Received: 2 April 2021 | Revised: 19 May 2021 | Accepted: 20 May 2021
DOI: 10.1002/ajh.26253

Prognostic impact of *KMT2A-AFF1*-positivity in 926 *BCR-ABL1*-negative B-lineage acute lymphoblastic leukemia patients treated in GIMEMA clinical trials since 1996

To the Editor:

The impact of *KMT2A-AFF1* rearrangement in pediatric-like, minimal residual disease (MRD)-based clinical trials and the effect of transplant in *KMT2A-AFF1* ALL are still debated.

By analyzing 926 *BCR-ABL1*-negative ALL treated in GIMEMA (Gruppo Italiano Malattie EMatologiche dell'Adulto) clinical trials since 1996, we documented that *KMT2A-AFF1*-positive ALL - accounting for 10.5% of cases - had a significantly shorter survival than *KMT2A-AFF1*-negative (20.3% vs 45.5%, $p = 0.003$), also after censoring for transplant. Within *KMT2A-AFF1*-positive patients, the only independent prognostic factor was allogeneic stem cell transplant (ASCT, HR: 0.318, $p = 0.002$), that confers a survival advantage to *KMT2A-AFF1*-positive patients.

The prognosis of adult B-lineage acute lymphoblastic leukemia (B-ALL), thought greatly improved over the years, is still suboptimal with survival rates approaching 50% at 5 years. The only subset that witnessed a dramatic improvement of outcome is *BCR-ABL1*-positive ALL that benefited from the introduction of tyrosine kinase inhibitors. Within *BCR-ABL1*-negative ALL, t(4;11)(q21;q23) is the most common chromosomal abnormality, accounting for roughly 10% of adult patients.[1] In ALL the *KMT2A-AFF1* fusion gene, derived from t(4;11)(q21;q23), is the most recurrent rearrangement of the promiscuous *KMT2A* gene and functions as a transcriptional activator.[2,3] *KMT2A-AFF1*/t(4;11)(q21;q23) leukemia is associated with a pro-B immunophenotype and it is recognized by the major cooperative groups as a subset with a particularly poor outcome.[4] For the latter reason, *KMT2A-AFF1*-positive ALL patients are managed more intensively and allocated to allogeneic transplant.[5,6] However, the datasets analyzed so far are too small to draw definitive conclusions on the role of *KMT2A-AFF1* in pediatric-like, minimal residual disease (MRD)-based clinical trials, and on the impact of transplant in this poor prognostic subgroup. To this respect, in the largest study conducted on patients enrolled in the UKALLXII/ECOG2993 clinical trial - including 88 *KMT2A-AFF1*-positive patients – patients undergoing ASCT had a survival advantage in comparison to those who received chemotherapy, though allograft was not an independent factor in multivariate analysis.[7] The PETHEMA group observed a trend towards a longer CR duration in *KMT2A-AFF1* undergoing HSCT vs those receiving chemotherapy.[8]

With regards to MRD-based protocols, the GRAALL study showed that *KMT2A-AFF1* fusion gene retains prognostic significance in a multivariate model - that included MRD as a covariate - for cumulative incidence of relapse.[9] Alongside, Issa et al. analyzed the impact of cytogenetic alterations in roughly 400 *BCR-ABL1*-negative ALL in the context of protocols contemplating MRD quantification.[10] The authors confirmed the negative impact of *KMT2A-AFF1*-positivity on survival but, in a multivariate model, *KMT2A-AFF1* rearrangement was not independently predictive of survival while MRD-positivity retained statistical significance.[10]

To get insights into these issues, in the present study we investigated a large cohort of *BCR-ABL1*-negative B-ALL - as assessed by molecular biology - to evaluate: (1) the incidence and clinico-biological features of *KMT2A-AFF1*-positive ALL; (2) the outcome of *KMT2A-AFF1*-positive in comparison with *KMT2A-AFF1*-negative ALL patients; (3) the clinico-biological parameters that affect *KMT2A-AFF1*-positive patients' prognosis. Between November 1996 and September 2016, 926 *BCR-ABL1*-negative B-ALL patients (median age 34.3 years) were enrolled in the GIMEMA clinical trials LAL0496 (n = 187), LAL2000 (n = 267), LAL0904 (n = 210), LAL1104 (n = 76), LAL1308 (n = 53), LAL1913 (n = 133) (Figure S1, Table S1) with a median follow-up of 25.4 months (range: 0.1–146.9). The clinico-biological features of the cohort of study are summarized in Table S2.

