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LETTER TO THE EDITOR

Long-term benefit of IGHV mutated patients in a real-life multicenter cohort of FCR-treated chronic lymphocytic leukemia

To the Editor,

Fludarabine, cyclophosphamide and rituximab (FCR) has been the standard of care for first-line chronic lymphocytic leukemia (CLL) treatment for more than a decade and the latest European Society for Medical Oncology guidelines still consider FCR a therapeutic option for immunoglobulin heavy-chain variable region gene (IGHV) mutated patients devoid of *TP53* disruption.[1] During the last few years, however, the therapeutic scenario of CLL has changed toward chemo-free strategies with pathway inhibitors.[2,3] Because the median age of patients at CLL diagnosis is 72 years, this leukemia affects mainly elderly individuals and its prevalence is closely linked to the population life expectancy.[4] In the past, the overwhelming majority of CLL cases were diagnosed in western countries where access to novel therapies is rapidly granted to patients with different reimbursement programs. However, a large fraction of the world population resides in middle/low income countries where access to medical care is subject to severe constraints.[5] Due to the steady increase in life expectancy, middle and low income countries are facing an increasing fraction of the cancer burden worldwide.[6] In middle and low income countries the median life expectancy was 66 and 53 years respectively in 2000, and increased to 72 and 64 years respectively in 2020.[7] Therefore, FCR (or bendamustine-rituximab in older patients) remains a viable option in many areas of the world in which pathway inhibitors are not yet available and/or are poorly accessible and affordable.

Exploiting a multicenter, real-life cohort of CLL patients treated with FCR, we previously showed that IGHV mutated patients devoid of *TP53* abnormalities and of 11q deletion can achieve a durable remission.[8] In the present study, we updated the follow-up of 301 CLL patients derived from the initial cohort.[8] At the time of the present analysis, the median follow-up of the cohort is 13.8 years that is doubled compared to our previous analysis and, to the best of our knowledge, represents the longest follow-up for a FCR-treated CLL cohort so far reported.[9,10] The study was approved by the Ethical Committee of the Ospedale Maggiore della Carità di Novara associated with the Università del Piemonte Orientale (study number Comitato Etico 120/19).

The patients' characteristics are reported in Table 1 and are consistent with those of patients treated with FCR with a median age of 61 years and with a median overall survival (OS) of 15.5 years.[9,10]

TABLE 1 Patients' characteristics

Characteristics	Values
Median age	61 (53–65)
Binet stage	
A	36 (12.0%)
B + C	265 (88.0%)
Gender	
Male	206 (68.4%)
Female	95 (31.6%)
IGHV mutational status	
Mutated	98 (32.6%)
Unmutated	203 (67.4%)
FCR model	
Low risk	82 (27.2%)
Intemediate risk	191 (63.5%)
High risk	28 (9.3%)

Abbreviations: FCR, fludarabine, cyclophosphamide and rituximab; IGHV, immunoglobulin heavy-chain variable region gene.

At the current update of follow-up (May 2022), low-risk patients characterized by mutated IGHV and devoid of 17p and 11q deletion continue to benefit from FCR, with 65.7% of patients who are alive 20 years after starting treatment (Figure 1A). In addition, the OS of this subgroup of patients continues to be superimposable to that of the general population matched for age and gender (Figure 1B), confirming that FCR does not associate with an excess of deaths in this category of patients. Conversely, the intermediate-risk group of patients characterized by IGHV unmutated genes and/or 11q deletion, as well as the high-risk group of patients characterized by 17p deletion, are confirmed to display a statistically inferior outcome compared to low-risk patient after FCR, as documented by a median OS of 13.4 years and of 10.0 years, respectively (Figure 1A,C and D). Remarkably, the outcome of IGHV unmutated and/or 11q deleted patients, at the current follow-up, did not significantly differ from that of 17p deleted patients ($p = 0.063$). Regarding secondary malignancies, that represent a significant concern after chemo-

