Eradication of Bone Marrow Minimal Residual Disease with First-line Chemoimmunotherapy May Prompt Early Treatment Discontinuation in Chronic Lymphocytic Leukemia

Running Title: MRD Eradication in CLL

Paolo Strati¹, Michael J. Keating¹, Susan M. O'Brien¹, Jan Burger¹, Alessandra Ferrajoli¹, Nitin Jain¹, Francesco Paolo Tambaro³, Zeev Estrov¹, Jeffrey Jorgensen², Pramoda Challagundla², Stefan H. Faderl¹, William G. Wierda¹

Department of Leukemia¹ and Department of Hematopathology², The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
Dipartimento di Patologia Generale³, Seconda Università degli Studi di Napoli, Napoli, Italy

Correspondence:
William G. Wierda, MD, PhD
Department of Leukemia, The University of Texas MD Anderson Cancer Center
1515 Holcombe Boulevard, Unit 428, Houston, TX 77030, USA.
E-mail: wwierda@mdanderson.org; Telephone: (713) 745-0428; Fax: (713) 794-1602
Previous presentation or disclaimer: presented in part at the 18th annual meeting of the European Hematology Association, Stockholm, Sweden, June 13-16, 2013, and at the 15th biannual meeting of the International Workshop on Chronic Lymphocytic Leukemia, Cologne, Germany, September 9-11, 2013

Scientific Category: lymphoid neoplasia, clinical trials and observations
Key Points

- MRD eradication is a desirable endpoint in CLL
- Early MRD eradication may prompt treatment discontinuation

Abstract

The high complete remission (CR) rate with first-line combined fludarabine, cyclophosphamide and rituximab (FCR) begs the question of the value of minimal residual disease (MRD)-negative status as a treatment endpoint. We report on 237 patients with CLL who received first-line FCR. MRD was prospectively assessed by 4-color flow cytometry in bone marrow after course 3 and at final response assessment. After course 3 and at final response assessment, 17% and 43% of patients were MRD-negative in bone marrow, respectively. A mutated immunoglobulin heavy chain variable (IGHV) gene and trisomy 12 were independently associated with MRD-negative status both after 3 courses of FCR and at final response assessment in multivariable analyses (MVA). MRD-negative status was independently associated with significantly longer PFS and OS in MVA (p=0.03 and 0.02, respectively). This association was confirmed also on landmark MVA at time of MRD assessment (p=0.04 and 0.05, respectively). MRD-negative patients had comparable PFS and OS, independent of the number of courses received or interim staging. Early MRD eradication may be a desirable goal, prompting consideration of early discontinuation of treatment. This study was registered at clinicaltrials.gov, identifier: NCT00759798.
Introduction

Combined fludarabine, cyclophosphamide and rituximab (FCR) was a significant advance in treatment of patients with chronic lymphocytic leukemia (CLL). First-line treatment with FCR results in a complete remission (CR) rate of 44-72% and overall response rate (ORR) of 90-95%; improved overall survival (OS) was demonstrated over FC\textsuperscript{1,2}.

Similar to other types of leukemia, minimal residual disease (MRD) eradication can be a realistic desirable treatment goal for CLL\textsuperscript{3-5}. Available methods to study MRD in CLL include consensus and allele-specific oligonucleotide (ASO)-polymerase chain reaction (PCR) for the clonal immunoglobulin heavy chain variable (IGHV) gene and multi-color flow cytometry\textsuperscript{6,7}. ASO-PCR and 4-color flow cytometry can reach the 0.01% sensitivity recommended by IWCLL/NCI-WG guidelines for clinical trials\textsuperscript{8}. The European Research Initiative on CLL (ERIC) established an international standardized approach for flow cytometric MRD evaluation, currently used at MD Anderson Cancer Center (MDACC)\textsuperscript{9,10}.

