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ORIGINAL REPORT

Quantitative PCR Analysis for *Bcl-2/IgH* in a Phase III Study of Yttrium-90 Ibritumomab Tiuxetan As Consolidation of First Remission in Patients With Follicular Lymphoma

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ABSTRACT

Purpose
The randomized First-Line Indolent Trial (FIT) was conducted in patients with advanced follicular lymphoma (FL), to evaluate the safety and efficacy of yttrium-90 (^{90}Y) ibritumomab tiuxetan given as consolidation of complete or partial remission. This study of minimal residual disease was undertaken in parallel, to determine the rate of conversion from *bcl-2* polymerase chain reaction (PCR) –detectable to –undetectable status and the corresponding effect on progression-free survival (PFS).

Patients and Methods
Blood samples from 414 patients (^{90}Y-ibritumomab, n = 208; control, n = 206) were evaluated using real-time quantitative polymerase chain reaction (RQ-PCR); 186 were found to have the *bcl-2* rearrangement and were thus eligible for inclusion in the RQ-PCR analysis.

Results
Overall, 90% of treated patients converted from *bcl-2* PCR–detectable to –undetectable disease status, compared with 36% in the control group. Treatment significantly prolonged median PFS in patients converting to *bcl-2* PCR-undetectable status (40.8 *v* 24.0 months in the control group; $P < .01$, hazard ratio [HR], 0.399). In patients who had *bcl-2* PCR-detectable disease at random assignment, treatment significantly prolonged median PFS (38.4 *v* 8.2 months in the control group; $P < .01$, HR, 0.293).

Conclusion
Eradication of PCR-detectable disease occurred more frequently after treatment with ^{90}Y-ibritumomab tiuxetan and was associated with prolongation of PFS.

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INTRODUCTION

Follicular lymphoma (FL) is the second most common histologic subtype of non-Hodgkin's lymphoma.[1] The clinical course of the illness is characterized by repeated responsiveness to treatment, followed almost inevitably by recurrence. With conventional therapy, the median survival was 9 to 10 years at the end of the 20th century, death typically being a consequence of refractoriness to treatment or complications thereof, regardless of whether transformation to diffuse large B-cell pathology had occurred.[2-5] However, recent data suggest that survival is improving with new treatment modalities administered at diagnosis and recurrence.[6-9]

In approximately 85% of patients, FL is associated with the t(14;18) translocation,[10-12] which can be used as a molecular marker of the disease. The translocation results in *bcl-2* oncogene rearrangement and *bcl-2/IgH* mRNA overexpression.[13-15] The major breakpoint cluster region (MBR) is the molecular site of translocation in approximately 70% of patients.[16-18]

Cells containing the t(14;18) translocation are detectable by polymerase chain reaction (PCR) assay.[19] Real-time quantitative PCR (RQ-PCR) analysis has enabled quantification of residual disease in bone marrow or blood,[16,20] and may be used to confirm remission or demonstrate subclinical recurrence.

Yttrium-90 (^{90}Y) -ibritumomab tiuxetan (Zevalin; Bayer Schering Pharma AG, Berlin, Germany) is an immunoconjugate composed of an anti-CD20 monoclonal antibody linked by the chelator tiuxetan to the pure beta-emitting radioisotope ^{90}Y.[21] It has been used as targeted radioimmunotherapy in patients with recurrent and refractory FL[22-26] and in newly diagnosed patients.[21]

The international, phase III, randomized First-Line Indolent Trial (FIT) was conducted to evaluate the safety and efficacy of ^{90}Y-ibritumomab when

