The prognostic impact of minimal residual disease in patients with chronic lymphocytic leukemia requiring first-line therapy

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ABSTRACT

A proportion of patients with chronic lymphocytic leukemia achieve a minimal residual disease negative status after therapy. We retrospectively evaluated the impact of minimal residual disease on the outcome of 255 consecutive patients receiving any front-line therapy in the context of a detailed prognostic evaluation, including assessment of *IGHV*, *TP53*, *NOTCH1* and *SF3B1* mutations. The median follow-up was 73 months (range, 2-202) from disease evaluation. The median treatment-free survival durations for patients achieving a complete response without or with minimal residual disease, a partial response and no response were 76, 40, 11 and 11 months, respectively (P<0.001). Multivariate analysis revealed that three variables had a significant impact on treatment-free survival: minimal residual disease (P<0.001), *IGHV* status (P<0.001) and β_2-microglobulin levels (P=0.012). With regards to overall survival, factors predictive of an unfavorable outcome were minimal residual disease positivity (P=0.014), together with advanced age (P<0.001), unmutated *IGHV* status (P=0.001), *TP53* mutations (P<0.001) and elevated levels of β_2-microglobulin (P=0.003). In conclusion, for patients requiring front-line therapy, achievement of minimal residual disease negativity is associated with significantly prolonged treatment-free and overall survival irrespective of other prognostic markers or treatment administered.

Introduction

Chronic lymphocytic leukemia (CLL) is an incurable disease with a heterogeneous clinical course. While some patients require early treatment and rapidly succumb to the disease, others have an indolent course that does not affect their lifespan.[1] In the last decades, the aim of therapy for patients with CLL has shifted from palliation[2] to disease eradication, particularly for younger patients who account for almost a third of the entire population with this disease.[3] Moreover, we are now able to predict the outcome of these patients more accurately using a plethora of prognostic markers such as molecular cytogenetics;[4] point mutations in a variety of genes, including *TP53*, *NOTCH1*, *SF3B1* and *POT1*;[5-9] DNA methylation,[10] immunoglobulin heavy chain gene (*IGHV*) mutational status;[11,12] CD38 and ZAP-70 expression;[12,13] serum β_2-microglobulin levels;[14] and clinical stage;[15,16] all of which have a significant impact on time to first treatment, overall survival, treatment-free survival or progression-free survival after therapy.

Modern chemoimmunotherapy regimens achieve much higher complete response rates than conventional chemotherapy, and a significant proportion of patients have no detectable disease in peripheral blood or bone marrow even when very sensitive immunophenotypic or molecular methods are used to look for residual disease. These patients are considered to have achieved a minimal residual disease (MRD) negative status.[17-20] Several phase II trials have demonstrated that patients achieving MRD negativity have a significantly longer survival than those who remain MRD positive, and this is true for patients treated with conventional chemotherapy,[21,22] monoclonal antibodies,[23] chemoimmunotherapy,[24] or stem cell transplantation.[25,26] Furthermore, a phase III trial performed by the German CLL Study Group (GCLLSG) recently revealed that patients obtaining MRD negativity had significantly longer progression-free and overall survivals, irrespectively of the treatment received.[18] Unfortunately, however, some of these studies were flawed by inappropriate statistical analysis, particularly the measurement of time-to-event outcomes from treatment initiation.[27]

Furthermore, there are several caveats to the use of MRD analysis in patients with CLL.[28] First, CLL remains incurable and at least 30% of patients who achieve MRD negativity after front-line therapy with fludarabine-cyclophosphamide (FC) or rituximab-FC eventually experience a disease relapse within 5 years.[18] Secondly, unlike the situation in acute promyelocytic leukemia or chronic myeloid leukemia,[29,30] there is no formal proof of a therapeutic benefit of re-treatment upon documentation of MRD positivity after an initial MRD-negative response compared to treatment at the time of clinical relapse. In fact, very few studies have demonstrated a clear benefit from MRD eradication or consolidation therapy in CLL,[31,32] and some of the strategies tested, although effective, resulted in significant toxicity.[33-35] Thirdly, it could be argued that MRD assessment is simply a surrogate for evalution of other adverse prognostic markers since, for instance, patients with a 17p

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deletion have a higher probability of remaining MRD-positive after therapy compared to patients without this chromosome abnormality.[18] For all these reasons, current guidelines for the management of patients with CLL recommend MRD assessment only within clinical trials with "curative intention".[36]

With all this facts in mind, we retrospectively evaluated the impact of MRD on the outcome of patients with CLL receiving any front-line therapy in the context of a very detailed prognostic evaluation, including recently described recurrent gene mutations.

