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Clinical Cancer Research

Personalized Medicine and Imaging

Minimal Residual Disease after Conventional Treatment Significantly Impacts on Progression-Free Survival of Patients with Follicular Lymphoma: The FIL FOLL05 Trial

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Abstract

Purpose: The role of the minimal residual disease (MRD) in follicular lymphoma is still debated. In this study, we assessed whether the *BCL2/IGH* rearrangement could have a prognostic role in patients receiving R-CHOP, R-FM, or R-CVP.

Experimental Design: DNAs from 415 patients among the 504 cases enrolled in the FOLL05 trial (NCT00774826) were centralized and assessed for the *BCL2/IGH* at diagnosis, at the end of treatment, and after 12 and 24 months.

Results: At diagnosis, the molecular marker was detected in 53% of cases. Patients without molecular marker or with a low molecular tumor burden ($<1 \times 10^{-4}$ copies) showed higher complete remission (CR) rate and longer progression-free survival (PFS; 3-year PFS 80% vs. 59%; $P = 0.015$). PFS was significantly conditioned by the PCR status at 12 and 24 months, with 3-year PFS of 66% for MRD^- cases versus 41% for those MRD^+ at 12 months ($P = 0.015$), and 84% versus 50% at 24 months ($P = 0.014$). The MRD negativity at 12 and 24 months resulted in an improved PFS both in CR and in partial remission (PR) patients (3-year PFS = 72% for cases CR/PCR^- vs. 32% for those CR/PCR^+ vs. 62% for those PR/PCR^- and 25% for patients in PR/PCR^+; $P = 0.001$). The prognostic value of MRD at 12 and 24 months of follow-up was confirmed also in multivariate analysis.

Conclusions: In this study, standardized molecular techniques have been adopted and applied on bone marrow samples from a large cohort. Data reported show that the MRD detection is a powerful independent predictor of PFS in patients with follicular lymphoma receiving conventional chemo-immunotherapy. *Clin Cancer Res; 20(24); 6398–405. ©2014 AACR.*

Introduction

The monitoring of minimal residual disease (MRD) in follicular lymphoma is a well-established predictor of outcome in the autologous transplantation scenario, in which the negative impact on survival of patients receiving $BCL2/IGH^+$ autologous stem cells and of the MRD persistence after transplantation have been already demonstrated (1–3). On the contrary, the role of MRD after conventional treatments is still debated (4, 5). The first critical point is what is the best technique for MRD assessment; indeed, more than half of patients affected by

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Prior presentation: The results of this study have been presented at the 2012 American Society of Hematology and at 2012 EHA annual meetings.

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Translational Relevance

Follicular lymphoma represents an indolent lymphoproliferative disease, but the incidence of relapse still interests more than one third of the responsive patients. Thus, the introduction of the rituximab maintenance and the monitoring of the minimal residual disease (MRD) could be useful for starting a preemptive therapy and delaying the clinical relapse. In this study, we assessed 415 patients affected by follicular lymphoma receiving R-CHOP, R-FM, or R-CVP by qualitative and quantitative PCR for *BCL2*/*IGH* rearrangement. We showed that: (i) the presence of the *BCL2*/*IGH* rearrangement in the bone marrow (BM) at diagnosis has got a predictive value on progression-free survival (PFS); (ii) a low molecular tumor burden at diagnosis positively impacts on the quality of response and PFS; (iii) the MRD negativity after 12 and 24 months off treatment correlates with a better outcome; (iv) R-CVP is the regimen offering a lower molecular disease clearance in comparison with R-CHOP and R-FM. Thus, a molecular assessment during the work-up of patients with follicular lymphoma could be considered as a sort of "dynamic" risk score that could lead to treat by rituximab patients losing the MRD negativity or to avoid maintenance in patients at very low risk of relapse.