Recently, several retrospective studies using different treatment regimens demonstrated the association between MRD-negative status (by different measures) and improved clinical outcomes\textsuperscript{11-13}. The German CLL study group (GCLLSG) CLL8 trial provided prospective evidence of the value of achieving MRD-negative status after treatment with FC or FCR\textsuperscript{14}. MRD was assessed by 4-color flow cytometry in blood before starting therapy, after 3 courses, 1 and 2 (final response assessment) months after the last
course, then every 3 months in follow up. In cases of CR, MRD was also assessed in bone marrow at final response assessment. In multivariable analysis (MVA) when also considering patient pretreatment characteristics, MRD-negative status was independently associated with longer PFS and OS. However, blood is a less sensitive site than bone marrow to detect MRD, especially during and for several months after treatment with regimens containing monoclonal antibody. Rawstron et al. highlighted the increased sensitivity of bone marrow over blood in evaluating for MRD after treatment with rituximab-containing regimens, such as with FCR in the CLL8 trial results of the GCLLSG (oral presentation, iwCLL 2013). A remaining question is whether there is additional benefit with continued FCR treatment in patients who achieve early (prior to 6 courses) MRD-negative status.

We prospectively evaluated pretreatment patient characteristics and response, including bone marrow MRD evaluation, in 237 patients with CLL who received first-line FCR and followed patients for disease progression and survival.
Patients and Methods

Patients

The study was approved by and conducted according to the Institutional Review Board of the University of Texas MDACC guidelines and was conducted in accordance with the principles of the Declaration of Helsinki. Between September 2008 and September 2012, 237 previously untreated patients with CLL requiring therapy according to IWCLL/NCI-WG indications, provided informed consent, received up to 6 courses of standard FCR, and were evaluated for response (IWCLL/NCI-WG criteria, including 4-color flow cytometry) and follow up on this prospective study.

All patients had pretreatment evaluation including medical history, physical examination, complete blood count, beta-2 microglobulin (B2M) level, blood chemistry, and bone marrow aspiration and biopsy. The latter included fluorescent in situ hybridization (FISH) for common CLL chromosome abnormalities, analysis of the mutation status of the IGHV gene, CD38 expression by flow cytometry and ZAP70 expression by immunohistochemistry. All patients had adequate baseline hepatic and renal function and received standard-dose FCR as previously described.

Minimal Residual Disease

Bone marrow MRD was quantified by flow cytometry in samples after course 3 and 2 months after the last course (response assessment). Four-color flow cytometry was performed according to the international standardized approach of ERIC. Quantitative MRD results were categorized as positive (higher than 0.01%) or negative (lower than
Assessment was performed on patients achieving complete or partial remission. Among patients who achieved bone marrow MRD-negative status after 3 courses of FCR, the median number of leukocytes evaluated in 4-color flow cytometry was 200,000 (range, 195,312-366,982); MRD evaluation was originally performed on 222 patients, but 28 MRD-negative cases were excluded due to not reaching adequate assay sensitivity (0.01%). Among patients achieving MRD-negative status at the end of treatment, the median number of leukocytes evaluated in 4-color flow cytometry was 200,000 (range, 200,000-366,982); MRD evaluation was originally available for 220 patients, but 59 MRD-negative cases were excluded due to not reaching adequate assay sensitivity (0.01%).

**Statistical analysis**

PFS was defined as time from start of treatment to progression, non-progressers were censored at last follow up. OS was defined as time from start of treatment to death or last follow up. Survival curves were calculated using the method of Kaplan and Meier, and univariable comparisons were made using the log-rank test. MVA was performed using Cox regression with forward and backward stepwise selection. A landmark analysis at time of MRD final assessment was performed for PFS and OS. Categorical and continuous variables were compared using the χ² or Fisher exact tests, and the Mann-Whitney test, as appropriate. Logistic regression was used for MVA of categorical variable (IBM SPSS 19). All p-values were 2-sided and considered significant if ≤0.05.
Results

Baseline and treatment characteristics

Baseline patient characteristics are shown (Table 1); 75% received more than 3 courses of FCR, 25% received 1-3 courses. CR was achieved in 65% of patients, CR with incomplete marrow recovery (CRi) in 7%, nodular partial remission (nPR) in 12%, and partial remission (PR) in 13%, for an ORR of 97%. Bone marrow MRD was negative in 17% of 194 patients evaluated after course 3. Bone marrow MRD was negative in 43% of 161 patients at final response assessment.

