RESEARCH LETTER

blood advances

TO THE EDITOR:

Haploidentical bone marrow transplantation for AML in remission after TBF conditioning: a long-term follow-up

Anna M. Raiola,[1] Carmen Di Grazia,[1] Alida Dominietto,[1] Stefania Bregante,[1] Sabrina Giammarco,[2] Riccardo Varaldo,[1] Federica Sorà,[2,3] Elisabetta Metafuni,[2] Maria A. Limongiello,[2] Antonella Laudisi,[1] Monica Passannante,[1] Eugenio Galli,[2] Massimiliano Gambella,[1] Simona Sica,[2,3] Andrea Bacigalupo,[2,3] Emanuele Angelucci,[1] and Patrizia Chiusolo[2,3]

[1]Divisione Ematologia e Terapia Cellulare, IRCCS Policlinico San Martino IST, Genova, Italy; [2]Dipartimento di Diagnostica per Immagini, Radioterapia Oncologica ed Ematologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; and [3]Sezione di Ematologia, Dipartimento di Scienze Radiologiche ed Ematologiche, Università Cattolica del Sacro Cuore, Rome, Italy

Allogeneic hemopoietic stem cell transplantation remains an important treatment modality for patients with acute myeloid leukemia (AML).[1-3] The donor is no longer an issue, following the introduction of high-dose posttransplantation cyclophosphamide (PTCY) in Baltimore for haploidentical (HAPLO) grafts.[4,5] The conventional timing of PTCY is day +3+4 after a HAPLO transplant, followed by a calcineurin inhibitor (CNI) and mycophenolate (MMF) on day +5.[4] We have shown that PTCY can be given on days +3+5, with a CNI starting on the day of transplant.[6,7] The European Blood and Marrow Transplantation group compared patients with leukemia receiving PTCY prophylaxis on days +3+4 with those receiving it on days +3+5, with a CNI given respectively on day +5 or on day 0[8]; relapse was significantly reduced in patients receiving PTCY on days +3+5 ($P = .02$), whereas transplant-related mortality (TRM) was comparable; leukemia free survival and graft relapse–free survival (GRFS) was superior for patients receiving PTCY on days +3+5.[8]

The conditioning regimen is another critical component of the transplant platform; we have suggested in a large retrospective study that the combination of thiotepa, busulfan, and fludarabine (TBF) significantly reduces the risk of relapse in patients with AML,[9] compared with a conventional 4-day IV busulfan and fludarabine.

We are now reporting the long-term follow-up of patients with AML in remission aged <65 years, prepared with a full dose myeloablative TBF regimen, and grafted with unmanipulated HAPLO bone marrow, followed by cyclosporine A (CSA) on day 0, MMF on day +1, and PTCY on days +3+5. Patients were grafted in 2 centers: Genova, San Martino, and Roma Gemelli.

This retrospective study was approved by the Ethical Committee Gemelli on 21 September 2023 (ID 5940) and conducted in accordance with the Declaration of Helsinki. It includes 98 patients with AML receiving unmanipulated HAPLO bone marrow graft between 2010 and 2021. Median patients age was 44 years (range, 17-64); median donor age was 34 years (range, 14-67). Patients were in first (n = 78) or second (n = 20) remission. The risk stratification of complete remission 1 (CR1) patients are the following: 59 of 78 CR1 patients (75%) had 1 (n = 37), 2 (n = 18), 3 (n = 4), or 4 (n = 1) risk factors, according to the European LeukemiaNet (ELN) 2017 classification.[10] These included primary induction failure (n = 18), AML with myelodysplastic-related changes (n = 12), leukocytes >30 × 10^9/L (n = 17), Flt3 ITD positivity (n = 15), blastic plasmacytoid–derived neoplasm (n = 2), t(6;9) (n = 1), t(9;22) (n = 1), del 7 (n = 2), complex cytogenetics (n = 6), TP53+ (n = 1), minimal residual disease positivity (n = 12), and therapy-related AML (n = 2).

