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Letters to *Blood*

TO THE EDITOR:

Complex karyotype in unfit patients with CLL treated with ibrutinib and rituximab: the GIMEMA LLC1114 phase 2 study

Gian Matteo Rigolin,[1,*] Ilaria Del Giudice,[2,*] Antonella Bardi,[1] Aurora Melandri,[1] Rocio Edith García-Jacobo,[1] Francesca Cura,[1] Sara Raponi,[2] Caterina Ilari,[2] Luciana Cafforio,[2] Alfonso Piciocchi,[3] Valentina Arena,[3] Gianluigi Reda,[4] Francesco Albano,[5] Stefano Molica,[6] Paolo Sportoletti,[7] Livio Trentin,[8] Monia Marchetti,[9] Mauro Nanni,[2] Nadia Peragine,[2] Paola Mariglia,[2] Marco Vignetti,[3] Anna Guarini,[10] Francesca Romana Mauro,[2,*] Robin Foà,[2,*] and Antonio Cuneo[1,*]

[1]Hematology Section, St Anna University Hospital, Ferrara, Italy; [2]Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome, Italy; [3]Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) Data Center, GIMEMA Foundation, Rome, Italy; [4]Hematology Unit, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy; [5]Hematology Section, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy; [6]Hematology, Azienda Ospedaliera Pugliese Ciaccio, Catanzaro, Italy; [7]Institute of Hematology and Center for Hemato-Oncological Research, Ospedale S Maria della Misericordia, University of Perugia, Perugia, Italy; [8]Hematology Unit, University of Padua, Padua, Italy; [9]Azienda Ospedaliera SS Antonio e Biagio e Cesare Arrigo, Alessandria, Italy; and [10]Department of Molecular Medicine, Sapienza University, Rome, Italy

In chronic lymphocytic leukemia (CLL), the presence of a complex karyotype, as defined by ≥3 chromosomal abnormalities in the neoplastic clone, has been shown to confer an adverse prognosis in retrospective series of untreated patients[1-3] and in patients treated with chemoimmunotherapy.[4,5] Recent guidelines[6] have therefore included conventional karyotyping among the desirable evaluations for prospective clinical trials. However, limited data are available on the prognostic impact of a complex karyotype in patients with CLL who received first-line treatment with novel agents.[7-9]

In this letter, we present the results from the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) LLC1114 phase 2 multicenter study, which aimed to assess the activity and safety of first-line ibrutinib plus rituximab in patients with CLL who were unfit for treatment.[10] The study enrolled 151 patients (supplemental Figure 1, available on the *Blood* Web site). The inclusion criteria were diagnosis of CLL according to the National Cancer Institute (NCI) criteria,[6] active disease requiring therapy (NCI criteria),[6] no previous treatment, total Cumulative Illness Rating Scale score of >6, and/or creatinine clearance <70 mL/min. Patients were excluded if they had a Richter's syndrome transformation or if they had HIV or an active hepatitis C virus or hepatitis B virus infection. The primary end point was progression-free survival (PFS) at 12 months from the start of treatment. Each patient in this study provided informed consent. This trial was registered at www.clinicaltrials.gov as #NCT02232386 and was approved by the local ethics committees.

Samples for cytogenetics testing were available for 121 (80%) of 151 patients. Chromosome analysis was successful in 98 (81%) of 121 patients (supplemental Table 1), as previously described.[11] Unlike the patient data in our previous study,[11] cytogenetics data capture in this study for nearly 20% of the patients was unsuccessful mainly because of sample centralization that may have negatively affected the mitotic yield. Demographics are presented in supplemental Table 2. Patients with and without cytogenetics data did not differ in terms of clinical and biological characteristics except for the median age (72 vs 77 years; $P = .023$). A complex karyotype (ie, ≥3 chromosomal abnormalities) was found in 26 (27%) of 98 patients, and 10 (38%) of 26 patients presented with a highly complex karyotype as defined by the presence of ≥5 chromosomal abnormalities.[3] Eighteen of these 26 patients with a complex karyotype (69%) presented unbalanced rearrangements, including extra chromosome material, derivatives, insertions, duplications, and marker chromosomes.[12] A *TP53* disruption (del17p and/or *TP53* mutation) was detected in 18 of the 98 patients with successful cytogenetics data capture (18%). Of these 18 patients with disrupted *TP53*, 10 also had a complex karyotype (56%). Wild-type *TP53* with a complex karyotype was found in 16 (16%) of 98 patients. Of the 26 patients with a complex karyotype (supplemental Table 3), 10 (38%) presented with a *TP53* disruption and 20 (77%) presented with an unmutated IGHV configuration. No significant differences were observed between patients with and without a complex karyotype when comparing the incidence of gene mutations involving *SF3B1*, *NOTCH1*, *BIRC3*, and *TP53*. Of note, 8 (80%) of 10 patients with a highly complex karyotype had a *TP53* disruption compared with 2 (13%) of 14 patients who did not have a highly complex karyotype ($P = .001$; supplemental Table 4).