Factors associated with achieving MRD-negative status

At final response assessment 70 (43%) patients were MRD-negative and 91 were MRD-positive; MRD-negative status was achieved in 62 (63%) patients in CR, 3 (33%) patients in CRi, and 5 (17%) patients in PR. None of the patients in nPR achieved a MRD-negative status. Pretreatment characteristics were evaluated to determine factors independently associated with MRD-negative status at final response assessment. In univariable analyses (UVA), factors associated with MRD-negative status were B2M less than 4 mg/L (p=0.03), mutated IGHV gene (p=0.02), trisomy 12 (+12)(p=0.004), absence of deletion 17p [del(17p)](p=0.04). Factors not significantly associated (p>0.05) with MRD-negative status in UVA included: age older than 65, Rai stage III-IV, ALC >75 K/uL; CD38 ≥30%; ZAP70 positivity; FISH: del(13q), negative, del(11q); receiving more than 3 courses of FCR. In MVA, factors independently associated with MRD-negative status at final response assessment were mutated IGHV gene (OR=2.5; 95% CI=1.2-5.2; p=0.01), and +12 (OR=2.5; 95% CI=1.5-4.2; p<0.001)(Table 2).
After course 3 of FCR, MRD-negative status was achieved in 34/194 (17%) patients. Pretreatment characteristics were evaluated to determine factors associated with MRD-negative status after course 3 of therapy. In UVA, factors associated with MRD-negative status were mutated \( IGHV \) gene \((p=0.02)\), ZAP70 negative \((p=0.04)\), and +12 \((p=0.05)\). Factors not significantly associated \((p>0.05)\) with MRD-negative status in UVA were age older than 65, Rai III-IV; ALC \(\geq\) 75 K/uL; B2M \(\geq\) 4 mg/L; CD38 \(\geq\)30%; FISH: del(13q), negative, del(11q), and del(17p). In MVA, factors independently associated with MRD-negative status after course 3 of FCR were mutated \( IGHV \) gene \((OR=2.7; 95\% \ CI=1.1-6.3; p=0.02)\), and +12 \((OR 2.7, 95\% \ CI=1.1-7.2; p=0.05)\)(Table 3).

**Progression-free and overall survival**

After a median follow up of 28 months (range, 4-53), the overall median PFS (30 patients progressed) and OS (18 deaths) cannot be estimated. Survival curves were compared according to pretreatment and treatment characteristics. Factors associated with a longer PFS in UVA were age younger than 65 years \((p=0.02)\), B2M lower than 4 mg/L \((p=0.04)\), mutated \( IGHV \) gene \((p=0.006)\), ZAP70 negative \((p=0.03)\), absence of del(17p) \((p<0.001)\), achievement of CR \((p<0.001)\) or overall remission \((p<0.001)\), and MRD-negative status at final response assessment \((p<0.001)\)(Figure 1A). PFS was not significantly associated \((p>0.05)\) in UVA with the following patient characteristics: Rai III-IV; ALC \(\geq\)75 K/uL; CD38 \(\geq\)30%; FISH: del(13q), no FISH abnormality, +12, and del(11q). The multivariable model for PFS included the following significant independent characteristics: MRD-negative status \((HR=0.1; 95\% \ CI=0.01-0.8; p=0.03)\), achievement...
of CR (HR=0.2; 95% CI=0.05-0.6; p=0.007) or overall remission (HR=0.1; 95% CI=0.03-0.5; p=0.003), and absence of del(17p) (HR=0.08; 95% CI=0.02-0.3; p<0.001) (Table 4).

Factors associated with a longer OS in UVA were age younger than 65 (p=0.02), B2M lower than 4 mg/L (p=0.03), absence of del(17p) (p=0.001), achievement of CR (p<0.001) or overall remission (p<0.001), and MRD-negative status at final response assessment (p=0.006)(Figure 1B). Variables not significantly associated (p>0.05) with OS in UVA were Rai III-IV; ALC ≥75 k/uL; unmutated IGHV gene; ZAP70 positive; CD38 ≥30%; FISH: del(13q), no FISH abnormality, +12, and del(11q).