Overall, 97/926 (10.5%) samples harbored the *KMT2A-AFF1* fusion gene, detected by RT-PCR. Molecular biology methods and statistical analyses details are described in supplemental material. The analysis of clinico-biological features at diagnosis revealed that *KMT2A-AFF1*-positive patients had a significantly higher median age (42 vs 32.5 years old, $p < 0.001$), were more likely to be female (60/97 vs 364/829, $p = 0.001$) and had a significantly higher median WBC count (70.7 vs 7.2×10^9/L, $p < 0.001$) than *KMT2A-AFF1*-negative patients (Table S2).

Next, we compared their outcomes. Firstly, the complete remission (CR) rate did not differ between *KMT2A-AFF1*-positive and -negative patients (87.5% vs 81.5%, $p = 0.188$).

The MRD evaluation after induction treatment was available for 197 patients (26 *KMT2A-AFF1*-positive and 171 *KMT2A-AFF1*-negative) enrolled in the most recent protocols (LAL0904, LAL1308, LAL1913, LAL1104) (Table S1, Figure S1). MRD was assessed by Q-RT-PCR in *KMT2A-AFF1*-positive as detailed in supplemental material. With the caveat that the number of patients evaluated for MRD was small, *KMT2A-AFF1*-positive and negative patients did not differ in the achievement of MRD-negativity: indeed, 18/26 (69.2%) *KMT2A-AFF1*-positive and 100/171 (58.5%) *KMT2A-AFF1*-negative patients were MRD-negative at the end of induction.

In spite of this, *KMT2A-AFF1*-positive patients had a significantly shorter survival than *KMT2A-AFF1*-negative in terms of median overall survival (OS, 20.3 vs 45.5 months, $p = 0.003$, Figure 1(A)), disease-free survival (DFS, 9.2 vs 34.3 months, $p < 0.001$) and event-free survival (EFS, 9.5 vs 21.9, $p = 0.03$).

