies that the possible minor break point–positive patients have been grouped in the *BCL2/IgH* major break point–negative group. Thus, we assume that if they had been included in the Bcl2/IgH-positive group, the differences in PFS between the pretreatment Bcl2/IgH PCR–positive versus *BCL2/IgH* PCR–negative patients (Fig 2) might even have been more pronounced.

It has been shown both in previously untreated and relapsed FL that rituximab maintenance has a clear clinical benefit after induction with either rituximab plus chemotherapy, chemotherapy alone, or rituximab monotherapy (reviewed in[32]). Although these studies demonstrated that rituximab maintenance can be safely given for up to 2 years, the optimal duration of maintenance (eg, until relapse?) has not yet been established. Although unfortunately in this study the number of samples from patients at the end of the 2 years of rituximab maintenance treatment was very limited, the strikingly rapid relapses in those patients who were still *BCL2/IgH* PCR–positive in PB and/or BM at that time point (Fig 5) might be taken as an argument in favor of continuation of rituximab maintenance beyond 2 years in these patients.

We conclude that for patients with relapsed/resistant FL, assessment of postinduction *BCL2/IgH* PCR levels in BM or PB is not useful for decisions on subsequent therapy, such as maintenance.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Robert E. Marcus, Roche, Genentech (C); Eva Kimby, Roche Advisory Board (C); Anton Hagenbeek, Roche Advisory Board (C) **Stock Ownership:** None **Honoraria:** Marinus H.J. van Oers, Roche; Richard Klasa, Roche; Robert E. Marcus, Roche, Genentech; Eva Kimby, Roche; Andrej Vranovsky, Roche **Research Funding:** Eva Kimby, Research Roche **Expert Testimony:** None **Other Remuneration:** Robert E. Marcus, Roche, Genentech

AUTHOR CONTRIBUTIONS

Conception and design: Marinus H.J. van Oers, Martine Van Glabbeke, Joop H. Jansen, Richard Klasa, Robert E. Marcus, Max Wolf, Eva Kimby, Anton Hagenbeek, Bert A. van der Reijden

Administrative support: Evelyn Tönnissen, Martine Van Glabbeke, Joop H. Jansen
Provision of study materials or patients: Marinus H.J. van Oers, Richard Klasa, Robert E. Marcus, Max Wolf, Eva Kimby, Andrej Vranovsky, Harald Holte, Anton Hagenbeek
Collection and assembly of data: Evelyn Tönnissen, Martine Van Glabbeke, Livia Giurgea, Joop H. Jansen, Robert E. Marcus, Max Wolf, Andrej Vranovsky, Bert A. van der Reijden
Data analysis and interpretation: Marinus H.J. van Oers, Evelyn Tönnissen, Martine Van Glabbeke, Livia Giurgea, Joop H. Jansen, Max Wolf, Anton Hagenbeek, Bert A. van der Reijden
Manuscript writing: Marinus H.J. van Oers, Bert A. van der Reijden
Final approval of manuscript: Marinus H.J. van Oers, Evelyn Tönnissen, Martine Van Glabbeke, Livia Giurgea, Joop H. Jansen, Richard Klasa, Robert E. Marcus, Max Wolf, Eva Kimby, Andrej Vranovsky, Harald Holte, Anton Hagenbeek, Bert A. van der Reijden

REFERENCES

1. Horning SJ: Natural history of and therapy for the indolent non-Hodgkin's lymphomas. Semin Oncol 20:75-88, 1993

2. Johnson PW, Rohatiner AZ, Whelan JS, et al: Patterns of survival in patients with recurrent follicular lymphoma: A 20-year study from a single center. J Clin Oncol 13:140-147, 1995

3. Forstpointner R, Dreyling M, Repp R, et al: The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: Results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 104:3064-3071, 2004

4. Hiddemann W, Kneba M, Dreyling M, et al: Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: Results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 106:3725-3732, 2005

5. Marcus R, Imrie K, Belch A, et al: CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. Blood 105:1417-1423, 2005

6. Salles GAG, Mounier N, de Guibert S, et al: Rituximab combined with chemotherapy and interferon in follicular lymphoma patients: Results of the GELA-GOELAMS FL2000 study. Blood. 2008;112: 4824-4831

7. Herold M, Haas A, Srock S, et al: Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: An East German Study Group Hematology and Oncology Study. J Clin Oncol 25:1986-1992, 2007

8. Buckstein R, Pennell N, Berinstein NL: What is remission in follicular lymphoma and what is its relevance? Best Practice & Research Clinical Haematology 18:27-56, 2005

9. Mandigers CM, Meijerink JP, Mensink EJ, et al: Lack of correlation between numbers of circulating t(14;18)-positive cells and response to first-line treatment in follicular lymphoma. Blood 98:940-944, 2001

10. Ghielmini M, Schmitz SF, Cogliatti SB, et al: Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly x 4 schedule. Blood 103:4416-4423, 2004

11. Rambaldi A, Carlotti E, Oldani E, et al: Quantitative PCR of bone marrow BCL2/IgH+ cells at diagnosis predicts treatment response and long-term outcome in follicular non-Hodgkin lymphoma. Blood 105:3428-3433, 2005