OS was independently associated with the following characteristics in MVA: MRD-negative status (HR=0.6; 95% CI=0.4-0.9; p=0.02), achievement of CR (HR=0.7; 95% CI=0.5-0.9; p=0.05), and absence of del(17p) (HR=0.2; 95% CI=0.04-0.7; p=0.02) (Table 3). MRD-negative status was not correlated with either PFS or OS when analyzing only patients who achieved CR with current follow up (Figure 1C-D). Of interest, among MRD-positive patients in PR, median MRD positivity was 18.8% (range, 0.02-95%), whereas among MRD-positive patients who were in CR, median MRD positivity was 0.56% (range, 0.03-83.1%).

**Landmark Analysis at MRD final assessment for Survival**

A landmark analysis for survival dated from final MRD assessment was performed. After a median follow-up of 24 months (range, 1-50), overall median PFS and OS by landmark analysis cannot be estimated. Survival curves were compared according to
baseline and treatment characteristics. Factors associated with a longer PFS in univariable landmark analyses were age younger than 65 (p=0.02), B2M lower than 4 mg/L (p=0.05), mutated $IGHV$ gene (p=0.006), ZAP70 negative (p=0.04), absence of del(17p) (p<0.001), achievement of CR (p<0.001) or overall remission (p<0.001), and MRD-negative status at final response assessment (p<0.001)(Figure 2A). Variables not significantly associated (p>0.05) with PFS in univariable landmark analyses were Rai III-IV; ALC ≥75 k/uL; CD38 ≥30%; FISH: del(13q), no FISH abnormality, +12, and del(11q).

The landmark multivariable model included MRD-negative status (HR=0.1, 95% CI=0.05-0.9; p=0.04), achievement of CR (HR=0.2; 95% CI=0.05-0.9; p=0.04), and absence of del(17p) (HR=0.05; 95% CI=0.01-0.2; p<0.001) independently associated with PFS (Table 5). Of interest, $IGHV$ mutation status was not significant in the multivariable landmark model for PFS.

Factors associated with a longer OS in univariable landmark analyses were age younger than 65 (p=0.03), B2M lower than 4 mg/L (p=0.03), absence of del(17p) (p=0.001), achievement of CR (p=0.001) or overall remission (p<0.001), and MRD-negative status at final response assessment (p<0.006)(Figure 2B). Variables not significant (p>0.05) in univariable landmark analyses for OS were Rai III-IV; ALC ≥75 K/uL; unmutated $IGHV$ gene; ZAP70 positive; CD38 ≥30%; FISH: del(13q), no FISH abnormality, +12, and del(11q). The multivariable landmark model included MRD-negative status (HR=0.7; 95% CI=0.5-0.9; p=0.05), achievement of CR (HR=0.3; 95% CI=0.1-0.8; p=0.02), and absence of del(17p) (HR=0.3, 95% CI=0.5-0.9; p=0.05) as independently associated with OS (Table 5).
Achieving early MRD-negative status

In order to potentially evaluate the significance of achieving early MRD-negative status, patients included in this study were divided according to the final number of FCR courses received and MRD status after course 3 and at final response assessment. Five patient groups were defined: patients who received 3 or fewer total courses of FCR and were MRD-negative (n=20) or -positive (n=30); patients who received more than 3 courses and were MRD-negative both after course 3 and at final response assessment (n=11), those who were MRD-positive at both time points (n=55), and those who were MRD-positive after course 3 but negative at final response assessment (n=32). Thirteen patients who had more than 3 courses of FCR but who hadn’t undergone MRD evaluation after course 3 were not included in the analysis. PFS and OS were compared among the 5 groups. No significant differences in baseline characteristics were observed among the 3 MRD-negative groups (see data supplement).