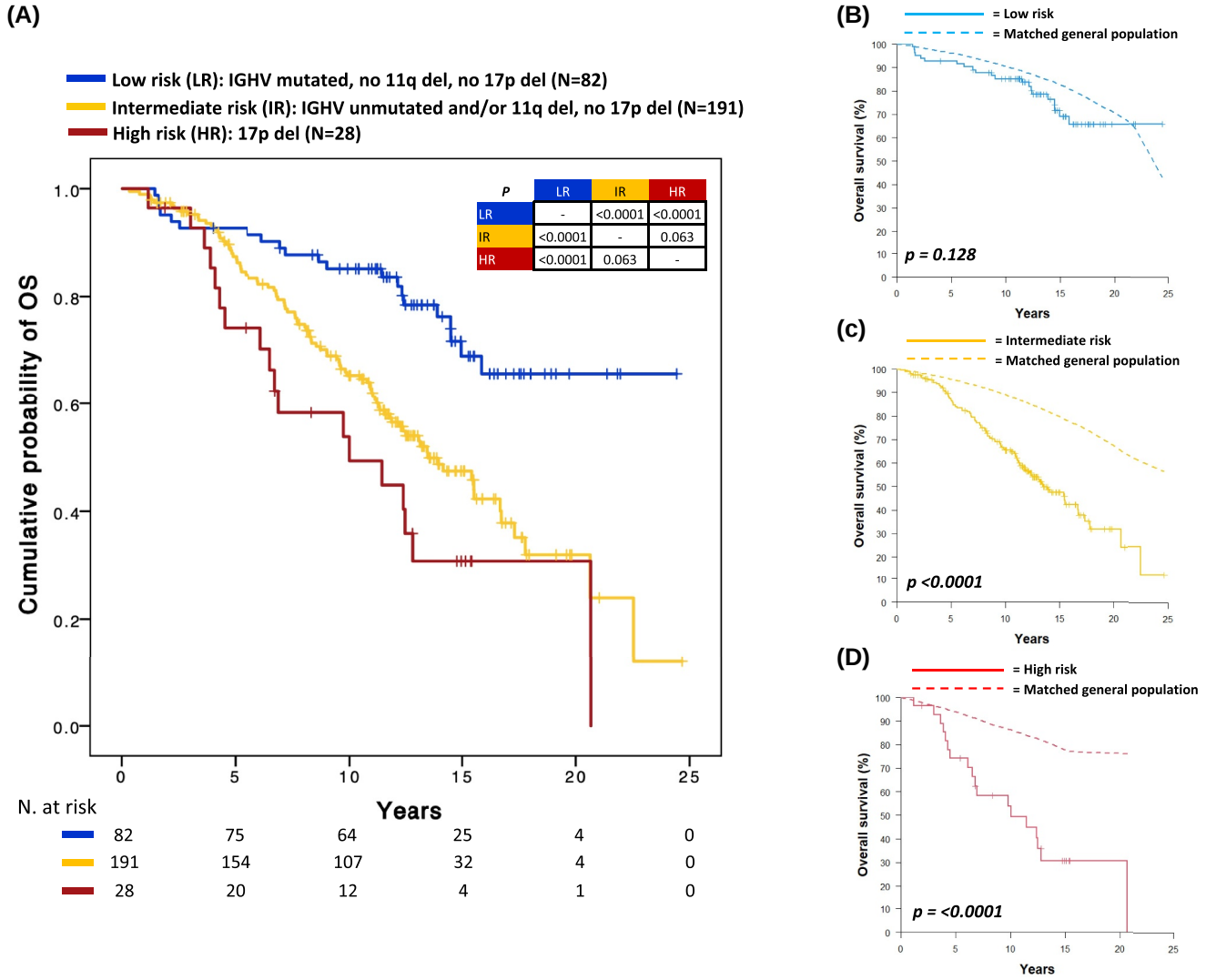

FIGURE 1 Estimates of survival in the Fludarabine, cyclophosphamide and rituximab (FCR) treated cohort. (A) overall survival (OS) estimates in the FCR treated cohort. High-risk cases harboring 17p deletion are color-coded in red. Intermediate-risk cases harboring unmutated IGHV genes and/or 11q deletion in the absence of 17p deletion are color-coded in yellow. Low-risk cases harboring mutated IGHV genes in the absence of 11q and 17p deletion are color-coded in blue. (B-D) Comparison of the OS survival in each risk group with the expected survival in the age, sex, and calendar year of treatment-matched general population. *p*-values are represented adjacent curves.

immunotherapy, they were more frequent ($p = 0.001$) in intermediate and high-risk patients (28.0%) compared to low-risk patients (10.0%) (Figure S1A). The lower prevalence of secondary malignances in low-risk patients may be related to a better disease control after FCR that reduced the need of second-line genotoxic therapies.