After a median follow-up 42.6 months (range, 2.1-56.7 months; interquartile range, 26.9-48.2 months), 17 events were recorded: 12 progressions (7 in patients with a complex karyotype) and 5 deaths (2 in patients with a complex karyotype). Median PFS was not reached (Figure 1A). At 36 months, the PFS was 79.9% (95% confidence interval [CI], 71.6% to 89.1%). In multivariable analysis (Table 1), the presence of a complex karyotype was significantly associated with a shorter PFS ($P = .009$; Figure 1B) along with a worse Eastern Cooperative Oncology Group performance status (ECOG PS) ($P = .048$). Patients with a highly complex karyotype had an unfavorable PFS similar to that of

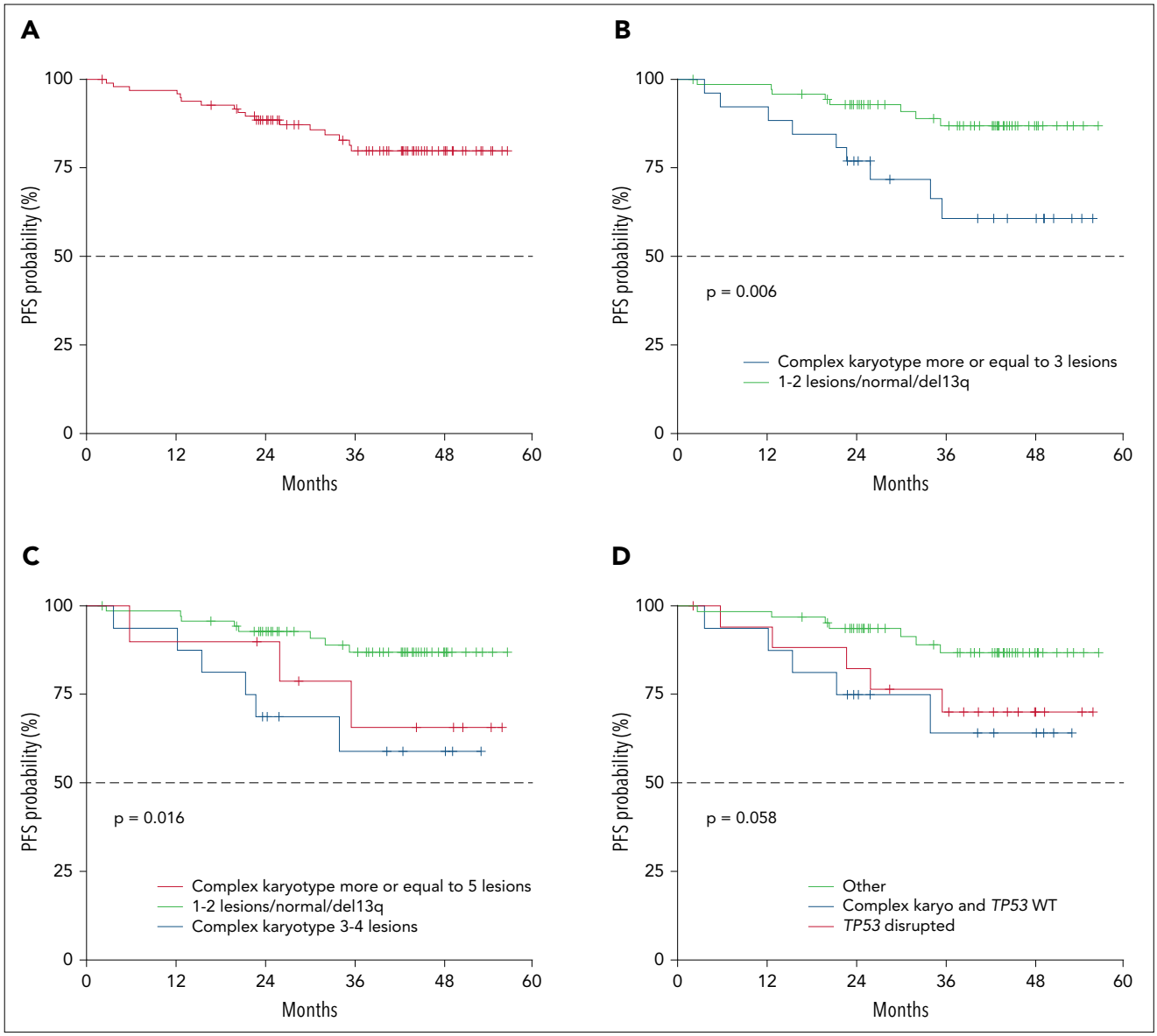

Figure 1. PFS probability. (A) Overall PFS, (B) PFS by complex karyotype, (C) by complex karyotype with 3-4 chromosomal lesions and with ≥5 chromosomal lesions (pairwise comparisons: complex karyotype ≥5 lesions vs 1-2 lesions/normal/del13q [*P* = .121], complex karyotype ≥5 lesions vs complex karyotype 3-4 lesions [*P* = .550], complex karyotype 3-4 lesions vs 1-2 lesions/normal/del13q [*P* = .006]), and (D) by *TP53* disruption and complex karyotype (pairwise comparisons: *TP53* disrupted vs complex karyotype and *TP53* wild type [WT] [*P* = .667], *TP53* disrupted vs other [*P* = .097], complex karyotype and *TP53* WT vs other [*P* = .026]).

patients who did not have a highly complex karyotype (Figure 1C), whereas patients with a complex karyotype without a *TP53* disruption showed a significantly worse PFS compared with patients without a complex karyotype (*P* = .026; Figure 1D). A similar PFS was also observed between patients with *TP53* disruption and those with a complex karyotype without *TP53* disruption (Figure 1D) and between patients who presented with a complex karyotype with or without *TP53* disruption (supplemental Figure 2).

The overall response rate (determined by partial response plus complete response) was 86%, with 27 patients (28%) achieving a complete response. The only biologic variable associated with a better response was IGHV mutational status, but it had no statistical significance (*P* = .07; supplemental Table 5).