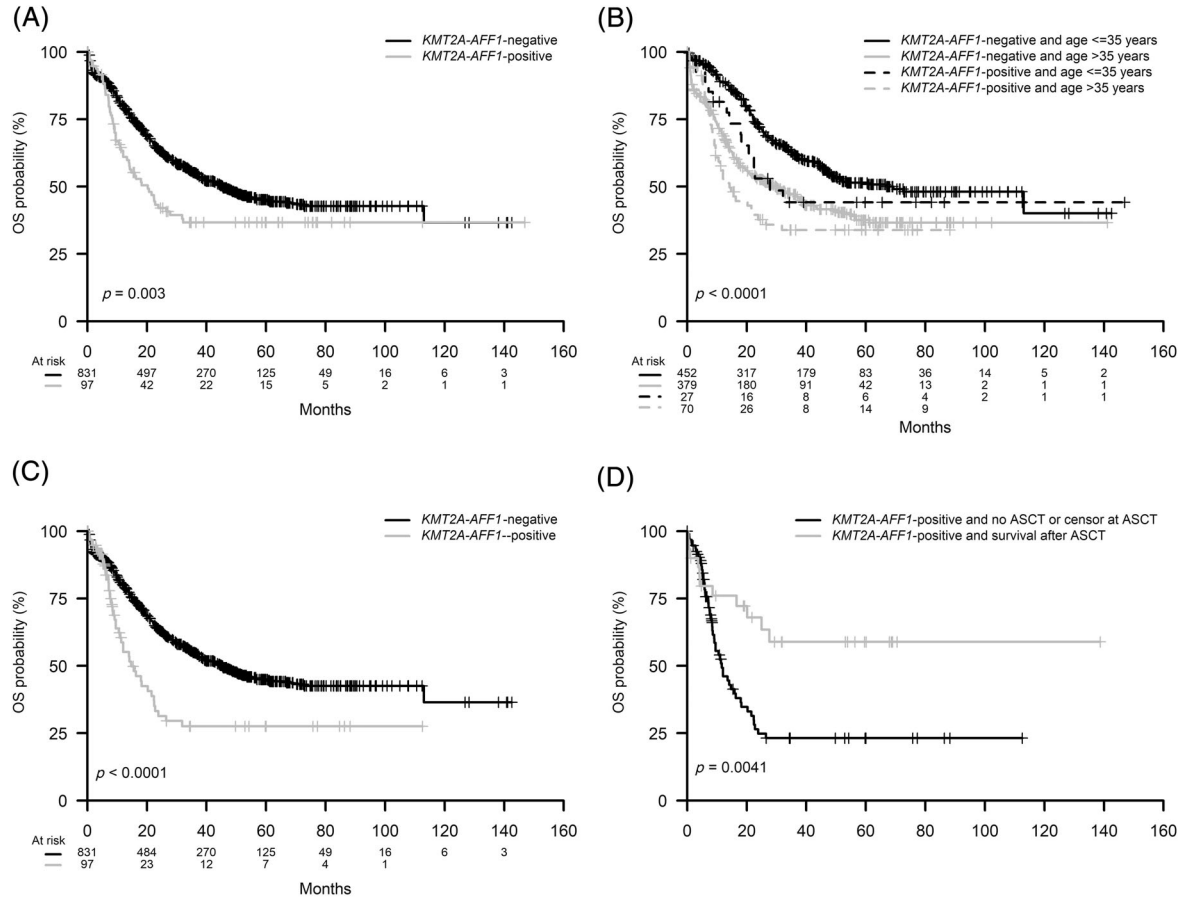

FIGURE 1 Survival curves of *KMT2A-AFF1*-positive and *KMT2A-AFF1*-negative patients. (A), OS; (B), OS stratified for age cohorts; (C), OS censored for ASCT. (D), OS of *KMT2A-AFF1*-positive patients for ASCT using the Simon-Makuch method

Remarkably, within the *KMT2A-AFF1*-positive subset, patients younger and older than 35 years old had a similar survival (27.8 vs 14.2 months, p value = 0.159, Figure 1(B)); while, within *KMT2A-AFF1*-negative ALL, patients younger than 35 years old had a significantly longer survival (69.3 vs 29.6, $p < 0.001$).

Of the *KMT2A-AFF1*-positive patients, 45 underwent transplant: in detail, 34 received an allograft and 11 an autograft. To take into consideration the effect of transplantation procedures, survival analyses were repeated after censoring for transplant. We found that *KMT2A-AFF1*-positive patients maintained a significantly worse median OS (12 vs 46.9 months, $p < 0.001$; Figure 1(C)), DFS (9.24 vs 37.3 months, $p < 0.001$) and EFS (9.5 vs 23.16 months, $p = 0.041$).

The effect of *KMT2A-AFF1*-positivity on OS was then adjusted for relevant clinico-biological parameters (i.e., age, gender, WBC and platelet counts, *TCF3-PBX1*), ASCT and treatment: *KMT2A-AFF1*-positivity retained statistical significance (HR: 1.338, 95%CI 1.006–1.779, $p = 0.045$) together with age and treatment (Table S3A).

When we focused on *KMT2A-AFF1*-positive ALL, by univariate and multivariate analyses, we found that the only independent prognostic factor in *KMT2A-AFF1*-positive ALL was ASCT (HR: 0.477, 95% CI 0.1241–0.946, $p = 0.034$, Table S3B), as highlighted in the Simon-Makuch plot (Figure 1(D)). In the subset of the patients evaluable for MRD (n = 26) we did not find any difference on survival outcome between MRD-negative vs MRD-positive patients.

Of note, when we analyzed *KMT2A-AFF1*-positive long-term survivors (i.e., patients alive at 60 months, n = 15), we found that 12 (80%) had undergone transplant. Age did not prove to be an independent prognostic factor, in keeping with Figure 1(B).

In 2010 Cimino and colleagues[6] described the outcome of 46 *KMT2A-AFF1*-positive ALL treated according to GIMEMA LAL0496 and LAL2000 clinical trials. In the present study, we extended the observation to the entire *BCR-ABL1*-negative B-ALL population and to the subsequent trials, including also those with a pediatric-inspired and MRD-based approach (LAL1308 and LAL1913). Firstly, we considered the entire *BCR-ABL1*-negative B-ALL population and we confirmed that *KMT2A-AFF1*-positive ALL accounts for roughly 10% of *BCR-ABL1*-negative ALL. When we analyzed the clinico-biological features of *KMT2A-AFF1*-positive in comparison with *KMT2A-AFF1*-negative ALL patients we found that *KMT2A-AFF1*-positivity is associated with a higher age, female gender and higher WBC count at diagnosis, in agreement with previously published data.[5]

The evaluation of the outcome showed that a high percentage of *KMT2A-AFF1*-positive patients - not significantly different from *KMT2A-AFF1*-negative patients - achieved a CR. Notwithstanding, *KMT2A-*

AFF1-positive patients had a significantly shorter survival, despite the higher rate of transplanted patients, as emerged after censoring for transplant. By performing a multivariate model on OS we found that *KMT2A-AFF1*-positivity, together with age and treatment, impacted on *BCR-ABL1*-negative ALL patients' outcome.

