High rate of minimal residual disease responses in young and fit patients with IGHV mutated chronic lymphocytic leukemia treated with front-line fludarabine, cyclophosphamide, and intensified dose of ofatumumab (FCO2)

Since its first use at the MD Anderson Cancer Center, FCR (fludarabine, cyclophosphamide, rituximab) chemoimmunotherapy has been considered the gold standard for the front-line treatment of young and fit patients with chronic lymphocytic leukemia (CLL).[1-3] Superior outcomes with this regimen have been observed in IGHV mutated (M-IGHV) compared to IGHV unmutated (UM-IGHV) patients.[3-5] Responses with undetectable minimal residual disease (uMRD) have been associated with a significantly longer progression-free survival (PFS) and overall survival (OS). Ofatumumab, a fully human anti-CD20 monoclonal antibody, revealed *in vitro* higher complement-mediated activity compared to rituximab. The clinical efficacy of ofatumumab as a single agent or combined with chemotherapy has been demonstrated in relapsed/refractory (R/R) patients as well as in treatment naïve (TN) patients with CLL.[6-8] In a meta-analysis that included six randomized trials, an improvement in the PFS, with no differences in the OS, was seen in the group of patients who received an ofatumumab-based treatment compared to the group of patients who received different regimens or who were only observed.[9]

In a study by Wierda *et al.*,[10] 50% of fit patients with CLL who received the front-line FC regimen combined with ofatumumab (FCO), given at a flat dose of 1000 mg, achieved a complete response (CR). Based on the efficacy of this regimen, the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) carried out a prospective, multicenter study (the LLC 0911 study) to evaluate the efficacy and safety of a front-line FCO regimen that was intensified with an additional dose of 1,000 mg of ofatumumab (FCO2). The primary endpoint of this study was the rate of CR obtained with the FCO2 regimen.

Between November 2013 and November 2015, 78 fit and young patients with CLL requiring front-line therapy according to the 2008 International Workshop CLL (iwCLL) criteria[11] were enrolled in this study. Age ≤65 years, Cumulative Illness Rating Score (CIRS) score up to 6, creatinine clearance of at least 60 mL/min, Eastern Cooperative Oncology Group (ECOG) performance status 0-1, were required for inclusion in the study. A central screening included immunophenotype, fluorescence *in situ* hybridization, the assessment of the IGHV and *TP53* mutation status.

Treatment consisted of six cycles of intravenous fludarabine (25 mg/m^2 daily) and cyclophosphamide (250 mg/m^2 daily) given on the first three days of each 28-day cycle. Ofatumumab was administered intravenously on day 14 of cycle 1 at the dose of 300 mg and on day 21 at the dose of 1000 mg. During the subsequent five cycles (cycles 2-6), ofatumumab was given at the dose of 1,000 mg on days 1 and 14 of each course. An additional dose of 1,000 mg of ofatumumab was given on day 28 of cycle 6. To prevent infusion reactions with ofatumumab, a premedication consisting of paracetamol 1,000 mg, chlorphenamine 10-20 mg, prednisolone 100 mg, or equivalent, was administered. All patients received *Pneumocystis Carinii* prophylaxis with co-trimoxazole and, as primary prophylaxis of granulocytopenia, pegfilgrastim on day 5 of each FCO2 course.

Table 1. Intention-to-treat response to the FCO2 regimen.

	N (%)
All patients	78 (100)
ORR	72 (92.3)
CR	60 (77)
PB and BM Flow-uMRD-CR[1]	36 of 78 (46.1)
PB and BM PCR-uMRD-CR[2]	17 of 78 (21.8)
PR	12 (15.4)
Failures[3]	6 (7.7)

FCO2: front-line fludarabine, cyclophosphamide, and intensified dose of ofatumumab; N: number; ORR: overall response rate; CR: complete response; MRD: minimal residual disease; Flow-uMRD: undetectable minimal residual disease by flow-cytometry; PCR: polymerase chain reaction; PCR-uMRD: undetectable minimal residual disease by PCR. [1]Peripheral blood (PB) and bone marrow (BM) Flow-uMRD in 36 of 60 (60%) patients with CR. [2]PB and BM PCR-uMRD in 17 of 60 (28.3%) patients with CR. [3]Failures: no response in five patients (stable disease, n=4; progressive disease, n=1) and unknown in 1.