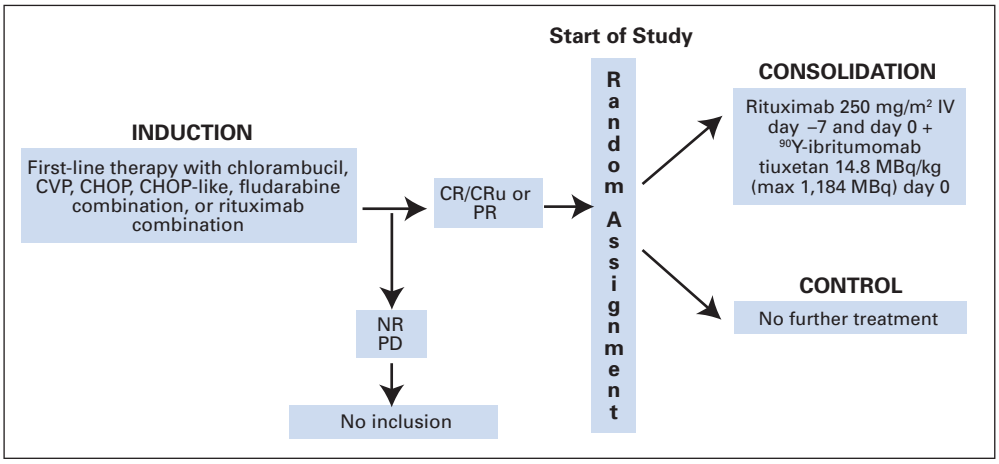

Fig 1. First-Line Indolent Trial (FIT) study design. CVP, cyclophosphamide, vincristine, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete response; CRu, unconfirmed complete response; PR, partial response; NR, no response; PD, progressive disease; ^{90}Y, yttrium-90; IV, intravenous.

used as consolidation of first complete or partial remission in patients with previously untreated, advanced-stage FL.[21] Patients were randomly assigned to either observation or treatment with ^{90}Y-ibritumomab within 3 months of completing initial induction therapy. The primary end point was progression-free survival (PFS) of the intent-to-treat (ITT) population. The results showed that ^{90}Y-ibritumomab was well tolerated with no unexpected toxicities and that it significantly prolonged PFS in patients with advanced FL by approximately 2 years.[21]

This report describes the secondary end point analysis from the clinical trial, examining change in molecular response, that is, from *bcl-2* PCR detectable to undetectable status. The aim was to investigate *bcl-2* PCR status before and after ^{90}Y-ibritumomab consolidation therapy, to document change in molecular response, and to determine whether a change in molecular response correlated with PFS.

PATIENTS AND METHODS

Patients

Patient eligibility criteria were reported in detail previously.[21] Briefly, eligible patients were age ≥ 18 years; had stage III or IV histologically confirmed CD20$^+$ FL (grade 1 or 2); had a WHO performance status of 0 to 2; were in CR, including unconfirmed CR, or PR; had less than 25% lymphoma involvement in bone marrow after first-line therapy; and had 6 to 12 weeks between the last dose of therapy and random assignment. All patients gave informed consent.

Study Design

Patients were randomly assigned to stop treatment (control) or consolidation with ^{90}Y-ibritumomab after completing first-line therapy (Fig 1). The ^{90}Y-ibritumomab consolidation group received rituximab 250 mg/m^2 intravenously on days −7 and 0, followed on day 0 by a single infusion of ^{90}Y-ibritumomab 14.8 MBq/kg (0.4 mCi/kg), not exceeding a total dose of 1,184 MBq. Baseline clinical characteristics are presented in Table 1.

The study was approved by the institutional review board at each participating center and conducted in accordance with the Declaration of Helsinki.

Samples

Blood samples were collected from 414 patients, 208 randomly assigned to ^{90}Y-ibritumomab and 206 randomly assigned to the control group. However, a *bcl-2* rearrangement was only detected in 186 patients, either at the time of random assignment (n = 127) or in subsequent follow-up (n = 59). The remaining patients were excluded from the RQ-PCR analysis, because a rearrangement was never detected, either because the lymphoma was genuinely *bcl-2* rearrangement negative, or because it had been rendered negative by the initial therapy and remained so for the duration of the study. Thus, the analysis of outcome for the 186 patients, 90 (48%) in the ^{90}Y-ibritumomab consolidation group and 96 (52%) in the control group, relates to bcl-2 status at the time of random assignment, as presented in Table 2. Blood samples were collected at random assignment; at week 14; at months 6, 12, and 24; and annually thereafter.

RQ-PCR Analysis

Samples were placed in EDTA tubes and transported at room temperature within 24 hours to St Bartholomew's Hospital, London, where MBR t(14;18) RQ-PCR analysis was performed using the ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA) as previously described.[16] Briefly, a 25-μL reaction mixture consisting of TaqMan Universal PCR Master Mix (PE Applied Biosystems), 300 nmol/L/L of primers, 200 nmol/L/L of probes,[27] and 0.25 to 0.50 μg DNA was used. MBR-positive cells were quantified relative to β-2 microglobulin positivity, used as a measure of total cell number.[16] Standard curves were created using the DoHH2 cell line, which contains the MBR t(14;18) translocation, and 10-fold dilutions made to generate standards ranging from 10 to 10^5 DoHH2 cells. Between 6 and 12 separate reactions (3 to 6 μg total DNA) were screened for each blood sample.