12. Gribben JG, Neuberg D, Barber M, et al: Detection of residual lymphoma cells by polymerase chain reaction in peripheral blood is significantly less predictive for relapse than detection in bone marrow. Blood 83:3800-3807, 1994

13. Lopez-Guillermo A, Cabanillas F, McLaughlin P, et al: The clinical significance of molecular response in indolent follicular lymphomas. Blood 91: 2955-2960, 1998

14. Haas R, Moos M, Karcher A, et al: Sequential high-dose therapy with peripheral-blood progenitor-cell support in low-grade non-Hodgkin's lymphoma. J Clin Oncol 12:1685-1692, 1994

15. Price CG, Meerabux J, Murtagh S, et al: The significance of circulating cells carrying t(14;18) in long remission from follicular lymphoma. J Clin Oncol 9:1527-1532, 1991

16. Lambrechts AC, Hupkes PE, Dorssers LC, et al: Clinical significance of t(14; 18)-positive cells in the circulation of patients with stage III or IV follicular non-Hodgkin's lymphoma during first remission. J Clin Oncol 12:1541-1546, 1994

17. Ladetto M, Corradini P, Vallet S, et al: High rate of clinical and molecular remissions in follicular lymphoma patients receiving high-dose sequential chemotherapy and autografting at diagnosis: A multicenter, prospective study by the Gruppo Italiano Trapianto Midollo Osseo (GITMO). Blood 100:1559-1565, 2002

18. Moos M, Schulz R, Martin S, et al: The remission status before and the PCR status after high-dose therapy with peripheral blood stem cell support are prognostic factors for relapse-free survival in patients with follicular non-Hodgkin's lymphoma. Leukemia 12:1971-1976, 1998

19. Apostolidis J, Gupta RK, Grenzelias D, et al: High-dose therapy with autologous bone marrow support as consolidation of remission in follicular lymphoma: Long-term clinical and molecular follow-up. J Clin Oncol 18:527-536, 2000

20. Gribben JG, Neuberg D, Freedman AS, et al: Detection by polymerase chain reaction of residual cells with the bcl-2 translocation is associated with increased risk of relapse after autologous bone marrow transplantation for B-cell lymphoma. Blood 81:3449-3457, 1993

21. Hardingham JE, Kotasek D, Sage RE, et al: Significance of molecular marker-positive cells after autologous peripheral-blood stem-cell transplantation for non-Hodgkin's lymphoma. J Clin Oncol 13: 1073-1079, 1995

22. Corradini P, Astolfi M, Cherasco C, et al: Molecular monitoring of minimal residual disease in follicular and mantle cell non-Hodgkin's lymphomas treated with high-dose chemotherapy and peripheral blood progenitor cell autografting. Blood 89:724-731, 1997

23. van Oers MH, Klasa R, Marcus RE, et al: Rituximab maintenance improves clinical outcome of relapsed/resistant follicular non-Hodgkin lymphoma in patients both with and without rituximab during induction: Results of a prospective randomized phase 3 intergroup trial. Blood 108:3295-3301, 2006

24. Reference deleted

25. Paszkiewicz-Kozik E, Kulik J, Fabisiewicz A, et al: Presence of t(14;18) positive cells in blood and bone marrow does not predict outcome in follicular lymphoma. MedOncol 2008

26. Leonard BM, Hetu F, Busque L, et al: Lymphoma cell burden in progenitor cell grafts measured by competitive polymerase chain reaction: Less than one log difference between bone marrow and peripheral blood sources. Blood 91:331-339, 1998

27. Lopez-Guillermo A, Cabanillas F, McLaughlin P, et al: Molecular response assessed by PCR is the most important factor predicting failure-free survival in indolent follicular lymphoma: Update of the MDACC series. Ann Oncol 11:137-140, 2001 (suppl 1)

28. Hirt C, Schuler F, Kiefer T, et al: Rapid and sustained clearance of circulating lymphoma cells after chemotherapy plus rituximab: Clinical significance of quantitative t(14;18) PCR monitoring in advanced stage follicular lymphoma patients. Br J Haematol 141:631-640, 2008

29. Morschhauser F, Radford J, Van HA, et al: Phase III trial of consolidation therapy with yttrium-90-ibritumomab tiuxetan compared with no additional therapy after first remission in advanced follicular lymphoma. J Clin Oncol 26:5156-5164, 2008

30. Colombat P, Salles G, Brousse N, et al: Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: Clinical and molecular evaluation. Blood 97:101-106, 2001

31. Schmitt C, Grundt A, Buchholtz C, et al: One single dose of rituximab added to a standard regimen of CHOP in primary treatment of follicular lymphoma appears to result in a high clearance rate from circulating bcl-2/IgH positive cells: Is the end of molecular monitoring near? Leuk Res 30:1563-1568, 2006

32. van Oers MH: Rituximab maintenance therapy: A step forward in follicular lymphoma. Haematologica 92:826-833, 2007