All patients received the following conditioning regimen: thiotepa (5 mg/kg) on day −6 and −5 (total dose 10 mg/kg), IV busulfan (BU) (3.2 mg/kg) on day −4, −3, and −2 (total dose 9.6 mg/kg), fludarabine

Submitted 22 September 2023; accepted 2 January 2024; prepublished online on *Blood Advances* First Edition 7 February 2024; final version published online 15 April 2024. https://doi.org/10.1182/bloodadvances.2023011759.

Data are available upon request from the corresponding author, Andrea Bacigalupo (andrea.bacigalupo@unicatt.it).

(FLU) (50 mg/m^2) on day −4, −3, and −2 (total dose 150 mg/m^2). BU pharmacokinetics levels were not assessed. GVHD prophylaxis was uniform and consisted of IV CSA 3 mg/kg from day 0 to day +20, adjusting for blood levels (200-400 ng/mL), and then orally until day +180; MMF (15 mg/kg every 12 hours) from day +1 to day +28 and cyclophosphamide (PTCY) 50 mg/kg on day +3 and +5. Granulocyte colony stimulating factor was started on day +6 until neutrophil recovery. The stem cell source was unmanipulated bone marrow for all patients, and the median dose of cells collected and infused on day 0 was 3.1 × 10^8/kg (range, 0.8-6.7). Donors were prioritized on the basis of younger age, whereas specific antibodies were not assessed before transplant. Supportive treatment, as well as diagnosis and therapy of GVHD, was given as per Institutional standard of care. No patients received maintenance therapy after transplant. Statistical analysis was carried out with NCSS 19 for Windows (Kaysville, UT). Disease-free survival (DFS) includes death and relapse as events.

Two patients died before day +20 and could not be evaluated. The cumulative incidence of trilineage engraftment at 100 days, including the 2 early deaths, was 95 of 98 patients (95% confidence interval [CI], 93-100). The median day to a neutrophil count of 0.5 × 10^9/L was day +18 (range, 13-76); for platelets 20 × 10^9/L, it was day +21 (range, 15-84). The cumulative incidence of acute GVHD grade 2 to 4 was 9% (95% CI, 5-17) (Figure 1A). In particular, 60 patients never developed acute GVHD; 9 patients developed GVHD grade 2 (2%) and 2 patients GVHD grade 3 (2%). The cumulative incidence of moderate-severe chronic GVHD was 17% (95% CI, 10-26) (Figure 1B); 15 patients had moderate and 3 patients severe chronic GVHD.

At a median follow-up for surviving patients of 5.8 years (range, 1.8-11), 19 patients (19%) died of transplant-related complications (n = 8) or relapse (n = 11). The overall cumulative incidence of TRM at 10 years is 8% (95% CI, 4-16) (Figure 1C). TRM was 5% for CR1 and 18% for CR2 patients (P = .1). TRM was 0%, 8%, and 13% for patients aged <30 years, 31 to 50 years, and >50 years, respectively (P = .2).

The 10-year CI of relapse is 14% (95% CI, 8-24; Figure 1D); it was 14% for CR1 patients and 15% for CR2 patients (P = .8).

At 10 years, the projected DFS is 75% (95% CI, 66-85) for all patients (Figure 2A); 80% vs 68% for CR1 vs CR2 patients (P = .2). In univariate analysis, age over the median (44 years) was the most significant risk factor of DFS (P = .1). GRFS at 10 years is 70% (95% CI, 61-79) (Figure 2B).