Methods

Patients, data collection and response criteria

All patients in this study signed informed consent and were recruited into the International Cancer Genome Consortium Chronic Lymphocytic Leukemia (ICGC-CLL) project, which was reviewed by our Institutional Review Board. From our database, we identified 327 consecutive patients who received any front-line therapy and were formally re-evaluated following National Cancer Institute–Working Group (NCI-WG) criteria.[36-38] Indications for treatment were also those recommended by the NCI-WG.[36-38] Patients were excluded from this analysis if they had received therapy elsewhere (n=48) or were treated before 1990 (n=24). Thus, the final number of patients evaluated was 255. All baseline characteristics were determined at the time of diagnosis and, in those patients in whom these markers were not available at that time, stored samples were used to obtain them *a posteriori*. A detailed explanation of molecular tests is available in the *Online Supplement*.

Treatments

In our institution, treatment for CLL has varied over the last two decades. Traditionally, chlorambucil and other alkylating agents were commonly used until the introduction of purine analogs, mostly fludarabine and cladribine, in the 1990s. From 1995 to 2004, we conducted several trials evaluating the role of FC-mitoxantrone,[21,22] while rituximab was not generally used until 2005.[17] Other treatments, such as corticosteroids, CHOP (cyclophosphamide, adriamycin, vincristine and prednisone) chemotherapy, alemtuzumab, bendamustine and interferon were also used in a small proportion of patients. For the purpose of this analysis, patients were grouped into those who received alkylating agents or other drugs, purine analogs without rituximab, and purine analogs with rituximab.

Minimal residual disease evaluation

MRD was assessed using sensitive flow cytometry of peripheral blood and/or bone marrow samples drawn 3 months after front-line therapy. The sensitivity of this technique was determined by dilutional studies, and established to be $<10^{-4}$, as previously reported.[39] Patients who achieved MRD negativity had 6-monthly MRD evaluations in peripheral blood and/or bone marrow until MRD became positive or the disease progressed, whichever occurred first. A detailed explanation of the method of evaluating MRD is available in the *Online Supplement*.

Statistical analysis

Patients were divided into four groups based on their response: complete response and MRD-negative ($<10^{-4}$), complete response and MRD-positive ($\geq 10^{-4}$), partial response and no response. The distribution of clinical and biological parameters among groups was compared using the Fisher exact or χ^2 test. Treatment-free survival and overall survival were calculated using a landmark analysis. All calculations were performed using either SPSS, version 18.0, or R, version 3.0.1. Two-sided *P* values <0.05 were considered statistically significant. A detailed explanation of the statistical methods is available in the *Online Supplement*.

Results

Baseline characteristics

The median age of the entire cohort was 58 years (range, 27-93 years), and the percentage of patients older than 70 years was 22%. According to Döhner's hierarchical model, 17/221 (8%) and 40/221 (18%) patients had 17p deletion and 11q deletion, respectively. *TP53* mutations were documented in 22/193 (11%) patients tested, and 12 of these cases were found in patients who did not have a 17p deletion on the other allele. *NOTCH1* and *SF3B1* mutations were detected in 39/244 (16%) and 28/204 (14%) patients, respectively. Full details of these mutations are shown in *Online Supplementary Table S1*. Other adverse prognostic factors, including unmutated *IGHV* genes and increased ZAP-70 or CD38 expression, were observed in 151/217 (70%), 113/211 (54%) and 106/232 (46%) patients, respectively (Table 1).