Overall, low-risk patients, defined by the presence of mutated IGHV genes in the absence of 17p and 11q deletion, rarely harbor gene mutations that further stratify their outcome.[11] To investigate this issue further, a subgroup of low-risk patients ($N = 35$) with available tumor genomic DNA before FCR start was subjected to a mutational analysis by targeted next generation sequencing using a panel of genes that are known to be frequently mutated in CLL. As expected, high-risk gene mutations, such as *TP53* and *BIRC3* lesions, were identified in only 1 patient each and both mutations were subclonal with a very low variant allele frequency (<2.5%). *SF3B1*, coding for a spliceosome component, was the most frequently mutated gene being recurrently affected in 6 patients (17.1%) but did not associate with a shorter survival. The *EGR2* gene, coding for a transcription factor that also mediates the *TP53* dependent apoptotic pathway, was found to be mutated in 3 patients (8.6%), and further stratifies the outcome of low-risk patients. In fact, *EGR2* mutated patients were associated with a 10-year OS of 0% compared to 87.4% for wild-type patients ($p < 0.001$) (Figure S1B). Remarkably, *EGR2* mutations showed a poor prognostic value only in low-risk patients whereas they had no impact on intermediate and high-risk patients.

Minimal residual disease (MRD) negativity after first line fixed-duration regimens, including both chemo-immunotherapy and venetoclax-obinutuzumab, reflects the depth of response and associates with longer outcome.[12,13] In this FCR treated cohort, we

identified a group of low-risk patients who did not progress at the current follow-up and were available for fresh blood sampling for MRD assessment ($N = 12$). Minimal residual disease was evaluated by immunophenotyping in a reference laboratory (Sapienza University, Rome, Italy) dedicated to MRD analysis in lymphoid malignancies. The median time between MRD sampling and FCR start was 12.3 years (range 8.9–15.4 years). Of these 12 patients, 8 (66.6%) were MRD-negative and therefore may represent a fraction of CLL that could be potentially considered as cured. The median level of MRD positivity of the 4 MRD-positive patients was 0.32% (range 0.05%–22.0%).

Overall, the updated analysis of our FCR-treated cohort confirms that FCR therapy induces long-term responses in fit patients with mutated IGHV and devoid of high-risk molecular features, such as 11q deletion and *TP53* disruption, with survival rates that are superimposable to those of the general population at a prolonged follow-up (13.8 years) from treatment. These findings may be of clinical relevance especially in geographic areas where the access to novel agents is limited for different reasons and in which the cancer burden, including the CLL burden, has been steadily rising and is expected to further increase in the upcoming years.[5,6] In order to identify low-risk patients who benefit the most from FCR, an essential requirement is represented by the molecular analysis of IGHV and *TP53* mutational status coupled to Fluorescent in situ hybridization karyotype. These assays may not be routinely performed in less-resourced countries, challenging an adequate risk stratification for therapy.[5] In this context, the creation of a network that allows to centralize samples for molecular analysis in few specialized and certified laboratories, may favor testing of CLL molecular predictors and enable therapy stratification.[14,15] Optimization of resources in less-resourced countries may thus allow to prioritize access to pathway inhibitors for high-risk patients with unmutated IGHV genes and *TP53* disruption that are refractory to FCR.