At 36 months, the estimated overall survival (OS) was 92.3% (95% CI, 87% to 98%) (supplemental Figure 3A). In univariable analysis, the presence of a complex karyotype was associated with a shorter OS, although this difference did not reach statistical significance (supplemental Figure 3B; supplemental Table 6). Patients who had a highly complex karyotype had a significantly worse OS when compared with those who did not (*P* = .027; supplemental Figure 3C). A similar OS was observed between patients with *TP53* disruption and those with a complex karyotype without *TP53* disruption (supplemental Figure 3D) and between patients presenting with a complex karyotype with and without *TP53* disruption (supplemental Figure 4).

On the whole, our analysis confirms that a complex karyotype can frequently be observed in treatment-naive patients with

Table 1. Univariable and multivariable analysis of PFS

Variable	Univariable analysis			Multivariable analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Female or male sex	0.47	0.15-1.44	.18	—		—
Age	1.02	0.96-1.09	.53	—		—
Performance status 1-2/0	2.54	0.98-6.59	.055	2.61	1.01-6.78	.048
Creatinine clearance ≤70/>70 mL/min	0.65	0.20-2.06	.46	—		—
β_2 microglobulin pathologic/normal	0.59	0.21-1.66	.32	—		—
LDH pathologic/normal	1.71	0.65-4.49	.28	—		—
Bulky disease ≥5/<5 cm	1.31	0.30-5.77	.72	—		—
Advanced-stage Rai III-IV Binet C/others	1.23	0.47-3.25	.67	—		—
IGHV mutated/unmutated	0.58	0.21-1.65	.31	—		—
Complex karyotype, no/yes	0.29	0.11-0.74	.010	0.28	0.11-0.73	.009
Cytogenetics						
Complex karyotype ≥5	—			—		—
1-2 lesions/normal/del13q	0.37	0-10-1.38	.14	—		—
3-4 lesions/normal/del13q	1.49	0.37-5.99	.57	—		—
TP53 disruption yes/no	1.82	0.64-5.18	.26	—		—
***TP53* and cytogenetics**						
No complex karyotype and *TP53* WT	—			—		—
Complex karyotype and *TP53* WT	3.45	1.09-10.9	.035	—		—
TP53 disruption	2.58	0.82-8.14	.11	—		—

HR, hazard ratio; LDH, lactate dehydrogenase.