Treatments and responses

Treatment schemes included chlorambucil (n=82 patients), CHOP or CHOP-like regimens (n=24), purine analogs as monotherapy (n=41), FC (± mitoxantrone) (n=46), rituximab-FC (± mitoxantrone) (n=51), and others (n=11) (Table 2). Ninety-six (38%) patients achieved a complete response. MRD was considered negative ($<10^{-4}$) in 44 cases and positive ($\geq 10^{-4}$) in 52 cases. In addition, 104 (41%) patients achieved a partial response and 55 (22%) had no objective response to therapy. Table 2 displays all response rates according to the different therapeutic regimens, and *Online Supplementary Table S2* shows the sensitivity of the technique. MRD negativity was documented in 31/51 (61%) patients treated with rituximab-FC(± mitoxantrone), 11/46 (24%) patients treated with FC(± mitoxantrone), ¼ (25%) patients treated with alemtuzumab and 1/41 (2%) patients treated with purine analogs (either fludarabine or cladribine) as monotherapy. There were no MRD-negative responses in patients treated with chlorambucil, CHOP or other drugs. The characteristics associated with a higher probability of obtaining a MRD-negative complete remission were absence of *NOTCH1* mutations (*P*=0.005), age less than 70 years (*P*=0.018) and type of therapy (alkylating agents or others *versus* purine analogs *versus* purine analogs plus rituximab; *P*<0.001). Of note, MRD status was not significantly associated with *IGHV* mutation status, Binet stage, fluorescence *in situ* hybridization (FISH) abnormalities, and CD38 or ZAP-70 expression (Table 1).

Treatment-free survival

The median follow-up was 73 (range 2-202), 73 (range 8-202) and 131 (24-266) months from disease evaluation after frontline therapy, landmark time (i.e. 9 months after initiation of therapy) and diagnosis, respectively. In the whole cohort, the median treatment-free survival for patients achieving an MRD-negative complete response was 76 months (95% CI, 42-109), whereas that of patients with an MRD-positive complete response was 40 months (95% CI,

11-69), patients with a partial response 11 months (95% CI, 7-15) and those who had no objective response 11 months (95% CI, 7-15) (P<0.001). In patients treated with purine analogs + rituximab, the median treatment-free survival was 80 (95% CI, 36-124) months for patients who achieved an MRD-negative complete response compared to 53 (95% CI, 29-78) months for patients achieving an inferior response (P=0.184). In patients treated with purine analogs, the median treatment-free survival for the same groups was 71 (95% CI, 17-125) and 16 (95% CI, 6-26) months, respectively (P=0.009).

Univariate analysis revealed that the following variables were significantly associated with a longer treatment-free survival after front-line therapy: mutated *IGHV* genes (P<0.001), absence of *NOTCH1* mutations (P<0.001), normal β_2-microglobulin levels (P<0.001), low *ZAP-70* expression (P=0.010), absence of *TP53* mutations (P=0.003), absence of 17p deletion by FISH (P=0.018), treatment with chemoimmunotherapy (P<0.001) and achievement of a MRD-negative status (P<0.001) (Figure 1 and Table 3). Cox regression analysis revealed that four variables had a significant independent impact on treatment-free survival: MRD-negative status [hazard ratio (HR) 3.28; 95% CI, 1.85-5.82; P<0.001], mutated *IGHV* genes (HR 2.11; 95% CI, 1.41-3.15; P<0.001), and normal β_2-microglobulin levels (HR 1.56; 95% CI, 1.10-2.23; P=0.012) (Table 3). All variables with a significant impact on treatment-free survival by multivariate analysis met the proportional hazards assumption.

Table 1. Patients' characteristics at diagnosis according to disease response after front-line therapy.

Characteristics	Total N. (%)	MRD-negative CR N. (%)	MRD-positive CR or PR or NR N. (%)	P value (χ^2 or Fisher exact test)
All patients	255 (100)	44 (17)	211 (83)	
Age, years (n=255)				0.018
< 70	198 (78)	40 (20)	158 (80)	
≥ 70	57 (22)	4 (7)	53 (93)	
Binet stage (n=255)				0.386
A	177 (69)	34 (19)	143 (81)	
B	61 (24)	7 (12)	54 (88)	
C	17 (7)	3 (18)	14 (82)	
β_2-microglobulin (n=216)				0.478
< 2.4 mg/dL	107 (50)	21 (20)	86 (80)	
≥ 2.4 mg/dL	109 (50)	17 (16)	92 (84)	
ZAP-70 expression (n= 211)			0.734	
< 20%	98 (46)	19 (19)	79 (81)	
≥ 20%	113 (54)	25 (22)	88 (78)	
CD38 expression (n=232)				0.401
< 30%	126 (54)	21 (17)	105 (83)	
≥ 30%	106 (46)	23 (22)	38 (78)	
FISH aberrations (n=221)				0.324
Low risk*	160 (74)	31 (19)	133 (81)	
11q deletion	40 (18)	11 (28)	29 (72)	
17p deletion	17 (8)	2 (12)	15 (88)	
TP53 gene (n=193)				0.172
Unmutated	171 (87)	40 (23)	131 (77)	
Mutated	22 (11)	2 (9)	20 (91)	
IGHV gene (n=217)				0.193
Unmutated	151 (70)	33 (22)	118 (78)	
Mutated	66 (30)	9 (14)	57 (86)	
NOTCH1 gene (n=244)				0.005
Unmutated	205 (84)	43 (21)	162 (79)	
Mutated	39 (16)	1 (3)	38 (97)	
SF3B1 gene (n=204)				0.805
Unmutated	176 (86)	38 (22)	138 (78)	
Mutated	28 (14)	5 (18)	23 (82)	
MYD88 gene (n=243)				0.075
Unmutated	237 (97)	41 (17)	196 (83)	
Mutated	6 (3)	3 (50)	3 (50)	
Treatment (n=255)				<0.001
Chemoinmunotherapy	51 (20)	31 (61)	20 (39)	
PA ± AA	87 (34)	12 (14)	75 (86)	
AA or others	117 (46)	1 (1)	116 (99)	