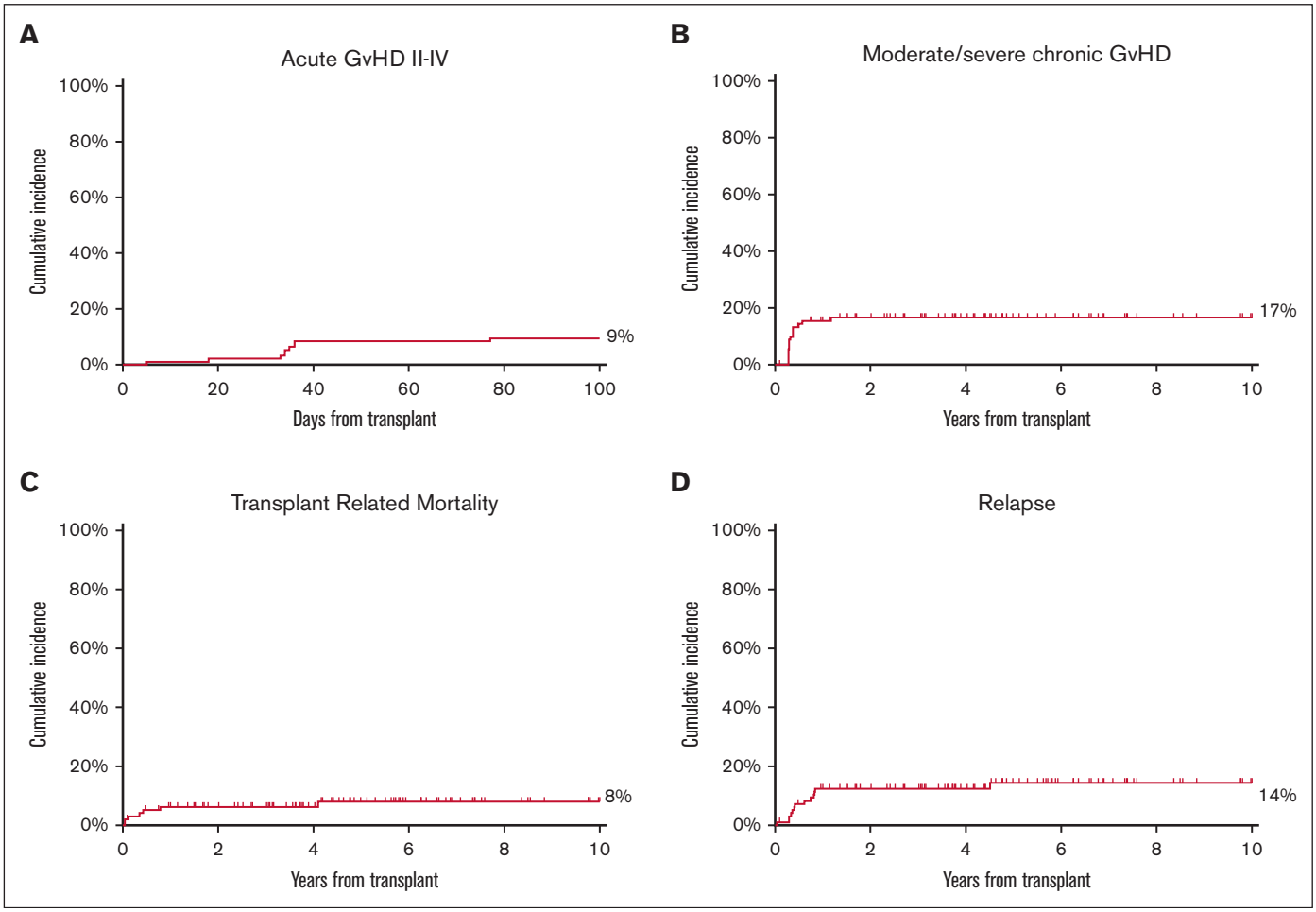

Figure 1. GvHD and competing outcome risks. (A-B) Cumulative incidence of acute GVHD and chronic GVHD (B); (C-D) Cumulative incidence of TRM (C) and relapse (D).