Response was assessed according to the iwCLL criteria.[11] In patients who achieved a CR, MRD was checked both in peripheral blood (PB) and bone marrow (BM) by a 6/4-color flow cytometry assay with a sensitivity of at least 10^{-4}.[12] MRD was further assessed by allele-specific oligonucleotide polymerase chain reaction (PCR) in the PB and BM of patients with no evidence of MRD by flow cytometry. According to the MRD levels, CR was sub-classified as follows: (i) MRD-positive CR in the presence of residual disease by flow cytometry in the PB and/or BM; (ii) CR with undetectable MRD by flow cytometry (Flow-uMRD-CR) in the absence of residual cytometric disease in both the PB and BM; (iii) CR with uMRD by flow cytometry and allele-specific oligonucleotide PCR (PCR-uMRD-CR) in the absence of MRD by flow cytometry and PCR in the PB and BM. In patients with a Flow-uMRD-CR or PCR-uMRD-CR, MRD was monitored during the follow-up every six months. The baseline clinical and biologic characteristics of patients and patient disposition are summarized in *Online Supplementary Table S1* and *Online Supplementary Figure S1*. Median follow-up of patients was 31 months; median age 55 years (range: 36-65 years). A *TP53* disruption, del17p and/or TP53 mutation, was detected in 11% of the cases, and 64% of patients were UM-IGHV.

Median number of administered cycles was six (range: 1-6). On an intention-to-treat (ITT) basis, 72 patients (92.3%) achieved a response with a CR in 60 (77%) (Table 1). The presence of *TP53* disruption was the only significant and independent variable with an impact on the achievement of CR (P=0.014) (*Online Supplementary Tables S2 and S3*). A Flow-uMRD-CR was achieved in 36 of 78 (46.1%) patients and a PCR-uMRD-CR in 17 of 78 (21.8%) (Table 1). In multivariate analysis (MVA), Binet stage was the only factor with statistical significance on the achievement of a Flow-uMRD-CR (P=0.042) while the IGHV mutational status was the only significant factor with an impact on the achievement of a PCR-uMRD-CR (*Online Supplementary Table S3*).

In the subset of patients without *TP53* aberrations, a CR was recorded in 84.4% of the cases, a Flow-uMRD-CR in 50% and a PCR-uMRD-CR in 23.4%. When the analysis was further restricted to the M-IGHV patients without *TP53* disruption, Flow-uMRD-CR and PCR-uMRD-CR rates were 68.2% and 45.4%, respectively, and significantly higher than those observed in UM-IGHV patients: 39% (P=0.036) and 12.2% (P=0.005), respectively (*Online Supplementary Table S4*). The IGHV