PR: partial remission; MRD: minimal residual disease; CR: complete remission; NR: no response; FISH: fluorescent in-situ *hybridization;* IGHV*: immunoglobulin heavy chain; PA: purine analogs; AA: alkylating agents. *Low-risk disease by FISH was defined by the absence of 17p or 11q deletions.*

Overall survival

Patients achieving a MRD-negative complete response had a median overall survival of 108 months (95% CI, 88-127) compared to 113 months (95% CI not estimated) in those who achieved a MRD-positive complete response, 62 months (95% CI, 54-70) in those who had a partial response and 69 months (95% CI, 49-89) in patients who did not have a response. This difference was statistically significant (P=0.001). In patients who received purine analgogs + rituximab, the median overall survival was not reached for either MRD-negative or MRD-positive patients, and there was no difference in terms of overall survival (P=0.68). In patients treated with purine analogs, the median overall survival was 111 months (95% CI, 96-125) for patients with an MRD-negative complete response compared to 93 (95% CI, 74-113) months for patients with an MRD-positive complete response, partial response or no response, but this difference was not statistically significant (P=0.381).

Other variables associated with a significantly longer median overall survival were mutated *IGHV* genes (185 *versus* 80 months, P=0.002), low-risk genomic aberrations (97 *versus* 73 *versus* 52 months, P=0.001), normal β_2-microglobulin levels (105 *versus* 61 months, P<0.001), absence of *TP53* mutations (99 *versus* 52, P=0.002), age younger than 70 years (98 *versus* 30 months, P<0.001) and type of therapy (not reached *versus* 98 *versus* 63 months for patients treated with chemoimmunotherapy, purine

Table 2. First-line treatment and response to therapy.

Therapy	All patients (N=255)	MRD-negative CR N. (%)	MRD-positive CR N. (%)	PR N. (%)	NR N. (%)
Chlorambucil	82	0 (0)	6 (7)	46 (56)	30 (37)
R-FC / R-FCM	51	31 (61)	13 (25)	3 (6)	4 (8)
FC/ FCM	46	11 (24)	18 (39)	17 (37)	0 (0)
Cladribine/ fludarabine monotherapy	41	1 (2)	9 (22)	23 (56)	8 (20)
CHOP, CHOP-like or bendamustine	24	0 (0)	4 (17)	11 (46)	9 (37)
Others	11	1 (10)	2 (18)	4 (36)	4 (36)

NR: no response; PR: partial remission; MRD: minimal residual disease; CR: complete remission; NR: no reponse or progression; FC: fludarabine and cyclophosphamide; FCM: fludarabine, cyclophosphamide and mitoxantrone; R: rituximab; CHOP: cyclophosphamide, adriamycin, vincristine and prednisone.

analogs and alkylating agents or others, respectively, *P*=0.001) (Figure 2). Other variables, such as CD38 or ZAP-70 expression, Binet stage, or the presence of *NOTCH1* or *SF3B1* mutations had no significant impact on overall survival. Cox regression analysis revealed that five variables had a favorable impact on overall survival: young age (HR 1.05; 95% CI 1.03-1.06; *P*<0.001), mutated *IGHV* genes (HR 2.06; 95% CI 1.35-3.13; *P*=0.001), normal β_2-microglobulin levels (HR 1.72; 95% CI 1.20-2.47; *P*=0.003), absence of *TP53* mutations (HR 2.39; 95% CI 1.48-3.85; *P*<0.001) and achievement of an MRD-negative status (HR 2.12; 95% CI 1.16-3.88; *P*=0.014) (Table 3). All variables with a significant impact on overall survival by multivariate analysis met the proportional hazards assumption.

