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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Marzia Cavalli6, Luca Arcaini7, Alessandra Tucci8, Giuseppe Alberto Palumbo9, Luigi Rigacci10, Alessandro Pulsoni5, Umberto Vitolo4, Carola Boccomini4, Daniele Vailisa11, Giovanni Bertoldero12, Gianluca Gaidano13, Pellegrino Musto14, Mario Petrini1, and Massimo Federico2

Patients with Follicular Lymphoma: The FIL FOLL05 Trial

Claudia Mannu5, Luigia Monitillo4, Barbara Mantoan4, Ilaria Del Giudice6, Irene Della Starza6, Marzia Cavalli6, Luca Arcaini7, Alessandra Tucci8, Giuseppe Alberto Palumbo9, Luigi Rigacci10, Alessandro Pulsoni5, Umberto Vitolo4, Carola Boccomini4, Daniele Vailisa11, Giovanni Bertoldero12, Gianluca Gaidano13, Pellegrino Musto14, Mario Petrini1, and Massimo Federico2

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 × 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
Progression-free survival (PFS). In another study conducted on relapsed/resistant patients did negatively condition the IGH eradication (12, 13).

Significantly increased the probability of achieving the MRD (3) regions, namely the minor cluster region (mcr), and the most of the remaining cases show the rupture in other inside the major breakpoint region (MBR; ref. 7), whereas detectable by the PCR (6). Usually, the breakpoint is correspondent fusion gene follicular lymphoma carry the t(14;18)(q32;q21) and the 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.

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.

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 with 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 I 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 immediate 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
follow-up. All qualitative molecular analyses were central-
ized at the molecular laboratory of the Division of Hema-
tology of the Pisa University (Italy). The four laboratories
composing the FIL–MRD network retrospectively per-
formed 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 con-
firmed 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 Ana-
yzer (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 lym-
phoma 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
enrollment, and between patients resulting PCR^- or
PCR^+ (Tables 1 and 2).

Both PCR positivity and BM infiltration at the enroll-
ment 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 treat-
ment allocation between cases with or without molecular 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
dichotomic variable) versus relapse (as dichotomic vari-
able) was performed, a BCL2/IGH copy number >1 × 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 × 10^-4 copies
relapsed versus 78% of patients with >1 × 10^-4 copies
(P = 0.033). The treatment allocation was not different between
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, 4.97; test for unequal HR: $P = 0.027$].

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: $P = 0.027$).

### 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.

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients Assessed</th>
<th>Patients Not Assessed</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
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<td>Number of patients</td>
<td>415</td>
<td>106</td>
<td>N.S.</td>
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<td>Median age, y</td>
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<td>68</td>
<td>N.S.</td>
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<td>Sex</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>47%</td>
<td>54%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Female</td>
<td>53%</td>
<td>46%</td>
<td></td>
</tr>
<tr>
<td>Histotype</td>
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<td></td>
<td></td>
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<tr>
<td>Grade 1</td>
<td>25%</td>
<td>35%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Grade 2</td>
<td>49%</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>Grade 3a</td>
<td>15%</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>Unclassified</td>
<td>11%</td>
<td>8%</td>
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</tr>
<tr>
<td>Ann Arbor stage</td>
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<td></td>
<td></td>
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<tr>
<td>II</td>
<td>8%</td>
<td>9%</td>
<td>N.S.</td>
</tr>
<tr>
<td>III</td>
<td>28%</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>64%</td>
<td>62%</td>
<td></td>
</tr>
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<td>ECOG performance status</td>
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<td></td>
<td></td>
</tr>
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<td>&gt;1</td>
<td>2.7%</td>
<td>2.8%</td>
<td>N.S.</td>
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<tr>
<td>FLIPI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>58%</td>
<td>64%</td>
<td>N.S.</td>
</tr>
<tr>
<td>3–5</td>
<td>42%</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td>FLIPI 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>64%</td>
<td>75%</td>
<td>N.S.</td>
</tr>
<tr>
<td>3–5</td>
<td>36%</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>$\beta$2-microglobulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;UNL</td>
<td>46%</td>
<td>44%</td>
<td>N.S.</td>
</tr>
<tr>
<td>BM involvement</td>
<td>57%</td>
<td>54%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Treatment allocation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>R–CVP</td>
<td>37%</td>
<td>32%</td>
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</tr>
<tr>
<td>R–FM</td>
<td>39%</td>
<td>35%</td>
<td></td>
</tr>
<tr>
<td>R–CHOP</td>
<td>32%</td>
<td>33%</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: N.S., “statistically not significant.”