follicular lymphoma carry the t(14;18)(q32;q21) and the correspondent fusion gene *BCL2*/*IGH* that could be easily detectable by the PCR (6). Usually, the breakpoint is inside the major breakpoint region (MBR; ref. 7), whereas most of the remaining cases show the rupture in other regions, namely the minor cluster region (mcr), and the 3′MBR (8). Breakpoints occurring in the MBR can be used for MRD purposes using standardized qualitative and quantitative PCR reactions (9), whereas PCR-based assays for translocations occurring at other breakpoints have not been yet extensively validated (10). In the remaining cases, patient-specific rearrangement of the immunoglobulins heavy chains (*IGH*) could be also detected, but this method is expensive, time-consuming, and offers a lower sensitivity (11).

The second issue concerns the best timing for the MRD assessment. In particular, it is unclear whether MRD would play any predictive role already at the end of treatment, or if its significance would be higher during the follow-up, before or after autotransplantation, or during the rituximab maintenance.

The introduction of rituximab (R) in the clinical practice and the possibility of consolidation with ^{90}Y-ibritumomab significantly increased the probability of achieving the MRD eradication (12, 13).

van Oers and colleagues (14) showed that the *BCL2*/*IGH*–positive status before treatment with R-CHOP in relapsed/resistant patients did negatively condition the progression-free survival (PFS). In another study conducted by the Italian Lymphoma Group, MRD resulted as a powerful outcome predictor in patients receiving rituximab maintenance (15).

Moreover, after the introduction of the qPCR during the last decade, the role of the "molecular tumor burden" has been also evaluated: Rambaldi and colleagues (16) reported that 70% of patients with low amount of *BCL2*/*IGH* copies achieved complete remission (CR) compared with only 26% of those with higher *BCL2*/*IGH* levels, with a significant advantage on the event-free survival.

Thus, in the 2005 the Fondazione Italiana Linfomi (FIL) decided to assess the MRD in patients with follicular lymphoma enrolled in the large phase III multicenter study FOLL05 (NCT00774826). In this trial, conducted between March 2006 and September 2010, 534 untreated patients affected by advanced follicular lymphoma were randomized to receive R-CHOP (that resulted to be the best regimen), R-CVP, or R-FM, as previously reported (17).

Here, we present the results of the molecular assessment of patients enrolled in the FOLL05 trial providing new insights on some still open issues about MRD in patients with follicular lymphoma receiving chemoimmunotherapy.

Patients and Methods

Study design and treatment

The prospective, randomized, multicenter phase III trial FOLL05 (NCT00774826) was conducted in 58 Italian centers, in accordance to the Declaration of Helsinki. The clinical trial included previously untreated patients, aged 18 to 75 years, with a histologic confirmed diagnosis of follicular lymphoma grade 1, 2, and 3a, Ann Arbor stages II to IV, ECOG (Eastern Cooperative Oncology Group) performance status 0 to 2, and active disease (18). In addition to the physical examination and total body CT scan, before enrollment all patients underwent bone marrow (BM) biopsy and aspirate for assessment of the *BCL2*/*IGH* fusion gene. Central pathology review was performed for all grade 3 follicular lymphomas or when the local pathologist did not specify grading. In each center all BM biopsies were assessed by immunohistochemistry (at least CD20, CD10, and CD5), to confirm the morphologic diagnosis of follicular lymphoma. All patients underwent an intermediate CT scan for assessment of response after cycle 3 and at treatment completion. Clinical response assessment was performed with physical examination, laboratory tests, and total body CT scan; BM biopsy and aspirate were required only for patients with initial BM involvement or *BCL2*/*IGH* positivity. Quality of response was defined according to the standardized international criteria (19). The conversion of the MRD negativity to the MRD positivity was not considered as relapse in the computation of PFS.