The RQ-PCR assay sensitivity was 1 in 10^5 cells, with at least 5 to 10 target copies of the rearrangement required to obtain highly reproducible results. The assay detection limit was 1 copy of the rearrangement in 1 to 2×10^5. To confirm authenticity, *bcl-2/IgH* products were amplified using RQ-PCR, omitting uracil-N-glycosylase from the reaction. RQ-PCR products were sequenced using an ABI 377 sequencer (PE Applied Biosystems).

The amount of residual disease at random assignment was based on numbers of *bcl-2/IgH*-positive cells, according to the schema of Rambaldi et al[28]: high (> 1 in 10^2 normal cells), intermediate (1 in 10^2 to 10^3 normal cells), low (1 in 10^3 to 10^5), and undetectable (< 1 in 10^5 normal cells).

Assessments

PFS was determined from date of random assignment to date of documented recurrence, disease progression, or death. Adverse events (AEs) were assessed throughout the study duration, with toxicity grading based on the National Cancer Institute Common Toxicity Criteria version 2.

Statistical Analysis

Efficacy evaluations were based on all randomly assigned patients (ITT population); safety evaluations were based on all randomly assigned patients for whom postrandom assignment data were available (safety population). Change in *bcl-2* PCR status (detectable to undetectable) was evaluated using descriptive statistics. PFS was analyzed by the Kaplan-Meier method, and a comparison of curves made using a log-rank test with a significance level of $\alpha = .05$.

Table 1. Patient Baseline Characteristics

Characteristic	RQ-PCR Population*				Overall Safety Population				
	Control		^{90}Y-Ibritumomab Tiuxetan		Control		^{90}Y-Ibritumomab Tiuxetan		
	No.	%	No.	%	No.	%	No.	%	*P*
No. of patients	96		90		205		204		
Male sex	54	56	44	49	103	50	97	48	.59
Median age, years	51		54		53		55		.14
Range	28-72		31-77		27-74		29-78		
Ann Arbor classification by stage									.15
I	0		0		0		1	< 1	
II	3	3	0		6	3	1	< 1	
III	35	36	30	33	63	31	72	35	
IV	58	60	60	67	136	66	130	64	
B symptoms									.59
No	77	80	68	76	162	79	156	77	
Yes	19	20	22	24	42	21	46	23	
Response after first-line therapy†									.76
CR/CRu	39	41	35	39	109	53	107	51	
PR	57	59	55	61	97	47	101	49	
bcl-2 PCR status in blood†									.04
Detectable	59	62	68	76	59	29	68	33	
Undetectable	37	39	22	24	132	64	128	62	
Unknown	—	—	—	—	15	7	12	6	

Abbreviations: RQ-PCR, real-time quantitative polymerase chain reaction; ^{90}Y, yttrium-90; CR, complete response; CRu, unconfirmed complete response; PR, partial response.

*One hundred eighty-six patients known to have a *bcl-2* rearrangement.

†Percentages in the overall safety population columns are based on the intent-to-treat population (control: n = 206; ^{90}Y-ibritumomab tiuxetan: n = 208).

RESULTS

Bcl-2 *PCR Status at Randomization*

At random assignment, 68% of patients (127 of 186) had *bcl-2* PCR–detectable disease: 54% (68 of 127) in the ^{90}Y-ibritumomab group and 46% (59 of 127) in the control group. Only 2% in each group had high levels; intermediate levels were found in 7% and 10%, and low levels in 67% and 49% of patients in the ^{90}Y-ibritumomab and control groups, respectively. *Bcl-2* PCR status at randomization according to first-line treatment is presented in Table 2.

Conversion From bcl-2 *PCR–Detectable to –Undetectable Disease*

In the ^{90}Y-ibritumomab group, median levels of *bcl-2* PCR–detectable cells decreased rapidly and remained low throughout the evaluation period, irrespective of the amount of MRD (high, intermediate, or low) before treatment. In contrast, in the control group, median levels of *bcl-2* PCR–detectable cells remained elevated (Fig 2).