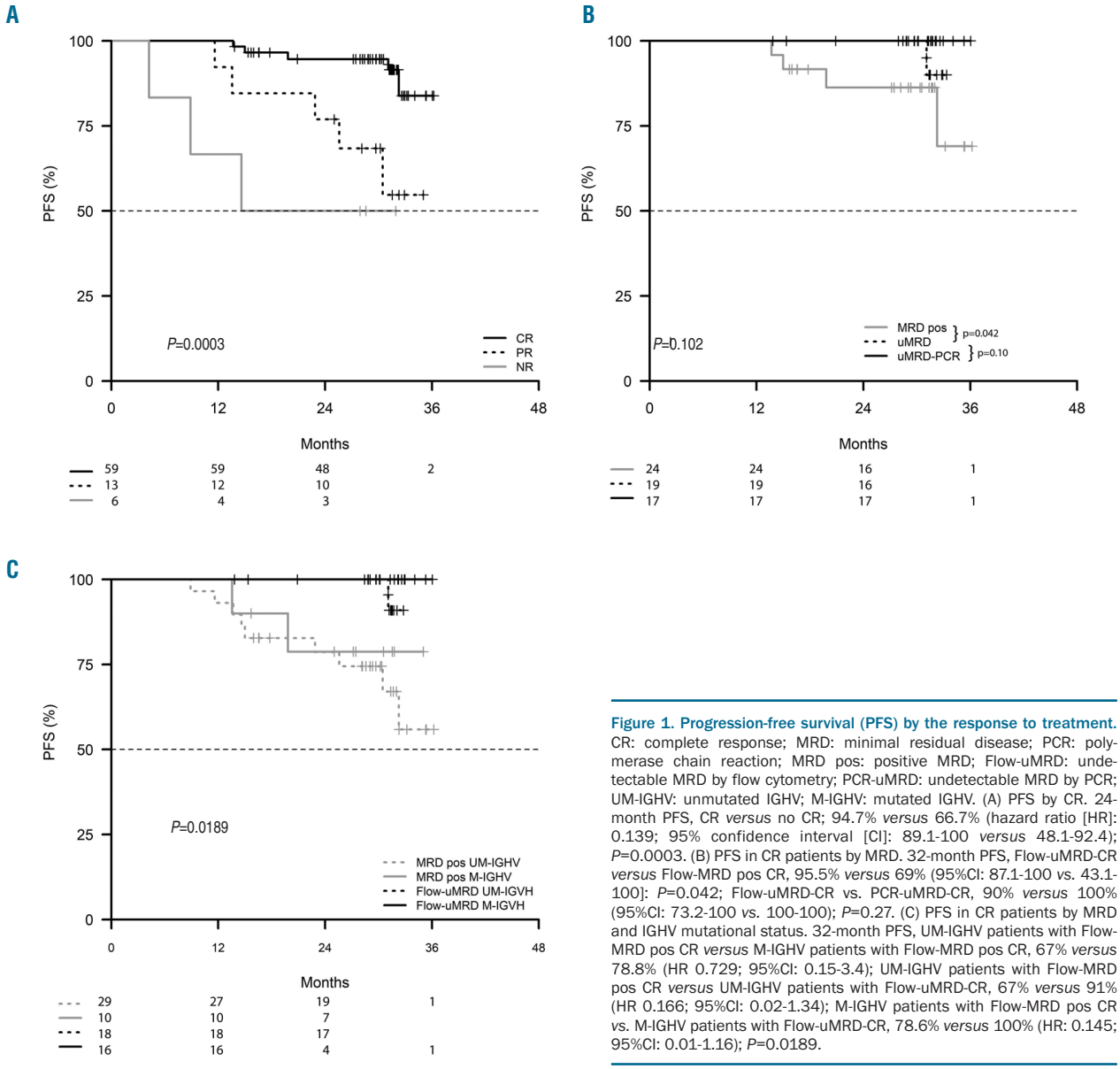

Figure 1. Progression-free survival (PFS) by the response to treatment. CR: complete response; MRD: minimal residual disease; PCR: polymerase chain reaction; MRD pos: positive MRD; Flow-uMRD: undetectable MRD by flow cytometry; PCR-uMRD: undetectable MRD by PCR; UM-IGHV: unmutated IGHV; M-IGHV: mutated IGHV. (A) PFS by CR. 24-month PFS, CR *versus* no CR; 94.7% *versus* 66.7% (hazard ratio [HR]: 0.139; 95% confidence interval [CI]: 89.1-100 *versus* 48.1-92.4); *P*=0.0003. (B) PFS in CR patients by MRD. 32-month PFS, Flow-uMRD-CR *versus* Flow-MRD pos CR, 95.5% *versus* 69% (95%CI: 87.1-100 *vs.* 43.1-100]: *P*=0.042; Flow-uMRD-CR vs. PCR-uMRD-CR, 90% *versus* 100% (95%CI: 73.2-100 *vs.* 100-100); *P*=0.27. (C) PFS in CR patients by MRD and IGHV mutational status. 32-month PFS, UM-IGHV patients with Flow-MRD pos CR *versus* M-IGHV patients with Flow-MRD pos CR, 67% *versus* 78.8% (HR 0.729; 95%CI: 0.15-3.4); UM-IGHV patients with Flow-MRD pos CR *versus* UM-IGHV patients with Flow-uMRD-CR, 67% *versus* 91% (HR 0.166; 95%CI: 0.02-1.34); M-IGHV patients with Flow-MRD pos CR *vs.* M-IGHV patients with Flow-uMRD-CR, 78.6% *versus* 100% (HR: 0.145; 95%CI: 0.01-1.16); *P*=0.0189.

mutational status was the only factor with a significant and independent impact on the achievement of both a Flow-uMRD-CR and a PCR-uMRD-CR in patients without *TP53* disruption (*Online Supplementary Table S3*).