Molecular assays

Qualitative *BCL2*/*IGH* rearrangement analysis was planned at baseline, at 6 weeks after the end of treatment, and then every 6 months during the second and third year of

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follow-up. All qualitative molecular analyses were centralized at the molecular laboratory of the Division of Hematology of the Pisa University (Italy). The four laboratories composing the FIL–MRD network retrospectively performed qPCR assays at diagnosis and at the end of therapy, after the inter-laboratory standardization of the used techniques.

DNA was extracted from BM mononuclear cells by the Wizard Genomic DNA Purification Kit (Promega). To amplify *BCL2*/*IGH* rearrangement, nested PCR reactions were performed as previously described (20).

The sensitivity of the qualitative PCR assays was confirmed by testing serial dilutions of DNA derived from the *BCL2*/*IGH*–positive DOHH-2 cell line, achieving a limiting dilution of $1{:}10^{-5}$. A second reaction for mcr breakpoint was also performed, as reported in literature (21).

qPCR was performed using the technique previously described by Ladetto and colleagues (22). Also in this case, standard curves were constructed using DNA extracted from the DOHH-2 cells. Even in this case, the sensitivity was $1{:}10^{-5}$.

To confirm the specificity of *BCL2*/*IGH* rearrangement, four-paired samples (at diagnosis and after treatment) were sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Kit 1.1 and the ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems).

Statistical analysis

All statistical analyses were performed using the SPSS 17.0 software (SPSS Inc.) at the FIL data center in Modena (Italy). Because about half of patients with follicular lymphoma relapse by the third year after treatment, and overall survival analyses are not possible in the time frame of the trial, PFS was chosen as the best surrogate of the efficacy of treatment. PFS was measured from the date of the study entry to the last follow-up, or to one of the following events: death from any cause, disease progression, and relapse. Survival curves were calculated using the Kaplan–Meier method, and statistical comparisons between curves were made using the log-rank test. *Post-hoc* comparisons were obtained using the Cox proportional hazard regression method. The χ^2 test, Fisher exact test, and Kruskal–Wallis test were used to compare variables when appropriate. For establishing the value of quantitative *BCL2*/*IGH* rearrangement on relapse, a ROC curve was performed. All statistical comparisons were two-sided. The date of the last molecular follow-up was December 30, 2012.

Results

Qualitative PCR positivity before therapy predicts the clinical response

Five hundred and thirty-four patients were enrolled into the FOLL05 trial by 58 Italian institutions; 30 patients were subsequently excluded, and the remaining 504 patients were analyzed. DNAs from 89 cases were not centralized in Pisa. Consequently, at baseline, 415 of the 504 eligible patients (82%) were assessed for *BCL2*/*IGH* rearrangement by qualitative PCR (see Supplementary Fig. S1): In 220 cases (53%) the molecular marker was found. The breakpoint was in the MBR in the 92% of cases and in the mcr in the remaining 8%. Overall, 227 patients were scored as showing BM infiltration after the local microscopy observation; in 40 of them (17.6%), we were not able to find the *BCL2*/*IGH*, probably for the presence of a rearrangement involving other rare breakpoints. On the other hand, in 127 of the 188 cases without BM infiltration at the microscopy (67.5%) the molecular marker was found, possibly due to a submicroscopic BM involvement.

Cytogenetic data about translocation between chromosome 14 and 18 were not available for this trial.

No significant differences were observed for the main clinical and prognostic features, and for treatment allocation, between cases with and without molecular assessment at enrollment, and between patients resulting PCR^- or PCR^+ (Tables 1 and 2).

Both PCR positivity and BM infiltration at the enrollment had a significant impact on the quality of response. The percentage of cases not achieving the complete response at the end of therapy was higher for patients PCR^+ or BM^+ in respect of cases without molecular marker or BM infiltration [61.9% for PCR^+ vs. 38.1% for PCR^- patients ($P = 0.027$); 32% for BM^+ vs. 21.8% for BM^- cases ($P = 0.021$)].

The 3-year PFS was significantly advantageous for patients BM^-/PCR^- versus those BM^+/PCR^+ (74% vs. 55%; $P = 0.04$).