Outcome of patients achieving minimal residual disease-negative status

Of 44 patients who achieved MRD negativity, 18 (41%) patients remained negative at last follow-up and 26 (59%) became MRD-positive. Moreover, 12/44 (27%) patients have required second-line treatment so far. The median times from disease evaluation to MRD positivity and second-line therapy were 37 months (95% CI, 33-41) and 73 months (95% CI, 59-97), respectively.

The presence of adverse FISH abnormalities (i.e. 17p or 11q deletion) was the only covariate significantly associated with a shorter time to MRD positivity (*P*=0.024). Moreover, adverse FISH and *TP53* mutations were both independently associated with a shorter time to second-line therapy (*P*=0.008 and *P*=0.006, respectively).

Discussion

Therapy for CLL has notoriously improved in the last decades. Alkylating agents traditionally used in CLL, such as chlorambucil, only achieved a 2-10% complete response rate,[40] with no MRD-negative responses.[41] Purine analogs significantly improved complete response rates but still less than 10% of patients achieved a MRD-negative status when these agents were given as monotherapy.[42] Combination chemotherapy (i.e. FC, with or without mitoxantrone) improved responses even more, obtaining complete response rates of 20-40%[40] and, for the first time, a quarter of patients treated with these combinations did not have detectable MRD at the time of response evaluation.[18,22] Unfortunately, none of these therapeutic options had a significant impact on overall survival[40] and, perhaps, the reason was that the percentage of patients obtaining MRD-negative responses was insufficient. With the advent of rituximab-containing combinations (i.e. chemoimmunotherapy), complete response rates have doubled, and so have MRD-negative rates.[17,18,20] Not surprisingly, several single-center and epidemiological studies have confirmed that front-line chemoimmunotherapy prolongs the overall survival of patients with CLL compared to that of historical cohorts of patients treated without rituximab.[24,43] Moreover, a phase III randomized trial recently showed that the addition of rituximab to FC front-line chemotherapy prolonged the overall survival compared to FC alone,[44] and also that MRD status after treatment was one of the most important predictors of survival.[18] Nevertheless, very few studies have performed a multivariate analysis evaluating the impact of MRD in