At 3 months, the number of *bcl-2* PCR–detectable cells had been reduced by at least 1 log in 76% of patients (52 of 68) treated with ^{90}Y-ibritumomab, and 75% (51 of 68) had converted to *bcl-2* PCR–undetectable status. At 6 months, six additional patients in the ^{90}Y-ibritumomab group had PCR-undetectable disease, for a final proportion of 84% (57 of 68 patients). In contrast, only 25% (15 of 59) and 5% of control patients (three of 59) had a 1-log reduction in *bcl-2* PCR–detectable cells at 3 and 6 months, respectively.

At the final evaluation (median of 2.4 years) for patients receiving ^{90}Y-ibritumomab, 43 (63%) of the 68 patients who had *bcl-2* PCR–detectable disease at random assignment maintained at least a 1-log reduction, and in 42 (62%), the disease remained undetectable by RQ-PCR. In contrast, in the control group at final evaluation (median of 1 year), only 22% of patients (13 of 59) had maintained a 1-log reduction, and only 20% (12 of 59) had undetectable disease.

Overall, 90% of patients (61 of 68) in the ^{90}Y-ibritumomab group converted from *bcl-2* PCR–detectable to –undetectable disease at some point, compared with 36% of patients (21 of 59) in the control group.

PFS

PFS for all patients included in the clinical trial. As previously reported, the median PFS for the trial ITT population was 3 years in the ^{90}Y-ibritumomab group, compared with 13 months in the control group ($P < .0001$; HR, 0.465).[21] PFS values of the corresponding two groups of patients included in the RQ-PCR analysis were similar (data not shown).

PFS according to PCR status. For the 68 patients who had *bcl-2* PCR–detectable disease at random assignment and received treatment with ^{90}Y-ibritumomab, the median PFS was 38 months, compared with 8 months for the 59 control patients with *bcl-2* PCR-detectable disease ($P < .01$; HR, 0.293; Fig 3). In contrast, there was no statistically significant difference in PFS between patients with PCR-undetectable disease at random assignment who received ^{90}Y-ibritumomab (median PFS, 37 months) and controls with PCR-undetectable disease (median PFS, 29 months; $P = .42$; HR, 0.735).

PFS of patients converting from bcl-2 PCR–detectable to–undetectable disease status. The median PFS for patients converting from *bcl-2*

Table 2. *bcl-2* PCR Status (in blood) at Randomization According to First-Line Treatment Regimen and Clinical Response to First-Line Therapy

bcl-2 PCR Status	No. Control (n = 96)	No. ^{90}Y-Ibritumomab Tiuxetan (n = 90)	No. Total (N = 186)
First-line treatment regimen			
Chlorambucil	12	10	22
Detectable	10	8	18
Undetectable	2	2	4
CHOP	32	31	63
Detectable	22	25	47
Undetectable	10	6	16
CHOP-like	10	12	22
Detectable	6	8	14
Undetectable	4	4	8
CVP	31	29	60
Detectable	17	23	40
Undetectable	14	6	20
Fludarabine combination	6	4	10
Detectable	3	4	7
Undetectable	3	0	3
Rituximab combination	5	4	9
Detectable	1	0	1
Undetectable	4	4	8
Clinical response to first-line therapy			
PR	57	55	112
Detectable*	42	44	86
%	74	80	
Undetectable*	15	11	26
%	26	20	
CR	39	35	74
Detectable†	17	24	
%	44	68	41
Undetectable†	22	11	
%	56	31	33

Abbreviations: PCR, polymerase chain reaction; ^{90}Y, yttrium-90; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CVP, cyclophosphamide, vincristine, and prednisone; CR, complete response; PR, partial response.

*Percentages are based on the total number of patients in PR (control, n = 57; ^{90}Y-ibritumomab tiuxetan, n = 55).

†Percentages are based on the total number of patients in CR (control, n = 39; ^{90}Y-ibritumomab tiuxetan, n = 35).