Molecular tumor burden before therapy significantly predicts both quality of response and PFS

At the study enrollment, the molecular tumor burden was assessed by qPCR in 105 cases of the 203 already positive for MBR breakpoint; this difference between cases assessed by qualitative and quantitative PCR was due to the residual availability of DNA. No significant differences were observed for clinical features and treatment allocation between cases with or without qPCR assessment (Table 3). The quantization of molecular tumor burden showed wide interpatients variability: the median value was 3×10^{-3} copies, ranging from 2×10^{-5} to 6 copies. The *BCL2*/*IGH* copy number did not correlate with stage, performance status, age (< or >65 year), or gender, but was significantly higher in patients presenting with high FLIPI and FLIPI2 score.

When a ROC analysis-computing *BCL2*/*IGH* copies (as continuous variable) versus relapse (as dichotomic variable) was performed, a *BCL2*/*IGH* copy number $>1 \times 10^{-4}$ was the most predictive value conditioning the quality of response and the relapse rate. Indeed, among patients with high molecular tumor burden, overall response rate (ORR) was significantly lower than in cases with low molecular tumor burden (38.9% vs. 76.6%; $P = 0.006$).

Moreover, only 22% of cases showing $<1 \times 10^{-4}$ copies relapsed versus 78% of patients with $>1 \times 10^{-4}$ copies ($P = 0.033$). The treatment allocation was not different between

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Table 1. Comparison of patients' characteristics between cases assessed or not by qualitative PCR for *BCL2/IGH* rearrangement

	Patients Qualitative PCR		
Characteristics	**Performed**	**Not performed**	***P***
Number of patients	415	106	
Median age, y	69	68	N.S.
Sex			
Male	47%	54%	N.S.
Female	53%	46%	
Histotype			
Grade 1	25%	35%	
Grade 2	49%	44%	N.S.
Grade 3a	15%	13%	
Unclassified	11%	8%	
Ann Arbor stage			
II	8%	9%	
III	28%	29%	N.S.
IV	64%	62%	
ECOG performance status			
>1	2.7%	2.8%	N.S.
FLIPI			
0–2	58%	64%	N.S.
3–5	42%	36%	
FLIPI 2			
0–2	64%	75%	N.S.
3–5	36%	25%	
β2-microglobulin			
>UNL	46%	44%	N.S.
BM involvement	57%	54%	N.S.
Treatment allocation			
R-CVP	37%	32%	
R-FM	39%	35%	N.S.
R-CHOP	32%	33%	

Abbreviation: N.S., "statistically not significant."

the two cohorts; moreover, cases displaying values $<1 \times 10^{-4}$ showed a clear advantage also in terms of PFS (3-year PFS 80% vs. 59% for cases with higher molecular tumor burden; $P = 0.015$; Fig. 1).

In the multivariate analysis, the molecular tumor burden significance was analyzed together with FLIPI, BM involvement, quality of response [CR vs. partial remission (PR) or stable disease], and arm of therapy (R-CVP vs. R-CHOP or R-FM) A high FLIPI score, missing the CR, and a high molecular tumor burden before therapy retained their negative impact on PFS [HR, 2.51; 95% confidence interval (CI), 2.44–4.3; $P = 0.009$, 0.010, 0.027, respectively; see Supplementary Table S1].

In particular, when the molecular tumor burden at diagnosis was analyzed in respect of the arm of randomization, the Mantel–Heanzel analysis confirmed that the high molecular tumor burden retained its negative impact on PFS independently from the arm of randomization (HR, 4.97; test for unequal HR: $P = 0.929$).

The impact of treatment on MRD

At the first time point of molecular observation (6 weeks after the end of therapy), 3 patients dropped out from the protocol and 63 samples were not sent to the referral molecular laboratory; thus, 154 of the 220 previously PCR^+ cases were reassessed by qualitative PCR: 109 (70.8%) achieved the PCR negativity.