A similar result was reported by Lafage-Pochitaloff et al.[11] who analyzed a large cohort of *BCR-ABL1*-negative patients treated in the GRAAL-2003/2005 trials: in this study, the only subgroups that displayed a significantly worse outcome were the *KMT2A-AFF1*-positive and 14q32/IGH. The latter result held true also after censoring for transplant.

Next, we focused on the *KMT2A-AFF1*-positive subset and we found that the only parameter that affects the outcome is ASCT. This issue is still a matter of debate: indeed, Marks and colleagues found that *KMT2A-AFF1*-positive patients undergoing ASCT had a longer survival than those treated with chemotherapy but ASCT did not prove to be an independent prognostic factor in a multivariate model.[7] To this regard, during the preparation of the current manuscript, the Acute Leukemia Working Party of the EBMT published the analysis of 151 *KMT2A-AFF1*-positive patients' outcome treated with ASCT.[12] The authors found that survival outcomes and relapse incidence (RI) were similar between *KMT2A-AFF1*-positive and normal karyotype B-ALL, when allografted. Moreover, a negative status of MRD pre-ASCT was the strongest prognostic factor of OS and RI in transplanted *KMT2A-AFF1*-positive patients.

In the current study we also compared different treatments. In particular, the survival of *KMT2A-AFF1*-positive patients treated in the protocol GIMEMA LAL1913 - that envisioned a pediatric-like regimen - did not significantly differ from the previous protocols (LAL0904, LAL2000 and LAL0496), thus meaning that a more intensive treatment does not confer a survival advantage to this high-risk subgroup. Though this matter is still debated,[13] our results are in agreement with the findings reported by Issa et al.[10] who found that *KMT2A-AFF1*-positive patients treated with hyper-CVAD or augmented BFM regimens had a similar dismal survival.

Interestingly, we observed that at 36 months the survival curve reaches a plateau, indicating that if we manage to transplant *KMT2A-AFF1*-positive patients we may give patients a survival advantage. However, in our cohort, a substantial proportion of *KMT2A-AFF1*-positive patients did not undergo ASCT. This event can be partly ascribed to the high percentage of early relapses: indeed, in *KMT2A-AFF1*-positive patients who did not receive ASCT, 23 out of 31 relapses (74%) occurred within 6 months.

Taken together, these findings show that in *KMT2A-AFF1* patients, an intensive treatment must be consolidated with transplant procedures.

One of the limitations of this study is the low number of patients with MRD quantification mainly because the majority of them was enrolled in old protocols not contemplating MRD. This issue made unfeasible a multivariate model adjusted for MRD quantification, reported by other groups.[9,10] Nonetheless, when we compared MRD-negative and MRD-positive *KMT2A-AFF1*-patients we did not find any difference in terms of survival.

Also, we were not able to include in the analysis the rarer *KMT2A* rearrangements because this information was not collected in the older protocols (LAL0496, LAL2000, LAL0904). In the most recent clinical trial - GIMEMA LAL1913 – two patients were *KMT2A-MLLT1*-positive, corresponding to 14.3% of *KMT2A*-rearranged cases.

Alternative strategies for the treatment of *KMT2A-AFF1*-positive patients under investigation are[14]: (i) DOT1L inhibitors that proved safe but moderately efficacious in a phase I trial,[15] (ii) BCL2 inhibitors that were effective in preclinical models of *KMT2A*-leukemia[16,17] (iii) menin-MLL1 inhibitors that induced complete remission or regression in the MLL1-rearranged leukemia models, including patient-derived xenograft (PDX) models[18] and (iv) immunotherapy that is regarded as promising approach.[19] To this respect, we will be able to assess the role of blinatumomab – a bispecific CD19-directed CD3 T cell engager antibody - in the GIMEMA LAL2317 that has recently closed to enrolment.[20] Another intriguing option is the use of chimeric antigen receptor (CAR) T-cells that have been approved for young relapsed/refractory ALL. However, the number of *KMT2A-AFF1*-positive patients enrolled in CAR-T studies is still limited.[21]

CONFLICT OF INTEREST

None of the authors has relevant conflicts of interest related to the content of this work.