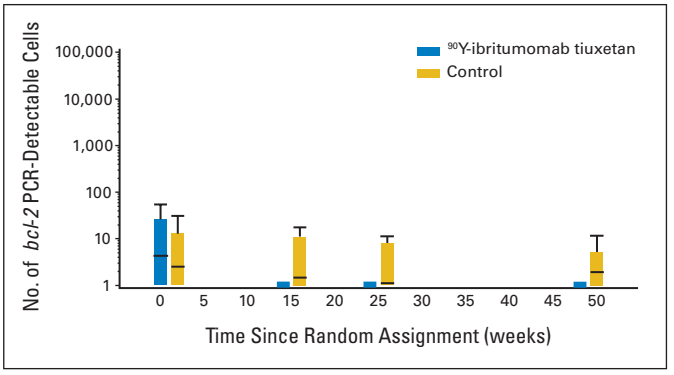

Fig 2. Number of *bcl-2* polymerase chain reaction (PCR) –detectable cells over time in patients receiving yttrium-90 (^{90}Y) –ibritumomab or no additional therapy. For each box plot, the line within the box indicates the median value observed; the box represents the range of values defined by the 25th and 75th percentiles; and the bars extend from the fifth to the 95th percentile.

PCR–detectable to –undetectable disease was 41 months for the ^{90}Y-ibritumomab group (n = 61), compared with 2 years for control patients in whom conversion was observed (n = 21; $P < .01$; HR, 0.399; Fig 4).

Recurrence

For patients who converted to *bcl-2* PCR–undetectable status, clinical evidence of recurrent lymphoma was observed in 39% (24 of 61) in the ^{90}Y-ibritumomab group, compared with 76% (16 of 21) in the control group. Clinical evidence of recurrence was not predicted by reappearance of the rearrangement in peripheral blood samples.

In the ^{90}Y-ibritumomab arm, only three patients had PCR-detectable disease persisting from random assignment to after treatment. All were in PR before treatment and progressed at 5.5, 6, and 6.5 months.

Correlations Between Clinical Status and bcl-2 PCR Status

At study entry. The *bcl-2* PCR status of patients at study entry, by clinical response to first-line therapy, is presented in Table 2. For patients in PR, the proportion with PCR-detectable disease was not significantly different between the ^{90}Y-ibritumomab and control groups (44 [80%] of 55 *v* 42 [74%] of 57, respectively; $P = .5$). However, for patients in CR, a significantly greater proportion had PCR-detectable disease in the ^{90}Y-ibritumomab group (24 [68%] of 35 *v* 17 [44%] of 39 in the control group, respectively; $P = .03$).

At clinical recurrence or progression. With regard to correlations between *bcl-2* PCR status and clinical recurrence or progression, recurrence was usually, but not necessarily, associated with a change in RQ-PCR–detectable disease. For patients in the ^{90}Y-ibritumomab group who entered the study in CR, 18 of 35 developed recurrent lymphoma and seven of 18 had PCR-undetectable disease at recurrence. For patients entering the ^{90}Y-ibritumomab group in PR, 23 of 55 progressed and eight of 23 had PCR-undetectable disease at that point. Similarly, among control group patients in CR at study entry, 23 of 39 developed recurrent disease and six of 23 had PCR-undetectable disease at that time. Not surprisingly, the highest number of progressions occurred in control-group patients entering in PR; 44 of 57 patients progressed and nine of 44 had PCR-undetectable disease at progression. As mentioned above, *bcl-2* PCR status did not necessarily predict recurrence (data not shown).

Prediction of PFS According to PCR or Clinical Status

With regard to whether PCR or clinical status was more or less predictive of outcome (PFS), the situation differs between the two groups. For patients in the control group, PCR and clinical status were equally predictive. PFS for patients entering the study in PR was 6.2 months[21] compared with 8.2 months for those entering the study with PCR-detectable disease (irrespective of clinical status). Similarly, for those entering the study in CR, PFS was 29.5 months[21] compared with 28.8 months for patients with PCR-undetectable disease.

In contrast, for patients in the ^{90}Y-ibritumomab group, PCR status at baseline (study entry) did not influence PFS. Thus, for patients in the ^{90}Y-ibritumomab group who entered the study with PCR-detectable disease, PFS was 38.4 months compared with 29.3 months[21] for those entering in PR. For patients entering the study with