To verify the identity of the molecular marker at the end of treatment with that observed at diagnosis, 4 patients (8 paired samples) were longitudinally sequenced: all tests confirmed the specificity of the *BCL2/IGH* rearrangement.

The MRD status at the end of therapy did not significantly correlate with the clinical features, quality of

Table 2. Comparison of patients' characteristics between cases with or without molecular marker at diagnosis

	Patients Qualitative PCR		
Characteristics	**Positive**	**Negative**	***P***
Number of patients	220	195	
Median age, y	69	68	N.S.
Sex			
Male	52%	55%	N.S.
Female	48%	45%	
Histotype			
Grade 1	39%	31%	
Grade 2	44%	43%	N.S.
Grade 3a	11%	16%	
Unclassified	6%	10%	
Ann Arbor stage			
II	6%	12%	
III	28%	30%	N.S.
IV	66%	58%	
ECOG performance status			
>1	3.0%	2.7%	N.S.
FLIPI			
0–2	56%	45%	N.S.
3–5	44%	55%	
FLIPI 2			
0–2	60%	70%	N.S.
3–5	40%	30%	
β2-microglobulin			
>UNL	46%	54%	N.S.
BM involvement	45%	55%	N.S.
Treatment allocation			
R-CVP	33%	33%	
R-FM	35%	34%	N.S.
R-CHOP	32%	33%	

Abbreviation: N.S., "statistically not significant."

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Table 3. Comparison of patients' characteristics between cases with or without qPCR assessment

	Patients qPCR		
Characteristics	**Assessed**	**Not assessed**	***P***
Number of patients	105	115	
Median age, y	68	67	N.S.
Sex			
Male	48%	52%	N.S.
Female	46%	44%	
Histotype			
Grade 1	53%	47%	
Grade 2	44%	56%	N.S.
Grade 3a	46%	54%	
Unclassified	33%	67%	
Ann Arbor stage			
II	50%	50%	
III	51%	49%	N.S.
IV	45%	55%	
ECOG performance status			
>1	3.8%	1.7%	N.S.
FLIPI			
0–2	54%	46%	N.S.
3–5	58%	42%	
FLIPI 2			
0–2	56%	44%	N.S.
3–5	58%	42%	
β2-microglobulin			
>UNL	58%	42%	N.S.
BM involvement	56%	44%	N.S.
Treatment allocation			
R-CVP	43%	57%	
R-FM	48%	52%	N.S.
R-CHOP	50%	50%	

N.S. means "statistically not significant"

response, or therapeutic arm: the percentage of cases initially PCR$^+$ that became MRD$^-$ after treatment was superimposable for patients receiving R-CHOP and R-FM (39% and 36%, respectively). Interestingly, only 25% of patients receiving R-CVP achieved the PCR negativity; even if statistically not significant ($P = 0.26$), this is in accordance to that already observed in the clinical trial, in which R-CVP resulted the arm with higher rate of events.

Concerning the impact of treatment on the *BCL2/IGH* molecular tumor burden assessed in 66 of previously PCR$^+$ cases, the mean observed reduction was about two logarithms; a lower molecular tumor burden reduction was measured in patients receiving R-CVP versus the remaining ones (decrease >3 log = 21.1% for R-CVP vs. 36.8% for R-FM and 42.1% for R-CHOP, $P = 0.07$).