There was no significant difference in PFS with current follow up among patients achieving MRD-negativity, regardless of number of courses and/or interim MRD status. The shortest PFS was observed for patients who received a total of 3 or fewer FCR courses and were MRD-positive (p=0.05), followed next by patients who received more than 3 total courses of FCR and were MRD-positive at final response assessment (Figure 3A). For patients who were MRD-negative after course 3 and who continued on treatment, there did not appear to be improved PFS for patients who stopped treatment versus those who achieved MRD-negative status upon continued treatment. A
significantly shorter OS was observed only for patients who were MRD-positive and received no more than 3 total courses of FCR \( (p<0.001) \) (Figure 3B).
Discussion

Achieving an MRD-negative remission has been validated as an important treatment endpoint for some leukemias\textsuperscript{3-5}. The improved outcomes in CLL achieved with chemoimmunotherapy\textsuperscript{1,2} raises the question about the value of achieving MRD-negative remission as a therapeutic endpoint in CLL. Four-color flow cytometry is feasible in blood or bone marrow and can achieve the 0.01\% sensitivity threshold requested by IWCLL/NCI-WG criteria for clinical trials\textsuperscript{8}.

Evaluation for MRD in blood versus bone marrow remains an issue for study. Historically, with chemotherapy- and chemoimmunotherapy-based treatment, bone marrow was the last site to clear of disease with response to treatment. High concordance between blood and bone marrow MRD status has been reported for regimens not including rituximab or alemtuzumab.\textsuperscript{9} Most data supporting the prognostic significance of blood MRD with rituximab-containing regimens don’t provide direct comparison with matched, paired bone marrow samples.\textsuperscript{14,17} Rawstron et al., analyzed 236 patients who received first-line FCR with paired bone marrow and blood MRD samples (oral presentation at iwCLL 2013, unpublished data), reporting a 75\% and a 85\% concordance at 3 and 6 months after the completion of therapy, respectively, with marrow showing residual disease in discordant cases where blood was negative. Comparison with very small sample size (12 patients) showed comparable median PFS for patients with discordant (positive bone marrow, negative peripheral blood) samples.\textsuperscript{18} Our study evaluates bone marrow MRD and does not provide comparative MRD data for bone marrow versus blood. We feel that in the absence of definitive data,
bone marrow should be the site for evaluation of MRD, especially in cases where blood is MRD-negative.

In this study, we prospectively evaluated bone marrow MRD status during treatment and at final response assessment in patients receiving first-line FCR treatment. MRD-negative status correlated with longer PFS and OS. Mutated *IGHV* gene and +12 were independently associated with achieving MRD-negative status both after 3 courses of FCR and at final response assessment. Inferior outcome with first-line FCR was previously reported for patients with unmutated *IGHV* gene. Higher level of CD20 expression associated with +12 CLL could favor a greater sensitivity to rituximab and improve outcome. Keeping in mind MRD eradication as a desirable goal, these findings support the use of first-line FCR for this genetic subgroup.

Similar to the CLL8 trial, our study showed that MRD independently correlated with PFS and OS. This was also confirmed by landmark analysis at final MRD assessment. Of interest, the independent prognostic role played by MRD-negative status was stronger in patients achieving PR than patients in CR. This is most likely related to the current relatively limited follow up period. Moreover, the low level of MRD-positivity observed among patients in CR with may be associated with the better outcome. Partial remission in patients who achieved MRD-negative status was related to persistent lymphadenopathy (defined by physical examination, confirmed by CT scan in a few patients). Given the favorable outcome among this group, the persistent lymphadenopathy may be scar tissue or fibrosis, and not disease related in these
patients. Biopsy would need to be performed to confirm and this observation illustrates the weakness that bone marrow MRD evaluation is only evaluating for residual disease at a single potential disease site, when the disease can reside in others not being assessed such as lymph nodes.