CLL[7,9] and that nearly two-thirds of patients with a complex karyotype may have no *TP53* disruption. In contrast to our findings in a previous study,[7] we found that in patients with CLL who received first-line treatment with ibrutinib plus rituximab, the presence of a complex karyotype was associated with worse PFS. The inclusion of unfit patients irrespective of age and, most importantly, the observation that two-thirds of patients with a complex karyotype had major structural abnormalities which have been recently associated with a worse outcome in patients with complex karyotype,[12] may account for the different results in this study. It is noteworthy that a recent analysis suggested that increasing karyotype complexity is independently associated with inferior survival for patients with CLL who were treated with ibrutinib.[13] Furthermore, the ECOG PS for our unfit patients represented an additional prognostic factor that had an unfavorable impact on overall effectiveness of the treatment. In patients with CLL who had comorbidities and who received first-line treatment with venetoclax and obinutuzumab, there were no statistically significant differences in PFS and OS between patients with and without complex karyotype.[9] Interestingly, very promising results have been observed with the combination of ibrutinib and venetoclax in older untreated patients with CLL who have high-risk genomic features and with undetectable bone marrow minimal residual disease in 4 (80%) of 5 patients with a complex karyotype after 12 of the 24 planned cycles of treatment.[8] Similarly, acalabrutinib-obinutuzumab or acalabrutinib monotherapy were recently shown to improve efficacy outcomes compared with chemoimmunotherapy in untreated patients with CLL who had high-risk genomic features, including complex karyotype.[14] These observations are particularly relevant in the era of targeted agents, although longer follow-ups in larger numbers of patients are warranted.

The strength of this analysis includes the duration of follow-up and the detailed genetic and molecular characterization in a homogeneously treated patient population. The relatively limited numbers of patients in specific biomarker subsets suggests that longer follow-up and further studies are required to confirm the impact of cytogenetic and mutational data on PFS and OS[15] and in the refinement of risk stratification in CLL.[13]

In summary, we have shown that in unfit patients with CLL who receive first-line treatment with ibrutinib plus rituximab, the presence of a complex karyotype might represent a biomarker

associated with a worse outcome. Further prospective clinical trials in larger series of patients are warranted to clarify whether patients with a complex karyotype represent a subset of patients with CLL who should be considered for treatment strategies that use combine targeted agents.

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Authorship

Contribution: G.M.R., I.D.G., M.V., F.R.M., R.F., and A.C. conceived and designed the study; A.B., A.M., R.E.G.-J., S.R., C.I., L.C., M.N., N.P., P.M., and A.G. performed biological studies; G.M.R., I.D.G., F.C., G.R., F.A., S.M., P.S., L.T., and M.M. acquired data and provided patient follow-up; G.M.R., I.D.G., A.P., V.A., R.F., and A.C. analyzed and interpreted data; A.P. and V.A. performed statistical analyses; A.C. wrote the first version of the manuscript; and all authors contributed to writing, approving, and reviewing the manuscript.

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ORCID profiles: G.M.R., 0000-0002-8370-5190; R.E.G.-J., 0000-0001-9099-3904; A.P., 0000-0001-8648-885X; V.A., 0000-0003-2782-2752; F.A., 0000-0001-7926-6052; S.M., 0000-0003-2795-6507; L.T., 0000-0003-1222-6149; N.P., 0000-0002-6338-3112; M.V., 0000-0003-1278-604X.

Correspondence: Antonio Cuneo, St Anna University Hospital, Via Aldo Moro, 8, 44124 Cona, Ferrara, Italy; e-mail: cut@unife.it.

Footnotes

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*G.M.R., I.D.G., F.R.M., R.F., and A.C. contributed equally to this work.

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