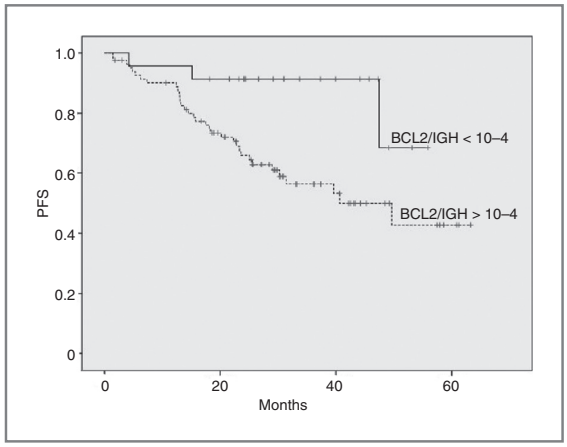

Figure 1. PFS from the randomization is significantly longer in patients with *BCL2/IGH* levels $<1 \times 10^{-4}$ before treatment (continuous dotted line; $P = 0.015$).

The conversion to MRD negativity just after treatment correlated with a lower probability of relapse and longer PFS, but it did not reach a statistical significance (relapse rate, 33% vs. 41%; $P = 0.363$; 3-year PFS 64.3% vs. 53.1; $P = 0.08$).

MRD negativity during follow-up has significant impact on PFS and retains its prognostic significance also in patients achieving partial response

At the molecular assessment performed after 12 months from the end of treatment, 63 cases were MRD$^-$, whereas 24 were still MRD$^+$; after 24 months, 46 cases became MRD$^-$, whereas 19 retained their MRD positivity. The allocation of patients in the three arms of therapy was not different between MRD$^+$ and MRD$^-$ cases.

PFS was significantly conditioned by the PCR status at 12 and 24 months, with 3-year PFS of 66% for PCR$^-$ cases

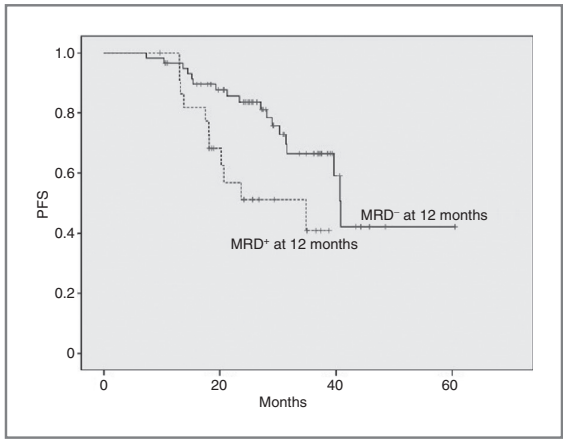

Figure 2. PFS from the randomization is significantly longer in patients without *BCL2/IGH* detectable after 12 months of follow-up (continuous dotted line; $P = 0.015$).

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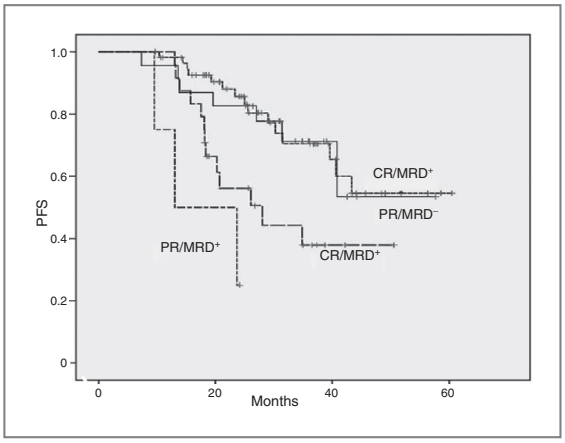

Figure 3. PFS from the randomization is significantly longer in patients without *BCL2/IGH* rearrangement during follow-up, independently of the quality of response; $P = 0.001$.

versus 41% for those PCR$^+$ at 12 months ($P = 0.015$; Fig. 2), and 84% versus 50% at 24 months ($P = 0.014$).

The MRD negativity at 12 and 24 months from the end of treatment resulted in an improved PFS both in CR and in PR patients (3-year PFS = 72% for cases CR/PCR$^-$ vs. 32% for those CR/PCR$^+$ vs. 62% for those PR/PCR$^-$ and 25% for patients in PR/PCR$^+$; $P = 0.001$; Fig. 3).