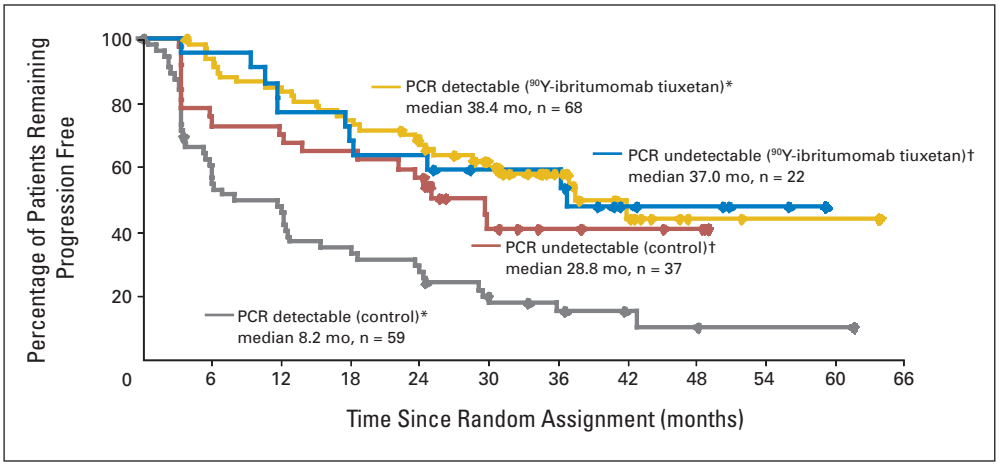

Fig 3. Kaplan-Meier plots of progression-free survival according to peripheral blood *bcl-2* polymerase chain reaction (PCR) status at random assignment. (*) Log-rank $P < .01$; hazard ratio (HR), 0.293; 95% CI, 0.184 to 0.464. (†) Log-rank $P = .42$; HR, 0.735; 95% CI, 0.350 to 1.544. ^{90}Y, yttrium-90.

PCR-undetectable disease, PFS was 37.0 months compared with 53.9 months[21] for those entering in CR.

DISCUSSION

This study was undertaken to determine the frequency and significance of molecular remission of FL, against the background of a randomized clinical trial testing the efficacy of consolidation of conventional induction chemotherapy with anti-CD20 targeted irradiation. It has been demonstrated that lack of *bcl-2* PCR–detectable disease at the completion of planned therapy, either induction alone in the control arm or after consolidation in the trial arm, is a very strong predictor of prolonged PFS, and that conversion from PCR–detectable to –undetectable status with ^{90}Y-ibritumomab is beneficial.

Before this study began, it was not known whether an ^{90}Y-labeled immunoconjugate would be effective as consolidation of remission in patients with FL, particularly those in CR after first-line therapy. These results suggest that consolidation of first remission with ^{90}Y-ibritumomab is associated with an additional degree of response at the molecular level, translating into prolonged PFS. The findings, in the largest study of its kind, are consistent with the published literature,[28-37] suggesting that persistence of *bcl-2* PCR–detectable disease is associated with an adverse prognosis and that eradication of PCR-detectable disease is itself a worthwhile aim.

Although conversion from *bcl-2* PCR–detectable to –undetectable status was observed in both the ^{90}Y-ibritumomab and control groups, conversion was much more frequent and had usually occurred by month 3 in the treatment group. Conversion was associated with significantly prolonged PFS in patients treated with ^{90}Y-ibritumomab, suggesting that consolidation with the radioimmunoconjugate enhances the molecular response, thereby prolonging median PFS. In the control arm, 36% converted to PCR-undetectable status after initial therapy. This is a well-described phenomenon occurring within the context of both fludarabine- and anthracycline-containing regimens,[28-33] with cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy resulting in a 2-log decrease in *bcl-2* rearrangement–containing cells.[28]

Because RQ-PCR was used to detect the presence or absence of *bcl-2/IgH+* cells, this study by definition had to be limited to patients known to have the rearrangement. However, the results (PFS) for patients in the RQ-PCR analysis mirror those for the clinical trial overall,[21] and there is no inherent reason for selection bias. Bone marrow samples were requested during follow-up, but few were received except at recurrence. Thus, no absolute comparison between blood and bone marrow could be made.