In our analysis, 20 patients achieved MRD-negative status after course 3 and stopped treatment, mostly owing to patients’ performance status, comorbidities, or myelosuppression. Their PFS outcome was comparable to patients who were MRD-negative and continued to receive additional FCR courses or patients who achieved MRD-negative status after receiving more than 3 courses of FCR, despite comparable baseline characteristics. Moreover, on MVA, receiving more than 3 courses of FCR did not correlate with a higher probability of achieving MRD-negative status. This raises the prospect of stopping treatment upon achieving MRD-negative status, rather than requiring administration of a defined number of courses. Such a strategy could reduce exposure to cytotoxic chemotherapy, thereby potentially reducing associated secondary complications like infection, myelosuppression, myelodysplastic syndrome and acute myeloid leukemia, and other malignancies. Although feasible, the FCR regimen can be associated with complications, particularly in elderly patients\textsuperscript{21}. Moreover, in young patients with a longer life expectancy, there is concern about second malignancies, including MDS and AML\textsuperscript{22,23}. This underscores the potential value of minimizing the total number of courses administered for maximum clinical benefit. Moreover, consolidation or maintenance treatment with less aggressive regimens may be considered for patients who remain MRD-positive after the completion of 6 courses of FCR. Bone marrow
evaluation after 3 cycles of treatment is not currently recommended by IWCLL guidelines and may cause discomfort. Moreover, this study has a limited follow up and number of events. These data will need to be confirmed in a large prospective randomized trial.
**Authorship Contributions**

W.G.W. designed, performed, and analyzed the trial, provided clinical care to patients, and wrote the paper; P.S. analyzed data, performed statistical analysis, and wrote the paper; M.J.K., S.O.B., J.B., A.F., N.J., F.P.T., Z.E. and S.F. provided clinical care to patients and coauthored the paper; J.J. and P.C. analyzed laboratory data and coauthored the paper.

**Conflict of Interest Disclosure**

The authors have no conflicts of interest to declare.
References


**Table 1. Patient Characteristics**

<table>
<thead>
<tr>
<th>Pretreatment Characteristic (N=237)</th>
<th>Number (%)</th>
<th>Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>144 (61)</td>
<td></td>
</tr>
<tr>
<td>Age ≥65 years</td>
<td>51 (21)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>59 (32-84)</td>
<td></td>
</tr>
<tr>
<td>Rai Stage III-IV</td>
<td>96 (40)</td>
<td></td>
</tr>
<tr>
<td>ALC (K/μL)</td>
<td>75 (1-425)</td>
<td></td>
</tr>
<tr>
<td>ALC ≥75 (K/μL)</td>
<td>119 (50)</td>
<td></td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>12 (6.9-16.5)</td>
<td></td>
</tr>
<tr>
<td>PLT (K/μL)</td>
<td>142 (12-398)</td>
<td></td>
</tr>
<tr>
<td>B2M ≥4 mg/L (n=231)</td>
<td>95 (41)</td>
<td></td>
</tr>
<tr>
<td>B2M (mg/L)</td>
<td>3.6 (1.3-14.1)</td>
<td></td>
</tr>
<tr>
<td>Unmutated IGHV gene (n=208)</td>
<td>126 (61)</td>
<td></td>
</tr>
<tr>
<td>CD38 ≥30% (n=225)</td>
<td>97 (43)</td>
<td></td>
</tr>
<tr>
<td>CD38 (%)</td>
<td>16 (0.1-99.9)</td>
<td></td>
</tr>
<tr>
<td>ZAP70 IHC Positive (n=214)</td>
<td>138 (64)</td>
<td></td>
</tr>
<tr>
<td>FISH (n=222)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>del(13q)</td>
<td>73 (33)</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>47 (21)</td>
<td></td>
</tr>
<tr>
<td>+12</td>
<td>39 (18)</td>
<td></td>
</tr>
<tr>
<td>del(11q)</td>
<td>47 (21)</td>
<td></td>
</tr>
<tr>
<td>del(17p)</td>
<td>16 (7)</td>
<td></td>
</tr>
</tbody>
</table>

N, number; ALC, absolute lymphocyte count; HGB, hemoglobin; PLT, platelet; B2M, beta-2 microglobulin; IGHV, immunoglobulin heavy chain variable gene; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization
Table 2. Multivariable Model for MRD-negative Status at End of Treatment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>MRD-Neg (n=70)</th>
<th>MRD-Pos (n=91)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutated IGHV gene</td>
<td>50</td>
<td>28 (56%)</td>
<td>22 (44%)</td>
<td>2.5 (1.2-5.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>+12</td>
<td>29</td>
<td>20 (69%)</td>
<td>9 (31%)</td>
<td>2.5 (1.5-4.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The ORs were adjusted from the multivariable logistic regression model. Variables not significant (p>0.05) in MVA: beta-2 microglobulin ≥4 mg/L; FISH positive for del(17p).