AUTHOR CONTRIBUTIONS

A.P. designed the research, analyzed data and wrote the manuscript; M.M. interpreted the results and wrote the manuscript; L.E., A.V. and M.M collected and analyzed samples; S.S. performed statistical analyses; A.M.T., F.A., A.S., M.K., M.B., R.C., C.V., F.F. and G.C. provided clinical data; F.C. collected and managed clinical data; S.C. and R.B. provided clinical data and critically revised the manuscript; P.F. and M.V. designed the research and critically revised the manuscript.

ETHICS STATEMENT

The GIMEMA clinical trials included in the present manuscript were approved and performed in accordance with appropriate regulatory requirements, and with approval of institutional review boards at individual enrolling institutions.

DATA AVAILABILITY STATEMENT

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

Alfonso Piciocchi[1], Monica Messina[1], Loredana Elia[2], Antonella Vitale[2], Stefano Soddu[1], Anna Maria Testi[2], Sabina Chiaretti[2], Marco Mancini[2], Francesco Albano[3], Antonio Spadano[4], Mauro Krampera[5], Massimiliano Bonifacio[5],

Roberto Cairoli[6], Calogero Vetro[7], Florinda Colella[1], Felicetto Ferrara[8], Giuseppe Cimino[9], Renato Bassan[10], Paola Fazi[1], Marco Vignetti[1,2]

[1]*GIMEMA Foundation Franco Mandelli Onlus, Rome, Italy*
[2]*Hematology, Department of Translational and Precision Medicine, Sapienza University, Rome, Italy*
[3]*Hematology, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy*
[4]*Department of Hematology, Transfusion Medicine and Biotechnology, "Santo Spirito" Civic Hospital, Pescara, Italy*
[5]*Department of Medicine, Section of Haematology, University of Verona, Verona, Italy*
[6]*Department of Hematology, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy*
[7]*Division of Hematology, Policlinico Rodolico-S. Marco, Catania, Italy*
[8]*Division of Hematology and Stem Cell Transplantation Unit, Cardarelli Hospital, Naples, Italy*
[9]*Hematology, Department of Translational and Precision Medicine, Sapienza University, ASL Latina, Presidio Ospedaliero Nord - Ospedale Santa Maria Goretti, Latina, Italy*
[10]*Hematology Unit, Ospedale dell'Angelo and Ospedale Ss Giovanni e Paolo, Mestre, Italy*

Correspondence
Monica Messina, GIMEMA Foundation, Rome, Italy, Via Casilina 5, 00182 Rome, Italy.
Email: m.messina@gimema.it

Clinical trial registration: NCT00439920, NCT00537550, NCT00458848, NCT00475280, NCT01156883, NCT02067143.

ORCID

Alfonso Piciocchi https://orcid.org/0000-0001-8648-885X
Monica Messina https://orcid.org/0000-0003-3531-4766
Francesco Albano https://orcid.org/0000-0001-7926-6052
Massimiliano Bonifacio https://orcid.org/0000-0003-0716-1686
Roberto Cairoli https://orcid.org/0000-0001-6372-2623
Calogero Vetro https://orcid.org/0000-0003-1922-5157
Felicetto Ferrara https://orcid.org/0000-0003-3721-5403
Renato Bassan https://orcid.org/0000-0001-8214-2894