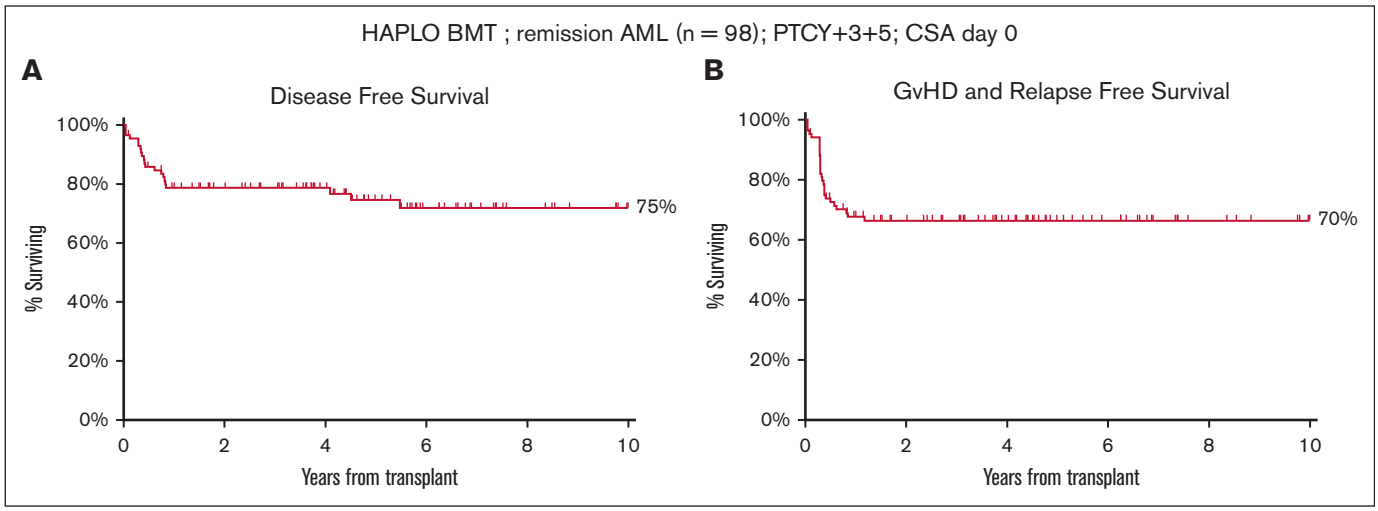

Figure 2. Major survival outcome. (A) DFS of all 98 patients; (B) graft-versus-host and relapse-free survival.

Conclusions

This retrospective study was carried out in patients with AML aged <65 years, tolerating a fully myeloablative TBF regimen, followed by a HAPLO bone marrow graft and GVHD prophylaxis with PTCY day +3+5 and CSA day 0, and MMF day +1. The 14% cumulative incidence of relapse at 10 years compares favorably with a 30% figure, reported with different conditioning regimens, including total body irradiation 12 Gray,[11] total body irradiation 8 Gray,[12] BU cyclophosphamide,[13] BU FLU,[13] and also when using a preparative regimen the week before the conditioning regimen.[14] These studies were conducted with different GVHD prophylaxis ranging from CSA + methotrexate,[11,12] with or without anti-thymocyte globulin,[13] or with alemtuzumab.[13] Despite these difference, the 30% relapse rate seems to be a recurrent figure in the literature, for remission AML posttransplant.

In our study, the rate of acute and chronic GVHD is low, and the TRM projected at 10 years is 8%; this may be due to the choice of using exclusively bone marrow as a stem cell source, as originally suggested by the Baltimore group.[4] Finally, we have chosen a modified PTCY schedule, given on days +3+5 with CSA starting on day 0 and MMF on day +1, before PTCY; this may further lead to improved graft-versus-leukemia, because on day +3, some alloreactive T cells are already under the control of CSA and are free to replicate after day +5.

The combination of reduced TRM, reduced relapse, as compared with data in the literature, results in a DFS, projected at 10 years of 75% and a GRFS of 70%.

Limiting factors are the retrospective nature of the study and the fact that 73 of the 98 patients had already been reported in the context of a larger group of >600 patients, with different conditioning regimens and stem cell sources.[15] On the other hand, in this study, we focus exclusively on long-term outcome of remission AML, receiving an identical conditioning regimen (TBF), marrow as a stem cell source, HAPLO donors, and same GVHD prophylaxis for all patients, which we report here for the first time.

We believe the choice of the stem cell source, conditioning regimen, and GVHD prophylaxis, all contribute to the very encouraging outcome.

Acknowledgments: This work was supported by an Associazione Italiana Ricerca contro il Cancro (Milan) grant (A.B.) and Fondazione Ricerca Trapianto Midollo Osseo (Genova).