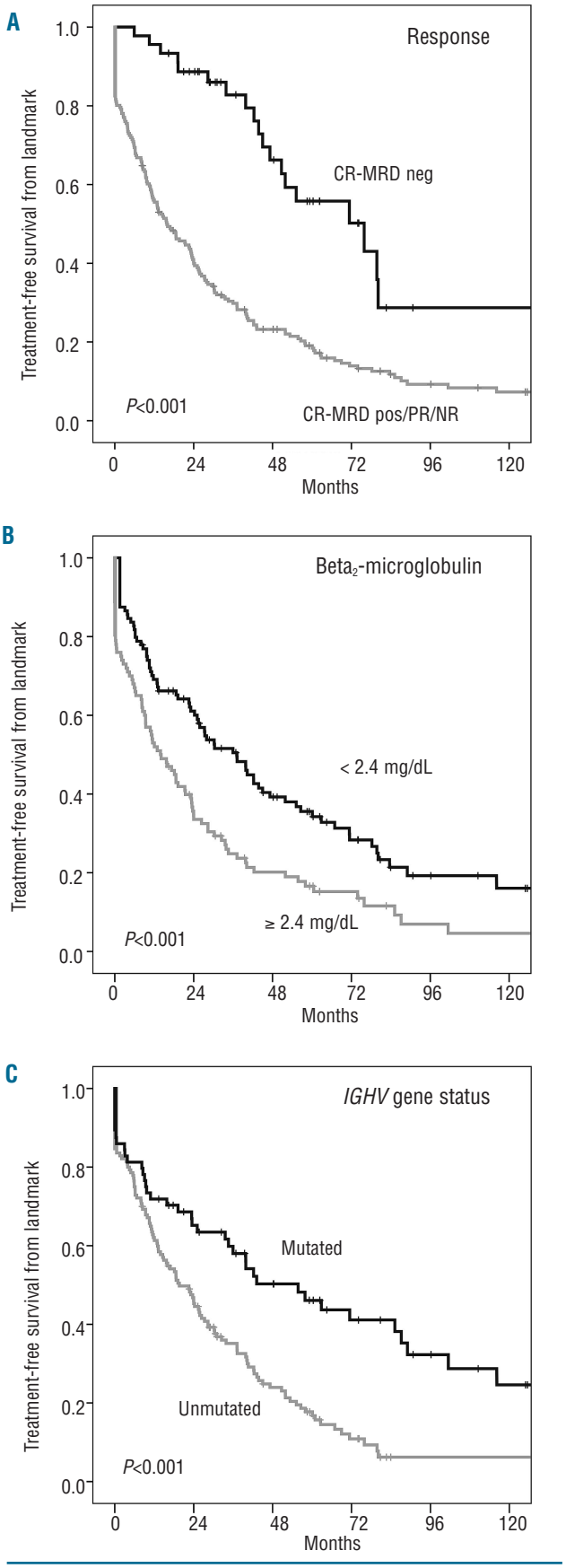

Figure 1. Treatment-free survival from landmark according to: (A) response to therapy; (B) β_2-microglobulin levels; and (C) *IGHV* mutational status. CR: complete response; PR: partial response; NR: no response.

the context of other prognostic biomarkers.

In the present study, we evaluated the prognostic value of MRD after first-line therapy in a group of patients with a very detailed prognostic evaluation, including assessment of *TP53*, *NOTCH1* and *SF3B1* mutations. This is particularly relevant since one of the major criticisms made to the implementation of MRD assessment in CLL is that patients in whom MRD can be eradicated could simply be biologically distinct from the remainder and, possibly, have a better prognosis.[18,23] In this study, we have confirmed that MRD negativity was a consistent predictor of both treatment-free survival and overall survival together with other well characterized prognostic factors, such as *IGHV* mutation status and β_2-microglobulin serum concentration. Patients achieving a MRD-negative complete response had the longest median treatment-free survival (76 months) compared to those who achieved a MRD-positive complete response, a partial response or who did not respond (40, 11 and 11 months, respectively). Moreover, and in contrast to the British study,[23] the difference in treatment-free survival between patients obtaining a MRD-positive complete response and those achieving a partial response was statistically significant (40 *versus* 11 months, $P<0.001$), probably because of the higher statistical power of our study. This result is, however, not surprising since in almost any malignancy the deeper the

Table 3. Univariate and multivariate analyses of the effect of MRD and other prognostic factors on treatment-free and overall survival.

	Treatment-free survival						Overall survival					
	Univariate analysis (Kaplan-Meier and log-rank test)			Multivariate analysis (Cox regression after multiple imputation)			Univariate analysis (Kaplan-Meier and log-rank test)			Multivariate analysis (Cox regression after multiple imputation)		
Variable	Median (months)	95% CI	*P* value	HR	95% CI	*P* value	Median (months)	95% CI	*P* value	HR	95% CI	*P* value
Age, years												
< 70	28	21-36	< 0.001	1.00	0.99-1.02	0.503	98	88-108	< 0.001	**1.05**	**1.03-1.06**	**< 0.001**
≥ 70	11	6-17					30	11-49				
Binet stage												
A	30	20-41	0.005	1.14	.87-1.52	0.334	90	68-113	0.551	-	-	-
B	18	8-22					87	67-108				
C	8	3-14					58	42-74				
β_2 microglobulin (mg/dL)												
< 2.4	37	25-49	< 0.001	**1.56**	**1.10-2.23**	**0.012**	105	85-125	< 0.001	**1.72**	**1.20-2.47**	**0.003**
≥ 2.4	14	7-21					61	51-70				
FISH												
Low risk*	30	20-40	0.018	1.18	0.83-1.71	0.355	97	83-111	0.001	1.47	0.96-2.24	0.075
11q deletion	25	18-33					73	39-106				
17p deletion	15	0-31					52	42-61				
TP53												
Unmutated	32	23-42	0.003	1.17	0.73-1.87	0.512	99	82-117	0.002	**2.39**	**1.48-3.85**	**<0.001**
Mutated	11	0-30					52	38-65				
ZAP70 expression												
Low	42	29-55	0.010	1.02	0.73-1.43	0.895	101	87-116	0.152	-	-	-
High	19	11-27					82	61-103				
IGHV gene												
Unmutated	20	13-26	<0.001	**2.11**	**1.41-3.15**	**< 0.001**	80	60-101	0.002	**2.06**	**1.35-3.13**	**0.001**
Mutated	58	32-80					185	85-284				
NOTCH1												
Unmutated	30	21-39	< .001	1.43	.96-2.13	0.080	97	84-109	0.062	-	-	-
Mutated	8	0-17					58	45-72				
SF3B1												
Unmutated	28	18-38	0.201	-	-	-	93	78-109	0.747	-	-	-
Mutated	23	15-32					99	67-132				
MRD												
Negative	76	42-109	< 0.001	**3.28**	**1.85-5.82**	**< 0.001**	108	88-127	0.012	**2.13**	**1.16-3.88**	**0.014**
Positive	16	10-22					78	62-95				
Treatment												
AA or others	13	6-20	<0.001	1.29	0.99-1.02	0.054	65	52-78	0.001	1.26	0.90-1.77	0.183
PA	23	15-31					98	74-122				
PA+R	80	38-123					NR	-				