N, number; MRD, minimal residual disease; Neg, negative; Pos, positive; OR, odds ratio; CI, confidence interval; IGHV, immunoglobulin heavy chain variable gene.
Table 3. Multivariable Model for MRD-negative Status After Course 3 of FCR

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>MRD-Neg (n=34)</th>
<th>MRD-Pos (n=160)</th>
<th>OR (95% CI) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutated IGHV gene</td>
<td>62</td>
<td>16 (26%)</td>
<td>46 (74%)</td>
<td>2.7 (1.1-6.3) 0.02</td>
</tr>
<tr>
<td>+12</td>
<td>32</td>
<td>10 (31%)</td>
<td>22 (69%)</td>
<td>2.7 (1.1-7.2) 0.05</td>
</tr>
</tbody>
</table>

The ORs were adjusted from the multivariable logistic regression model.
Variables not significant (p>0.05) in MVA: ZP70 positive
N, number; MRD, minimal residual disease; Neg, negative; Pos, positive; OR, odds ratio; CI, confidence interval; IGHV, immunoglobulin heavy chain variable gene.
Table 4. Multivariable Models for PFS and OS by Baseline and Response Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median PFS (months)</th>
<th>HR (95% CI)</th>
<th>p-value</th>
<th>Median OS (months)</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of del(17p)</td>
<td>NR</td>
<td>0.08</td>
<td>&lt;0.001</td>
<td>NR</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.02-0.3)</td>
<td></td>
<td></td>
<td>(0.04-0.7)</td>
<td></td>
</tr>
<tr>
<td>Complete Remission</td>
<td>NR</td>
<td>0.2</td>
<td>0.007</td>
<td>NR</td>
<td>0.7</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.05-0.6)</td>
<td></td>
<td></td>
<td>(0.5-0.9)</td>
<td></td>
</tr>
<tr>
<td>Overall Remission</td>
<td>NR</td>
<td>0.1</td>
<td>0.003</td>
<td>NR</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.03-0.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRD Response-Negative</td>
<td>NR</td>
<td>0.1</td>
<td>0.03</td>
<td>NR</td>
<td>0.6</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.01-0.8)</td>
<td></td>
<td></td>
<td>(0.4-0.9)</td>
<td></td>
</tr>
</tbody>
</table>

The HRs were adjusted from the multivariable Cox regression model. Variables not significant (p>0.05) on MVA for PFS: age ≥65 years; beta-2 microglobulin ≥4 mg/L; unmutated IGHV gene; ZAP70 positive. Variables not significant (p>0.05) on MVA for OS: age ≥65 years; beta-2 microglobulin ≥4 mg/L, overall remission. PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; OS, overall survival; NR, not reached; NS, not significant; MRD, minimal residual disease.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median PFS (months)</th>
<th>HR (95% CI) p-value</th>
<th>Median OS (months)</th>
<th>HR (95% CI) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of del(17p)</td>
<td>NR</td>
<td>0.05 (0.01-0.2)</td>
<td>&lt;0.001</td>
<td>NR</td>
</tr>
<tr>
<td>Complete Remission</td>
<td>NR</td>
<td>0.2 (0.05-0.9)</td>
<td>0.04</td>
<td>NR</td>
</tr>
<tr>
<td>MRD Response-Negative</td>
<td>NR</td>
<td>0.1 (0.01-0.9)</td>
<td>0.04</td>
<td>NR</td>
</tr>
</tbody>
</table>

The HRs were adjusted from the multivariable Cox regression model.