REFERENCES

1. Moorman AV. The clinical relevance of chromosomal and genomic abnormalities in B-cell precursor acute lymphoblastic leukaemia. *Blood Rev*. 2012;26(3):123-135.
2. Meyer C, Burmeister T, Gröger D, et al. The MLL recombinome of acute leukemias in 2017. *Leukemia*. 2018;32(2):273-284.
3. Thomas M, Gessner A, Vornlocher HP, Hadwiger P, Greil J, Heidenreich O. Targeting MLL-AF4 with short interfering RNAs inhibits clonogenicity and engraftment of t(4;11)-positive human leukemic cells. *Blood*. 2005;106(10):3559-3566.
4. Chiaretti S, Vitale A, Cazzaniga G, et al. Clinico-biological features of 5202 patients with acute lymphoblastic leukemia enrolled in the Italian AIEOP and GIMEMA protocols and stratified in age cohorts. *Haematologica*. 2013;98(11):1702-1710.
5. Moorman AV, Harrison CJ, Buck GA, et al. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/eastern cooperative oncology group (ECOG) 2993 trial. *Blood*. 2007;109:3189-3197.
6. Cimino G, Cenfra N, Elia L, et al. The therapeutic response and clinical outcome of adults with ALL1(MLL)/AF4 fusion positive acute lymphoblastic leukemia according to the GIMEMA experience. *Haematologica*. 2010;95(5):837-840.
7. Marks DI, Moorman AV, Chilton L, et al. The clinical characteristics, therapy and outcome of 85 adults with acute lymphoblastic leukemia and t(4;11)(q21;q23)/MLL-AFF1 prospectively treated in the UKALLXII/ECOG2993 trial. *Haematologica*. 2013;98(6):945-952.
8. Motlló C, Ribera JM, Morgades M, et al. Frequency and prognostic significance of t(v;11q23)/KMT2A rearrangements in adult patients with acute lymphoblastic leukemia treated with risk-adapted protocols. *Leuk Lymphoma*. 2017;58(1):145-152.
9. Beldjord K, Chevret S, Asnafi V, et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. *Blood*. 2014;123(24):3739-3749.
10. Issa GC, Kantarjian HM, Yin CC, et al. Prognostic impact of pretreatment cytogenetics in adult Philadelphia chromosome-negative acute lymphoblastic leukemia in the era of minimal residual disease. *Cancer*. 2017;123(3):459-467.
11. Lafage-Pochitaloff M, Baranger L, Hunault M, et al. Impact of cytogenetic abnormalities in adults with Ph-negative B-cell precursor acute lymphoblastic leukemia. *Blood*. 2017;130(16):1832-1844.
12. Esteve J, Giebel S, Labopin M, et al. Allogeneic hematopoietic stem cell transplantation for adult patients with t(4;11)(q21;q23) KMT2A/AFF1 B-cell precursor acute lymphoblastic leukemia in first complete remission: impact of pretransplant measurable residual disease (MRD) status. An analysis from the acute leukemia working party of the EBMT. *Leukemia*. 2021. https://doi.org/10.1038/s41375-021-01135-2. [Epub ahead of print].
13. Ludwig WD, Rieder H, Bartram CR, et al. Immunophenotypic and genotypic features, clinical characteristics, and treatment outcome of adult pro-B acute lymphoblastic leukemia: results of the German multicenter trials GMALL 03/87 and 04/89. *Blood*. 1998;92(6):1898-1909.
14. Bassan R, Bourquin JP, DeAngelo DJ, Chiaretti S. New approaches to the Management of Adult Acute Lymphoblastic Leukemia. *J Clin Oncol*. 2018;JCO2017773648. https://doi.org/10.1200/JCO.2017.77.3648. [Epub ahead of print].
15. Stein EM, Garcia-Manero G, Rizzieri DA, et al. The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. *Blood*. 2018;131(24):2661-2669.
16. Benito JM, Godfrey L, Kojima K, et al. MLL-rearranged acute lymphoblastic Leukemias activate BCL-2 through H3K79 methylation and are sensitive to the BCL-2-specific antagonist ABT-199. *Cell Rep*. 2015;13(12):2715-2727.
17. Bourquin JP, Fischer U, Forster M, et al. Genomic and drug response profiling of fatal TCF3-HLF-positive pediatric acute lymphoblastic leukemia identifies recurrent mutation pattern and novel therapeutic options. *Nat Genet*. 2015;47:1020-1029.
18. Klossowski S, Miao H, Kempinska K, et al. Menin inhibitor MI-3454 induces remission in MLL1-rearranged and NPM1-mutated models of leukemia. *J Clin Invest*. 2020;130(2):981-997.
19. Winters AC, Bernt KM. MLL-rearranged Leukemias-an update on science and clinical approaches. *Front Pediatr*. 2017;5:4.