The 36-month PFS was 76.4% (*Online Supplementary Figure S2A*). The only variable with a significant and independent impact on PFS was the presence of a *TP53* disruption (*Online Supplementary Tables 3* and *5*; *P*=0.002). After excluding patients with *TP53* disruption, none of the baseline factors revealed an impact on PFS (*Online Supplementary Table S6*). A significantly higher PFS was observed in patients who achieved a CR (*P*=0.0003). Moreover, a significantly higher PFS was seen in patients who achieved a CR with Flow-uMRD (*P*=0.042) (Figure 1A and B). All M-IGHV patients and 91% of UM-IGHV patients with a Flow-uMRD-CR were progression-free at 32 months (Figure 1C). All 17 patients (11 M-IGHV and 6 UM-IGHV) who achieved a PCR-uMRD-CR were projected as progression-free at 32 months. After a median time of 40 months (range: 28-56 months) from the initial response, residual disease was still absent in 11 of 13 patients at the last re-assessment of MRD by PCR. The 36-month OS was 94.7% (*Online Supplementary Figure S2B*). A significantly inferior survival probability was observed in patients with *TP53* disruption (*P*<0.001) and ≥5cm enlarged nodes (*P*=0.0015) (Figure 2). However, in MVA *TP53* disruption emerged as the only significant factor with an impact on OS (*Online Supplementary Tables S3* and *S7* and *Online Supplementary Figure S3A*). Patients who achieved a CR with Flow-uMRD showed a significantly superior survival than those with residual disease (*P*=0.055) (Figure 2). All CR patients with Flow-uMRD (19 patients) or PCR-uMRD (17 patients) were still alive at 32 months.

Adverse events recorded during treatment are listed in *Online Supplementary Table S8*. No unexpected toxicities were observed. Despite the prophylactic use of growth factors, grade ≥3 granulocytopenia leading to fludarabine and cyclophosphamide dose reduction was observed in 33 patients (42.3%). However, a severe infection was experienced by 21 (27%) patients. Taken together, the results of this study show that the FC regimen combined with a double dose of ofatumumab was associated with a high rate of CR and Flow-uMRD-CR in young and fit