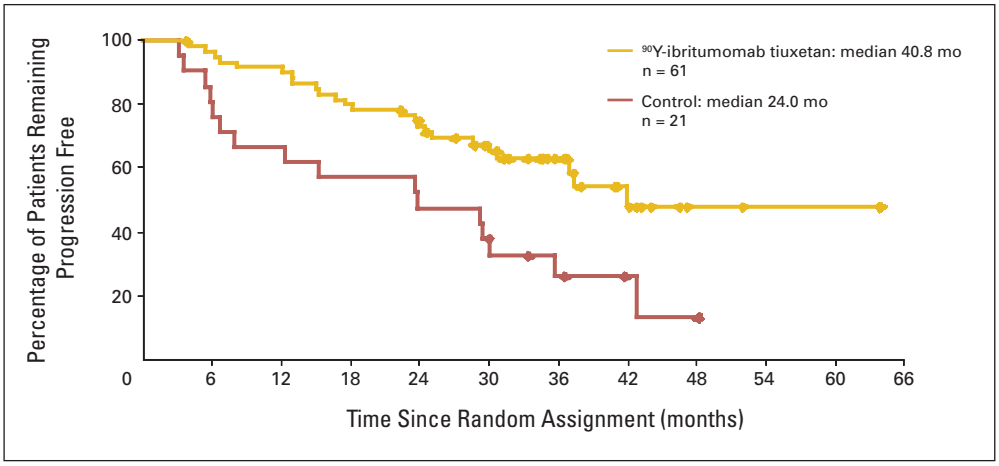

Fig 4. Kaplan-Meier plots of progression-free survival in patients converting from *bcl-2* polymerase chain reaction (PCR) –detectable to PCR-undetectable status after random assignment. *Bcl-2* PCR status was assessed in peripheral blood by real-time quantitative PCR. Log-rank $P < .01$; hazard ratio, 0.399; 95% CI, 0.212 to 0.751. ^{90}Y, yttrium-90.

Only a small number of patients (treated later in the study period) received a chemotherapy plus rituximab–containing regimen; the trial was not powered to detect differences in outcome for this subgroup. Future studies will assess outcome in patients treated in this way, who might subsequently receive radioimmunotherapy as consolidation. The usefulness of RQ-PCR monitoring with such sequential treatment is unknown.

Concern has been expressed that, particularly in the context of minimal residual disease, rituximab may prevent the radioimmunoconjugate from accessing the CD20 antigen, thereby compromising the efficacy. Illidge et al[38] have addressed this specific issue in a recent report showing that prior rituximab did not appear to block the binding of subsequent ^{131}I anti-CD20 radioimmunotherapy, which proved highly efficient and led to significantly longer time to progression than the last qualifying therapy, including a rituximab-containing regimen. Also, while it is conceivable that some of the efficacy could be attributable to predosing with unlabeled anti-CD20, it is highly unlikely that a total dose of 500 mg/m^2 (which represents only one third of the conventional cumulative rituximab dose) could solely explain the 2-year PFS improvement observed after ^{90}Y-ibritumomab consolidation.

At 3 months, PCR-detectable cell levels were substantially lower in the treatment group than the control group, but this could merely reflect B-cell depletion by the antibody. However, B-cell recovery would be expected by 6 months after treatment and at this point, the PCR levels were still much lower in the treated group.

Because samples were taken during follow-up, correlations between clinical and RQ-PCR status could be studied. In the majority of patients clinical recurrence was associated with PCR-detectable disease, though this was not always the case. The answer to whether clinical or PCR status was a better predictor of outcome depended on whether patients received consolidation. Treatment with ^{90}Y-ibritumomab abrogated the negative effect of having PCR-detectable disease.

Achieving *bcl-2* PCR–undetectable status has been shown to correlate with improved clinical outcome in patients with recurrent FL after treatment with fludarabine-containing regimens,[29] high-dose therapy supported by autologous hematopoietic progenitor cells,[34,35] rituximab-containing regimens,[28,31] and radioimmunotherapy.[36,37] An extremely high response rate associated with a very high conversion rate has also been reported after treatment of newly diagnosed patients with radioimmunotherapy alone.[37] In keeping with these reports, the FIT results suggest that ^{90}Y-ibritumomab consolidation improves the quality of response at both the clinical and molecular levels, thereby prolonging response duration.

Consolidation therapy is given to improve on the response to initial therapy. Targeted irradiation is an attractive means by which to improve on the response rate, being administered to the patient only once and generally (though not always) without serious adverse effects. Longer follow-up is needed to determine whether the advantage seen in median PFS will translate into prolonged OS. The results also confirm that achievement of *bcl-2* PCR–undetectable disease should be an aim of treatment in patients with FL.

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.

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