When the analysis was restricted to the cohort of patients who achieved CR but then relapsed, the MRD negativity at 12 and 24 months still retained its favorable prognostic significance [3-year PFS = 88% for MRD$^-$ cases vs. 52% for those still MRD$^+$ at 12 months ($P = 0.046$), and 91% vs. 52% at 24 months ($P = 0.034$)].

When PCR negativity at 12 and 24 months was considered in multivariate analysis together with FLIPI, BM involvement, quality of response (CR vs. PR) or stable disease, and arm of therapy (R-CVP vs. R-CHOP or R-FM), only the BM involvement at 12 months retained its poor prognostic role in addition to the MRD persistence (BM$^+$: HR, 3.23; 12 months MRD$^-$: HR, 0.38; $P = 0.010$ and 0.016, respectively; see Supplementary Table S2). At 24 months, only the persistence of the *BCL2/IGH* rearrangement conditioned the long-term outcome (24 months MRD$^-$: HR, 0.26; 95% CI, 0.07–0.92; $P = 0.036$; see Supplementary Table S3).

Moreover, when the prognostic role of the MRD at 12 and 24 months was analyzed in respect of the arm of therapy, the Mantel–Heanzel test confirmed that the MRD negativity was a good prognostic factor, independently from the type of treatment (HR, 0.44; test for unequal HR: $P = 0.604$).

Finally, from the 26 cases still MRD$^+$ after treatment, 13 (50%) achieved the MRD negativity during follow-up; on the other hand, 21 of the 45 cases MRD$^-$ after induction (46%) presented at least one positive PCR during follow-up. There was a good trend to a higher percentage of patients relapse free at 36 months in the cohort of the MRD$^{+/-}$ cases (76%) versus cases MRD$^{-/+}$ (46%) versus patients always MRD$^+$ (21%); $P = 0.08$.

When 10 cases with both molecular and clinical relapse were evaluated, in 9 of them the molecular relapse preceded the clinical one for a median of 5 months (range, 2–8).

Discussion

The results of this large, prospective, and randomized trial support the usefulness of the MRD evaluation in patients with follicular lymphoma treated with conventional chemoimmunotherapy. Four main findings arose from this study: (i) the presence of the *BCL2/IGH* rearrangement in the BM at diagnosis has got a predictive value on PFS; (ii) a low molecular tumor burden at diagnosis positively impacts on the quality of response and PFS; (iii) the MRD negativity after 12 and 24 months off treatment correlates with a better outcome; (iv) R-CVP is the regimen offering a lower molecular disease clearance in comparison with R-CHOP and R-FM.

It is relevant that our study compares favorably with other reports because of its larger number of patients: indeed, the majority of reports included not more than 100 cases (12–14). Our study analyzed 415 cases before treatment (105 by qPCR), and 207 during the further 24 months of follow-up, for overall 1,100 PCR reactions.

Another relevant item is represented by the study design: although other studies put together results coming from both peripheral blood and BM, often regardless of the presence of the molecular marker at diagnosis (14), in our study only patients PCR$^+$ at diagnosis (always assessed on BM) were considered for the statistical analysis. This could be relevant, because differences higher than 1 log have been reported in favor of BM samples (23). Moreover, qPCR assays were conducted according to the European EURO-MRD guidelines (24).

BCL2/IGH rearrangement was detected at diagnosis in 53% of our patients; this percentage could be considered too low for a marker with prognostic implications; nevertheless, it is worth to consider that in the next future the availability of primers and probes for detecting the rare *BCL2/IGH* breakpoints will increase the number of follicular lymphoma cases with a molecular marker at diagnosis. Moreover, the percentage of cases with a molecular marker is superimposable to that previously reported by other authors (48%–54%; refs. (14, 15, 22).