20. Bassan R, Chiaretti S, Della Starza I, et al. Preliminary results of the GIMEMA LAL2317 sequential chemotherapy-blinatumomab front-line trial for newly diagnosed adult Ph-negative B-lineage ALL patients. EHA library. 324522; S114.
21. Britten O, Ragusa D, Tosi S, Kamel YM. MLL-rearranged acute leukemia with t(4;11)(q21;q23)-current treatment options. Is there a role for CAR-T cell therapy? *Cell*. 2019;8(11):1341.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Received: 10 May 2021 | Revised: 21 May 2021 | Accepted: 24 May 2021
DOI: 10.1002/ajh.26257

Interpretation of retrospective data evaluating high-dose methotrexate as central nervous system prophylaxis in diffuse large B-cell lymphoma; caution required

To the Editor:

There remains a lack of consensus regarding the efficacy of high-dose methotrexate (HD-MTX) prophylaxis to reduce the risk of central nervous system (CNS) relapse in newly diagnosed patients with systemic diffuse large B-cell lymphoma (DLBCL). The clinical controversy reflects the lack of high-quality evidence supporting optimal prophylaxis strategies, coupled with the poor outcomes of patients who experience CNS relapse. Interest in this area has been rejuvenated recently following the publication of a number of retrospective studies[1–6] investigating this critical question. We commend Puckrin and colleagues[7] for attempting to address the role of HD-MTX in a retrospective evaluation of their regional practice.

Although the authors acknowledge many of the limitations associated with a study of this nature, we feel that the results presented by Puckrin et al. merit a more nuanced and balanced discussion. This is particularly important when interpreting the significance of rare events, in the context of an uncontrolled modest-sized retrospective dataset exposed to a number of biases.

There are a number of key considerations that we hope offer further perspective and balance to the interpretation of data published in this paper.

1. The criteria used to determine a recommendation for CNS prophylaxis changed during the course of the study period which introduces potential bias from the outset in terms of patient selection and therefore interpretation of results. This is particularly important given that the decision to offer HD-MTX was clinician-choice rather than uniform practice.
2. Notably, adherence to the regional guidance was only 35.3%, conferring a substantial risk of clinician and/or centre bias. This is reflected by more patients in the HD-MTX-treated cohort with very high-risk clinical features (kidney/adrenal involvement: 28.8% vs 19.3%) together with more adverse biological features (eg, double-hit lymphoma 39.1% vs 20.6%). It is recognized that a CNS-IPI score of 4–6 represents a group with substantial heterogeneity of CNS relapse risk, with kidney/adrenal involvement and multiple extra-nodal sites amongst the highest risk sub-groups. We are concerned about the ability of the propensity score analysis to adequately adjust, with appropriate weighting, for all the relevant factors in high-CNS-risk patients.
3. Importantly, insufficient data are presented on the nature of CNS relapse events. Isolated CNS relapse represents a distinct clinical scenario from CNS lymphoma occurring concurrently with systemic DLBCL relapse. These two different clinical scenarios are almost certainly pathobiologically incomparable; isolated CNS relapse is likely to reflect relapse of an occult clone that has taken sanctuary in the CNS early in the course of the disease, whereas CNS disease concurrent with systemic relapse cannot be delineated from the failure to control systemic, often chemotherapy-resistant, disease. This is relevant as the intention of HD-MTX prophylaxis is solely to reduce the risk of isolated CNS relapse and has no known impact on reducing the risk of systemic DLBCL relapse. Hence only patients with isolated CNS relapse can be considered when analyzing the effectiveness or otherwise of HD-MTX prophylaxis. This important issue is often overlooked in published analyses of CNS prophylaxis strategies.[4–7]
4. Amongst the denominator study population (n = 906), there were only 18 isolated CNS events (1.9%); a substantially larger cohort would be required with adequate power to detect reliable differences (if any) associated with the use of HD-MTX. Puckrin and colleagues did not present an a priori statistical analysis plan. The size of the cohort receiving HD-MTX (n = 115) is small given the low event rate and potential for selection bias, reflected in the wide confidence intervals presented in the manuscript. Moreover, only six patients with an *isolated* CNS event had received HD-MTX prophylaxis (5.2%). It is therefore not possible to draw any firm conclusions as to the effectiveness or otherwise of HD-MTX prophylaxis.
5. In their conclusions, the authors suggest that the use of more intensive regimens in patients with high CNS-IPI disease (n = 35 of 326 high-risk patients), with/without consolidative autologous stem cell transplantation (ASCT) in first response (n = 68), may be a more effective strategy to reduce the risk of CNS relapse than HD-MTX alone. However, to attempt to draw this conclusion from the data presented is fraught with bias and confounding factors. Most (73%) of patients undergoing ASCT had also received HD-MTX and the more intensive regimens commonly incorporate HD-MTX and/or other CNS-penetrant agents. Moreover, as acknowledged by the authors in the results section, any analysis of