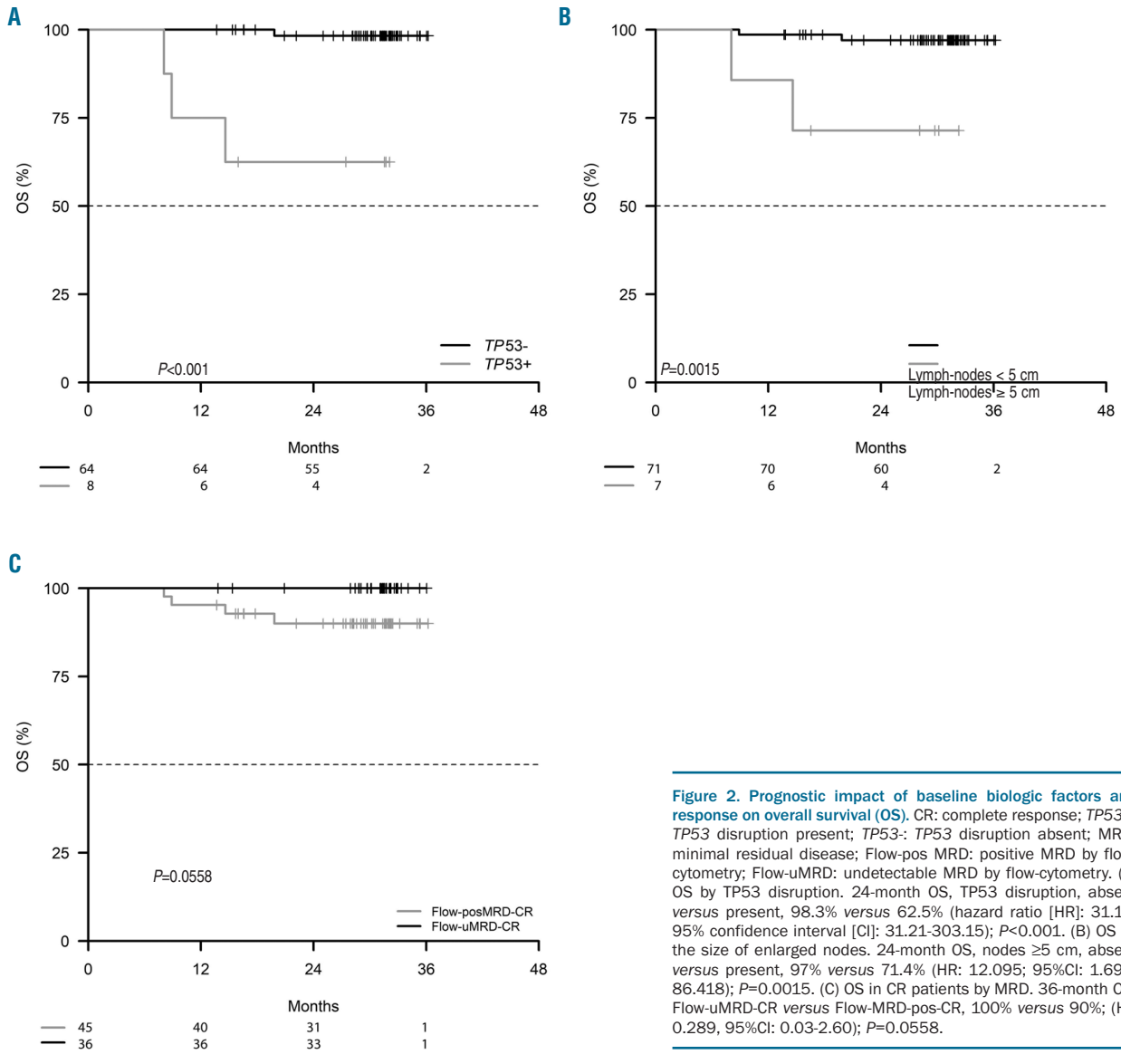

Figure 2. Prognostic impact of baseline biologic factors and response on overall survival (OS). CR: complete response; *TP53+*: *TP53* disruption present; *TP53-*: *TP53* disruption absent; MRD: minimal residual disease; Flow-pos MRD: positive MRD by flow-cytometry; Flow-uMRD: undetectable MRD by flow-cytometry. (A) OS by TP53 disruption. 24-month OS, TP53 disruption, absent *versus* present, 98.3% *versus* 62.5% (hazard ratio [HR]: 31.19; 95% confidence interval [CI]: 31.21-303.15); *P*<0.001. (B) OS by the size of enlarged nodes. 24-month OS, nodes ≥5 cm, absent *versus* present, 97% *versus* 71.4% (HR: 12.095; 95%CI: 1.693-86.418); *P*=0.0015. (C) OS in CR patients by MRD. 36-month OS, Flow-uMRD-CR *versus* Flow-MRD-pos-CR, 100% *versus* 90%; (HR 0.289, 95%CI: 0.03-2.60); *P*=0.0558.

patients with CLL. IGHV-M patients without *TP53* disruption had the highest benefit from the FCO2 chemoimmunotherapy; about two-thirds of them achieved a Flow-uMRD-CR and were progression-free at 32 months. These findings confirm the favorable outcomes of M-IGHV patients treated with the FCR regimen[3-5] and the survival benefit of patients who obtain an uMRD at response.[3-5,13] Direct cross-comparisons between the results of this study and those of other trials with the FCR regimen,[1-3] or with the FC schedule combined with obinutuzumab,[14] or a single dose of ofatumumab,[10] are methodologically incorrect. These studies differ on many points: the number and age of treated patients, inclusion criteria, selection of patients who had an MRD assessment, and supportive measures. In the absence of a randomized study, the FCR regimen remains the standard chemoimmunotherapy approach for fit and young patients with CLL and no deletion 17p. However, recent studies highlight the superiority of front-line chemo-free regimens over conventional chemoimmunotherapy. In the randomized ECOG E1912 study,[15] young and fit patients with CLL who received front-line treatment with ibrutinib and rituximab showed a significantly higher PFS and OS than those treated with FCR. A superior PFS than that observed with FCR was seen in UM-IGHV patients, while it was less evident in M-IGHV patients. Given the favorable outcomes with front-line chemoimmunotherapy in young and fit patients, IGHV mutated and without *TP53* disruption, the role of novel agents in this subset of patients should be better defined.

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