Variables not significant (p>0.05) on MVA for PFS: age ≥65 years; beta-2 microglobulin ≥4 mg/L; unmutated IGHV gene; ZAP70 positive; overall remission.

Variables not significant (p>0.05) on MVA for OS: age ≥65 years; beta-2-microglobulin ≥4 mg/L; overall remission.

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; OS, overall survival; NR, not reached; MRD, minimal residual disease.
Figure Legends

Figure 1. Survival by MRD Status. A. Median PFS not reached in both groups; relapses among MRD-positive patients were 23/91; relapses among MRD-negative patients were 1/70 (p<0.001). B. Median OS not reached in both groups; deaths among MRD-positive patients were 11/91; there were no deaths among the 70 MRD-negative (p=0.006). C. PFS according to MRD status and IWCLL/NCI-WG. Relapses among MRD-positive patients in CR were 3/37; relapses among MRD-negative patients in CR were 1/62 (p=0.23). Relapses among MRD-positive patients in PR were 20/54; there were no relapses among the 8 MRD-negative patients in PR were (p=0.03). D. OS according to MRD status and IWCLL/NCI-WG response. Deaths among MRD-positive patients in CR were 1/37; there were no deaths among the 62 MRD-negative patients in CR were (p=0.27). Deaths among MRD-positive patients in PR were 10/54; there were no deaths among the 8 MRD-negative patients in PR (p=0.17).

Figure 2. Landmark analysis at MRD final assessment for survival by MRD status. A. Median PFS not reached in both groups; relapses among MRD-positive patients were 23/91; relapses among MRD-negative patients were 1/70 (p<0.001). B. Median OS not reached in both groups; deaths among MRD-positive patients were 11/91; there were no deaths among MRD-negative patients (p=0.006).

Figure 3. PFS and OS according to MRD/therapy groups. A. Patients achieving MRD-negative status after 3 or more courses of FCR, irrespective of MRD status at intermediate staging, had similar PFS (p=NS). Patients with MRD-negative status had a longer PFS than patients with MRD-positive status receiving 3 (p<0.001) or more
(p=0.05) courses of FCR. B. Patients achieving MRD-negative status after 3 or more courses of FCR, irrespective of MRD status at intermediate staging, or patients MRD-positive receiving more than 3 courses of FCR, had similar OS (p=NS). Patients with MRD-negative status or MRD-positive but receiving more than 3 courses of FCR had a longer OS than patients with MRD-positive status receiving only 3 courses of FCR (p<0.001). NS: not significant.
Figure 1

A. MRD- vs. MRD+: p < 0.001

B. MRD- vs. MRD+: p = 0.006

C. CR MRD- vs. CR MRD+: p = 0.23
PR MRD- vs. PR MRD+: p = 0.03

D. CR MRD- vs. CR MRD+: p = 0.27
PR MRD- vs. PR MRD+: p = 0.17
Figure 2

For personal use only.

A. Proportion Not Progressed

- MRD- 70 1
- MRD+ 91 23

p < 0.001

B. Proportion Alive

- MRD- 70 0
- MRD+ 91 11

p = 0.006
Figure 3

(A) Proportion Not Progressed vs. PFS (months)
- MRD3-: 20 events, p=NS
- MRD3+: 30 events, p=0.05
- MRD3-/end-: 11 events, p<0.001
- MRD3+/end-: 32 events
- MRD3+/end+: 55 events

(B) Proportion Alive vs. OS (months)
- MRD3-: 25 events, p=NS
- MRD3+: 30 events
- MRD3-/end-: 11 events
- MRD3+/end-: 32 events
- MRD3+/end+: 55 events
Eradication of bone marrow minimal residual disease with first-line chemoimmunotherapy may prompt early treatment discontinuation in chronic lymphocytic leukemia

Paolo Strati, Michael J. Keating, Susan M. O'Brien, Jan Burger, Alessandra Ferrajoli, Nitin Jain, Francesco Paolo Tambaro, Zeev Estrov, Jeffrey Jorgensen, Pramoda Challagundla, Stefan H. Faderl and William G. Wierda