AUTHOR CONTRIBUTIONS

Riccardo Moia, Davide Rossi, Robin Foà, Gianluca Gaidano, designed the study, interpreted data, and wrote the manuscript; Riccardo Moia, Riccardo Dondolin, Donatella Talotta, and Samir Mouhssine, performed statistical analysis; Maria Stefania De Propris, performed MRD analysis; Francesca Perutelli, Gianluigi Reda, Veronica Mattiello, Gian Matteo Rigolin, Marina Motta, Jacopo Olivieri, Renato Fanin, Omar Perbellini, Isacco Ferrarini, Francesca Romana Mauro, Ilaria Del Giudice, Luca Laurenti, Annamaria Tomasso, Massimo Gentile, Anna Maria Frustaci, Alessandra Tedeschi, Alessandro Gozzetti, Caterina Stelitano, Carlo Visco, Carol Moreno, Francesco Forconi, Roberto Marasca, and Marta Coscia collected clinical and molecular data and contributed to manuscript revision.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Eichhorst B, Robak T, Montserrat E, et al. Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2021;32(1):23-33. https://doi.org/10.1016/j.annonc.2020.09.019
2. Fischer K, Al-Sawaf O, Bahlo J, et al. Venetoclax and obinutuzumab in patients with CLL and coexisting conditions. *N Engl J Med*. 2019;380(23):2225-2236. https://doi.org/10.1056/nejmoa1815281
3. Shanafelt TD, Wang XV, Hanson CA, et al. *Long-term Outcomes for Ibrutinib-Rituximab and Chemoimmunotherapy in CLL: Updated Results of the E1912 Trial*. Blood; 2022.
4. Teras LR, DeSantis CE, Cerhan JR, Morton LM, Jemal A, Flowers CR. 2016 US lymphoid malignancy statistics by World Health Organization subtypes. *CA Cancer J Clin*. 2016;66(6):443-459. https://doi.org/10.3322/caac.21357
5. Chiattone CS, Gabus R, Pavlovsky MA, Akinola NO, Varghese AM, Arrais-Rodrigues C. Management of chronic lymphocytic leukemia in less-resourced countries. *Cancer J*. 2021;27(4):314-319. https://doi.org/10.1097/ppo.0000000000000533
6. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249. https://doi.org/10.3322/caac.21660
7. The World Bank. Life Expectancy at Birth - Middle Income & Low Income Countries. Accessed August 17, 2022. https://data.worldbank.org/indicator
8. Rossi D, Terzi-di-Bergamo L, De Paoli L, et al. Molecular prediction of durable remission after first-line fludarabine-cyclophosphamide-rituximab in chronic lymphocytic leukemia. *Blood*. 2015;126(16):1921-1924. https://doi.org/10.1182/blood-2015-05-647925
9. Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. *Blood*. 2016;127(2):208-215. https://doi.org/10.1182/blood-2015-06-651125
10. Thompson PA, Tam CS, O'Brien SM, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia. *Blood*. 2016;127(3):303-309. https://doi.org/10.1182/blood-2015-09-667675
11. Diop F, Moia R, Favini C, et al. Biological and clinical implications of BIRC3 mutations in chronic lymphocytic leukemia. *Haematologica*. 2020;105(2):448-456. https://doi.org/10.3324/haematol.2019.219550
12. Wierda WG, Rawstron A, Cymbalista F, et al. Measurable residual disease in chronic lymphocytic leukemia: expert review and consensus recommendations. *Leukemia*. 2021;35(11):3059-3072. https://doi.org/10.1038/s41375-021-01241-1
13. Al-Sawaf O, Zhang C, Lu T, et al. Minimal residual disease dynamics after venetoclax-obinutuzumab treatment: extended off-treatment follow-up from the randomized CLL14 study. *J Clin Oncol*. 2021;39(36):4049-4060. https://doi.org/10.1200/jco.21.01181
14. Agathangelidis A, Chatzidimitriou A, Chatzikonstantinou T, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: the 2022 update of the recommendations by ERIC, the European Research Initiative on CLL. *Leukemia*. 2022;36(8):1961-1968. https://doi.org/10.1038/s41375-022-01604-2
15. Malcikova J, Tausch E, Rossi D, et al. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-update on methodological approaches and results interpretation. *Leukemia*. 2018;32(5):1070-1080. https://doi.org/10.1038/s41375-017-0007-7

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