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive</th>
<th>Negative</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
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<td>Number of patients</td>
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<td>N.S.</td>
</tr>
<tr>
<td>Median age, y</td>
<td>69</td>
<td>68</td>
<td>N.S.</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>52%</td>
<td>55%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Female</td>
<td>48%</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>Histotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>39%</td>
<td>31%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Grade 2</td>
<td>44%</td>
<td>43%</td>
<td></td>
</tr>
<tr>
<td>Grade 3a</td>
<td>11%</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>Unclassified</td>
<td>6%</td>
<td>10%</td>
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<tr>
<td>Ann Arbor stage</td>
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<td></td>
</tr>
<tr>
<td>II</td>
<td>6%</td>
<td>12%</td>
<td>N.S.</td>
</tr>
<tr>
<td>III</td>
<td>28%</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>66%</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>3.0%</td>
<td>2.7%</td>
<td>N.S.</td>
</tr>
<tr>
<td>FLIPI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>56%</td>
<td>45%</td>
<td>N.S.</td>
</tr>
<tr>
<td>3–5</td>
<td>44%</td>
<td>55%</td>
<td></td>
</tr>
<tr>
<td>FLIPI 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>60%</td>
<td>70%</td>
<td>N.S.</td>
</tr>
<tr>
<td>3–5</td>
<td>40%</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>$\beta$2-microglobulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;UNL</td>
<td>46%</td>
<td>54%</td>
<td>N.S.</td>
</tr>
<tr>
<td>BM involvement</td>
<td>45%</td>
<td>55%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Treatment allocation</td>
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<tr>
<td>R–CVP</td>
<td>33%</td>
<td>33%</td>
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<tr>
<td>R–FM</td>
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<td>34%</td>
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</tr>
<tr>
<td>R–CHOP</td>
<td>32%</td>
<td>33%</td>
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</tbody>
</table>

Abbreviation: N.S., “statistically not significant.”

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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, $P = 0.07$).

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 1. PFS from the randomization is significantly longer in patients with $BCL2/IGH$ levels $<1 \times 10^{-4}$ before treatment (continuous dotted line; $P = 0.015$).](image1.png)

![Figure 2. PFS from the randomization is significantly longer in patients without $BCL2/IGH$ detectable after 12 months of follow-up (continuous dotted line; $P = 0.015$).](image2.png)

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients qPCR Assessed</th>
<th>Not assessed</th>
<th>$P$</th>
</tr>
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<td>N.S.</td>
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<tr>
<td>Male</td>
<td>48%</td>
<td>52%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Female</td>
<td>46%</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>Histotype</td>
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<tr>
<td>Grade 1</td>
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<td>47%</td>
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</tr>
<tr>
<td>Grade 2</td>
<td>44%</td>
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<tr>
<td>Grade 3a</td>
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<td>54%</td>
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</tr>
<tr>
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<td>67%</td>
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<tr>
<td>II</td>
<td>50%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>51%</td>
<td>49%</td>
<td>N.S.</td>
</tr>
<tr>
<td>IV</td>
<td>45%</td>
<td>55%</td>
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<tr>
<td>ECOG performance status</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>3.8%</td>
<td>1.7%</td>
<td>N.S.</td>
</tr>
<tr>
<td>FLIPI</td>
<td></td>
<td></td>
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<tr>
<td>0-2</td>
<td>54%</td>
<td>46%</td>
<td>N.S.</td>
</tr>
<tr>
<td>3-5</td>
<td>58%</td>
<td>42%</td>
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<tr>
<td>FLIPI 2</td>
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<tr>
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</tr>
<tr>
<td>3-5</td>
<td>58%</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>$\beta_{2}$-microglobulin &gt;UNL</td>
<td>58%</td>
<td>42%</td>
<td>N.S.</td>
</tr>
<tr>
<td>BM involvement</td>
<td>56%</td>
<td>44%</td>
<td>N.S.</td>
</tr>
<tr>
<td>Treatment allocation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-CVP</td>
<td>43%</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>R-FM</td>
<td>48%</td>
<td>52%</td>
<td>N.S.</td>
</tr>
<tr>
<td>R-CHOP</td>
<td>50%</td>
<td>50%</td>
<td></td>
</tr>
</tbody>
</table>

N.S. means “statistically not significant”

and 84% versus 50% at 24 months (P = 0.034).

When PCR negativity at 12 and 24 months was consid-
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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 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)).

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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-EUIDRACT NUM:

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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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Galimberti, S. Luminari, E. Ciabatti, F. Guerrini, C. Mannu, I.D. Giudice, L. Arcaini, A. Tucci, G.A. Palumbo, L. Rigacci, A. Pulsoni, U. Vitolo, C. Boccomini, D. Vallisa, G. Bertolero, P. Musto
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Performed PCR and interpretation of data: I.D. Starza, M. Cavalli

Acknowledgments
The authors thank Dr. Rossana Testi for her precious samples management and for her help.

Grant Support
This study was partially supported by grants from the Associazione Angelica Serra per la Ricerca sul Cancro, Modena, from AIL, Pisa, and by an unrestricted grant from “Ministero della Salute, Dipartimento dell’Innovazione—Direzione Generale Ricerca Scientifica e Tecnologica” (Progetto di Ricerca Finalizzata 2008, IRCCS CROB Rionero CIUP 165308000900000). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 17, 2014; revised August 28, 2014; accepted September 21, 2014; published OnlineFirst October 14, 2014.

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Mineral Residual Disease in Follicular Lymphoma


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