PR: partial remission; MRD: minimal residual disease; CR: complete remission; ND; no response or disease progression; FISH: fluorescent in-situ *hybridization;* IGHV*: immunoglobulin heavy chain; PA: purine analogs; AA: alkylating agents; R: rituximab; NR: not reached. *Low risk disease by FISH was defined by the absence of 17p or 11q deletions. Statistically significant results are shown in bold.*

response obtained with therapy, the longer the patient remains in remission and, therefore, free of therapy.[29,30]

MRD status also had a significant impact on overall survival, and this effect, too, was independent of other well-established prognostic factors. In keeping with the results of both the British and German studies,[18,23] patients obtaining MRD negativity at the 10^{-4} level had a significantly longer overall survival than that of patients obtaining an inferior response (not reached *versus* 81 months, *P*=0.007). When we specifically evaluated patients who responded to therapy, there was a trend towards a longer overall survival for MRD-negative patients (108 *versus* 78 months, *P*=0.012), but this significant difference was lost when patients achieving a MRD-negative complete response were compared to those achieving a MRD-positive complete response (*P*=0.82). These results indicate that MRD negativity appeared at least as relevant to the survival of the patients as obtaining a complete response by NCI-WG criteria, and possibly more important than the treatment they received to attain that goal.[18] Furthermore, MRD sta-