Contribution: A.B., A.M.R., and P.C. designed the study and wrote the manuscript, and all authors provided clinical care and reviewed the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: A.D., 0000-0001-9143-3714; S.G., 0000-0002-1382-5496; F.S., 0000-0002-9607-5298; E.M., 0000-0002-4780-1273; E.G., 0000-0002-2839-916X; A.B., 0000-0002-8169-7289; E.A., 0000-0002-6512-6080; P.C., 0000-0002-1355-1587.

Correspondence: Andrea Bacigalupo, Hematology Department, Fondazione Policlinico Universitario A. Gemelli, Largo Gemelli 8, Rome 00168, Italy; email: andrea.bacigalupo@unicatt.it.

References

1. Loke J, Buka R, Craddock C. Allogeneic stem cell transplantation for acute myeloid leukemia: who, when, and how? *Front Immunol.* 2021; 12:659595.
2. Kaleka G, Schiller G. Immunotherapy for acute myeloid leukemia: allogeneic hematopoietic cell transplantation is here to stay. *Leuk Res.* 2022;112:106732.
3. Hilberink J, Hazenberg C, van den Berg E, et al. Not type of induction therapy but consolidation with allogeneic hematopoietic cell transplantation determines outcome in older AML patients: a single center experience of 355 consecutive patients. *Leuk Res.* 2019;80: 33-39.
4. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematological malignancies using

nonmyeloablative conditioning regimen and high dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14(6):641-650.

5. Passweg JR, Baldomero H, Bader P, et al. Use of haploidentical stem cell transplantation continues to increase: the 2015 European Society for Blood and Marrow Transplant activity survey report. *Bone Marrow Transplant*. 2017;52(6):811-817.
6. Raiola AM, Dominietto A, Ghiso A, et al. Unmanipulated haploidentical bone marrow transplant and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning. *Biol Blood Marrow Transplant*. 2013;19(1):117-122.
7. Chiusolo P, Bug G, Olivieri A, et al. A modified post-transplant cyclophosphamide regimen, for unmanipulated haploidentical marrow transplantation, in acute myeloid leukemia: a multicenter study. *Biol Blood Marrow Transplant*. 2018;24(6):1243-1249.
8. Ruggeri A, Labopin M, Battipaglia G, et al. Timing of post-transplantation cyclophosphamide administration in haploidentical transplantation: a comparative study on behalf of the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant*. 2020;26(10):1915-1922.
9. Sora F, Grazia CD, Chiusolo P, et al. Allogeneic hemopoietic stem cell transplants in patients with acute myeloid leukemia (AML) prepared with busulfan and fludarabine (BUFLU) or thiotepa, busulfan, and fludarabine (TBF): a retrospective study. *Biol Blood Marrow Transplant*. 2020;26(4):698-703.
10. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
11. Clift RA, Buckner CD, Appelbaum FR, Sullivan KM, Storb R, Thomas ED. Long-term follow-up of a randomized trial of two irradiation regimens for patients receiving allogeneic marrow transplants during first remission of acute myeloid leukemia. *Blood*. 1998;92(4):1455-1456.
12. Fasslrinner F, Schetelig J, Burchert A, et al. Long-term efficacy of reduced-intensity versus myeloablative conditioning before allogeneic haemopoietic cell transplantation in patients with acute myeloid leukaemia in first complete remission: retrospective follow-up of an open-label, randomised phase 3 trial. *Lancet Haematol*. 2018;5(4):e161-e169.
13. Rambaldi A, Grassi A, Masciulli A, et al. Busulfan plus cyclophosphamide versus busulfan plus fludarabine as a preparative regimen for allogeneic haemopoietic stem-cell transplantation in patients with acute myeloid leukaemia: an open-label, multicentre, randomised, phase 3 trial. *Lancet Oncol*. 2015;16(15):1525-1536.
14. Craddock C, Jackson A, Loke J, et al. Augmented reduced-intensity regimen does not improve postallogeneic transplant outcomes in acute myeloid leukemia. *J Clin Oncol*. 2021;39(7):768-778.
15. Raiola AM, Angelucci E, Sica S, Bacigalupo A. Haploidentical bone marrow transplants with post transplant cyclophosphamide on day + 3 + 5: the Genova protocol. *Blood Rev*. 2023;62:101031.