Obviously, we have to consider that the *BCL2/IGH* rearrangement was detected in 2% to 23% of healthy donors (25, 26). In our study, all data come from the BM analysis only and the specificity of rearrangement was confirmed also by DNA sequencing.

About the clinical impact of the qualitative PCR at diagnosis, 61.9% of PCR$^+$ did not reach CR versus 38.1% of the PCR$^-$ cases.

As second item, we demonstrated that qPCR, already at diagnosis, is able to predict the outcome of patients with follicular lymphoma. Some authors reported that levels of

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BCL2/IGH $<1 \times 10^{-3}$ did not improve quality of response (14), whereas others showed that cases with low molecular tumor burden at diagnosis achieved CR more frequently than those with high molecular tumor burden (15). In our study, we demonstrated that cases displaying values $<1 \times 10^{-4}$ showed a clear advantage in terms of PFS (3-year PFS 80% vs. 59% for cases with higher molecular tumor burden; $P = 0.015$) and ORR (76.6% vs. 38.9%; $P = 0.006$).

Moreover, we reported that the disappearance of the molecular marker after therapy positively conditioned the outcome, with a statistical significance in the long-term follow-up. The lower significance of the molecular marker disappearance at the end of treatment could be justified by the short interval between the last cycle of rituximab and the MRD assessment; indeed, the long-lasting activity of the anti-CD20 antibody could underestimate the MRD^+ cases.

Interestingly, our data sustain the role of the MRD negativity not only in patients reaching CR, but also in those with partial response: 3-year PFS was 62% for patients in PR and MRD^- versus 32% for patients in CR but still MRD^+ after 12 months of follow-up. This is interesting, because it seems to be a real proof of the importance of the MRD in follicular lymphoma. Moreover, our data are comparable with those reported by the Nordic Group in mantle cell lymphoma and by our group in follicular lymphoma, in which MRD was highly predictive for prolonged response duration also in cases achieving PR (27, 28).

The fourth finding of this study is that patients treated with R-CVP had an inferior clearance of the molecular disease; these data are in perfect accordance with those from the clinical trial in which PFS and time-to-treatment-failure were shorter for the R-CVP arm (17).

In conclusion, our study sustains the importance of the *BCL2/IGH* detection at diagnosis and the utility of the MRD monitoring during the follow-up of patients with follicular lymphoma.

All of us agree that FLIPI and FLIPI2 are very good prognostic factors in follicular lymphoma (29, 30); nevertheless, a molecular assessment during the work-up of these kind of patients could be considered such as a sort of "dynamic" risk score that could lead to treat by rituximab patients losing MRD negativity or to avoid maintenance in patients at very low risk of relapse.

PET scan is another tool that proved to be highly predictive for outcome in follicular lymphoma. In our study, we had a small subset of patients in which both tools were used. This panel of patients is too small to be conclusive, but we noticed that PCR was able to discriminate two prognostic subgroups among PET^- cases, with MRD negativity associated with longer PFS (Luminari S; unpublished data).

Moreover, we could speculate that the predictive value of MRD could be jeopardized by the use of rituximab maintenance that is now the standard of care in follicular lymphoma. However, a recent study from our group in old patients receiving R-FND followed by a brief consolidation with rituximab and a random between rituximab maintenance or observation showed power that MRD still retained an excellent prognostic discrimination among patients receiving rituximab maintenance (15). Moreover, our data suggest that a preemptive strategy similar to that used by the Nordic group in mantle cell lymphoma might appear of interest for future studies in follicular lymphoma, as also shown by the retrospective experience from our group (28).

In this line, the FIL recently started a new large randomized phase III trial based on the MRD and PET status assessment after R-CHOP induction (FOLL12-EUDRACT NUMBER: 2012-003170-60, Clinical trial.gov NCT00774826).

Disclosure of Potential Conflicts of Interest

U. Vitolo reports receiving speakers bureau honoraria from Celgene and Roche. No potential conflicts of interest were disclosed by the other authors.

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