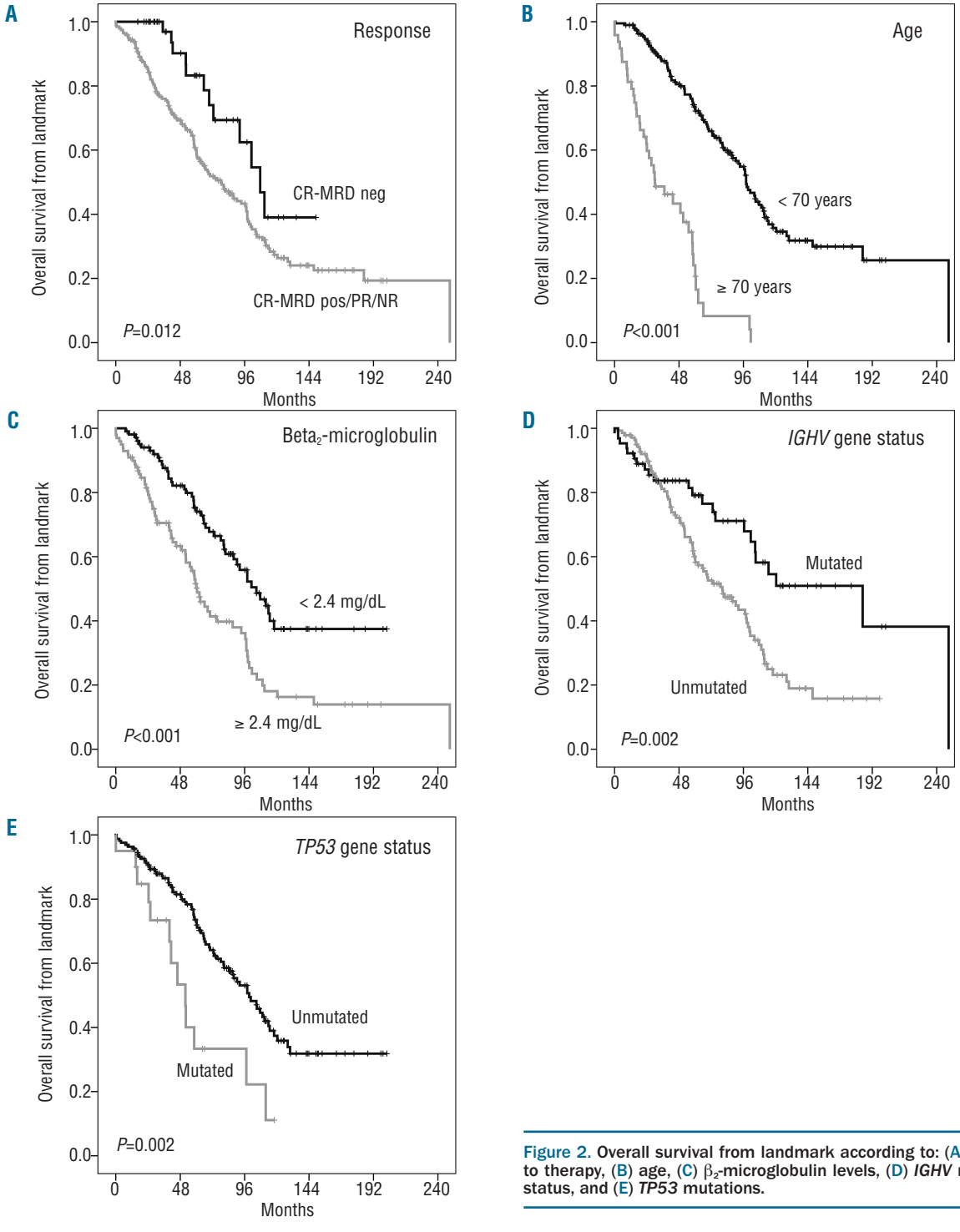

Figure 2. Overall survival from landmark according to: (A) response to therapy, (B) age, (C) β_2-microglobulin levels, (D) *IGHV* mutational status, and (E) *TP53* mutations.

tus was significantly more relevant than other well-characterized prognostic markers, including FISH aberrations, ZAP-70 expression or gene mutations (*NOTCH1* or *SF3B1*). Another interesting finding of our study was the fact that *TP53* mutations had an independent prognostic impact on overall survival even when evaluated alongside FISH aberrations, confirming results from other groups and suggesting that *TP53* sequencing should be incorporated into the laboratory work-up of patients with CLL.[5,45-47]

Current clinical guidelines for the management of CLL do not recommend the evaluation of MRD in routine practice, but only within clinical trials that "aim toward achieving long-lasting complete responses".[36] We believe that there is already enough evidence to recommend the assessment of MRD status in all patients who achieve a complete remission after front-line therapy since the prognostic power of this test is at least comparable to that of other tests generally recommended by current guidelines, such as FISH cytogenetics.[36] MRD status after therapy could be used in deciding how closely a patient should be followed-up, and also in selecting those patients who might benefit from inclusion in clinical trials evaluating maintenance or consolidation strategies.[48,49] Regarding the technique used to evaluate MRD in CLL, MRD-flow cytometry does not require pre-treatment samples and is becoming the technique of choice in view of its sensitivity and reproducibility, even in the context of multicenter trials.[19,50] Furthermore, several problems associated with four-color panels, such as the large amounts of time and sample required for an accurate analysis of patients with low level disease, have been partially circumvented with the advent of modern six-color combinations. With these new advances, MRD-flow could be implemented in most diagnostic laboratories with additional savings in terms of time, labor and reagents.[50]

There are, however, several considerations that should be highlighted. First, MRD-flow cytometry has changed several times over the years and has only been standardized in the last decade, even though its sensitivity has remained constant. Moreover, treatment groups were heterogeneous and relatively small, compared to those in recent randomized trials, due to the retrospective nature of this study, and this might have reduced the statistical power of the variable "treatment" in our multivariate models. Furthermore, we only had MRD results for patients who achieved a complete response and, therefore, we were unable to analyze the outcome of patients with MRD-negative partial responses together with those with MRD-negative complete responses as recommended by other groups.[18,28]

In conclusion, in this study we observed that, for patients with CLL requiring front-line therapy, achievement of a MRD-negative status is associated with significantly prolonged treatment-free survival irrespective of other prognostic markers or treatment given. We also observed a significant benefit in terms of overall survival, but this effect was less clear. These results could have potential implications when deciding the most appropriate treatment for patients with the disease, and could also set the stage for future MRD-directed therapy in CLL.

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Authorship and Disclosures

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