Histamine dihydrochloride and low-dose interleukin-2 has anti-leukemic efficacy in *NPM1*-mutated and myelomonocytic/monocytic acute myeloid leukemia

Immunotherapy with histamine dihydrochloride and low-dose interleukin-2 (HDC/IL-2) has been reported to reduce the risk of relapse in younger adults with acute myeloid leukemia (AML)[1] but the benefit of HDC/IL-2 in molecular subgroups of AML is not known. This study reports analyses of minimal/measurable residual disease (MRD)[2] in serial bone marrow (BM) and peripheral blood (PB) samples of patients with *NPM1*-mutated or core binding factor (CBF) AML. Data from a registry comprising patients who received immunotherapy with HDC/IL-2 in the post-consolidation phase were compared with outcomes of matched contemporary patients in German-Austrian registries. Results point towards reduction of molecular relapse among HDC/IL-2-treated patients with leukemic cells of monocytic origin and carrying *NPM1* mutations.

Patients with AML frequently attain morphological disappearance of leukemic cells and restored normal hematopoiesis (defined as complete remission, CR)[2] after initial induction chemotherapy. Despite ensuing cycles of consolidation and/or maintenance chemotherapy, hematological relapses in CR are common with high mortality.[3] Patients at high or intermediate risk of relapse may benefit from allogeneic stem cell transplantation (allo-SCT) in the early phase of treatment[4] but upfront allo-SCT is mostly not practiced in patients at lower relapse risk, including *NPM1*-mutated or CBF-AML (carrying *CBFB-MYH11* or *RUNX1-RUNX1T1* rearrangements). Combinatorial immunotherapy with HDC/IL-2 aims to achieve immune-mediated elimination of residual leukemic cells in patients who are not eligible for allo-SCT.[1] HDC/IL-2 is approved within the European Union for remission maintenance with preferential efficacy in patients <60 years old.[5] *Post hoc* analyses of phase III trial results suggested pronounced relapse prevention in (i) AML of French-American-British (FAB) classes M4/M5 (myelomonocytic/monocytic AML),[6] (ii) patients who achieve CR after the first course of chemotherapy,[7] and (iii) patients with normal karyotype AML.[8,9] The past decennia have seen the implementation of molecular analysis of residual leukemia in CR for prognostication and for supporting treatment decisions. Assessment of MRD in BM or PB has proven useful to determine the efficiency of upfront chemotherapy and to detect arising or increasing leukemia in the CR phase. Here we report results from a German/Austrian registry of AML patients in first CR with *NPM1*-mutated or CBF-AML who were serially monitored for MRD before, during and after treatment with HDC/IL-2. or comparison with outcomes among contemporary controls, matched patient data were retrieved from the AMLSG BiO, AML-CG 2004 and AML-CG 2008 registries.[10,11] Matching was performed by an independent party that did not have access to outcome data and included type of AML, risk group by World Health Organization criteria, French-American-British (FAB) class of leukemic cells and source of MRD at first analysis. Planned matching for previous chemotherapy was, however, not possible as patients treated with HDC/IL-2 had received a median of two previous cycles of intensive chemotherapy (induction and consolidation) *versus* five in the matched control group (P<0.0001, t test). Prior allo-SCT was an exclusion criterion. Quantitative assessment of the MRD markers *NPM1*^{mut}, *CBFB-MYH11* and *RUNX1-RUNX1T1* was performed by standardized real-time polymerase chain reaction. Sampling of BM and PB for MRD assessment was recommended every third month after the completion of chemotherapy. Patients (N=14) and matched controls (N=21) were MRD-negative at the end of previous chemotherapy. Patient characteristics are summarized in Table 1. The study

Table 1. Patient characteristics.

Characteristics	HDC/IL-2[a] N=14	Matched controls N=21
Sex, N (%) Men Women	 8 (57) 6 (43)	 10 (48) 11 (52)
Age in years, median (range)	45 (19-59)	46 (23-60)
NPM1^{mut}, N (%)	10 (71)[c]	15 (71)
CBF-AML[b], N (%)	4 (29)	6 (29)
FAB class, N (%) M1 M4/M5	 5 (36) 9 (64)	 7 (33) 13 (62)
Prior induction cycles, median (mean)	1 (1.1)[d]	2 (1.7)[f]
Prior consolidation cycles, median (mean)	1 (1.6)[e,h]	3 (3.2)[g,h]

[a]Histamine dihydrochloride/low-dose interleukin-2 (HDC/IL-2). [b]Core binding factor acute myeloid leukemia (CBF-AML); [c]including *FLT3*-ITD (N=4; 29%). [d]Sequential high-dose cytarabine and mitoxantrone (S-HAM, 71%); thioguanine, cytarabine and daunorubicin/S-HAM (TAD-9/S-HAM, 7%); or 3+5+7 (21%) induction chemotherapy. For further information, see Braess *et al.*[11] [e]Thioguanine, cytarabine, and daunorubicin (TAD-9, 79%) or high-dose cytarabine (HD-AraC; 21%). [f]Six control patients (29%) received 1 induction cycle with S-HAM. The remaining 15 patients (71%) received double induction with TAD followed by HAM on day 21. For further detail, see Braess *et al.*[11] [g]TAD-9 consolidation treatment. For further information, see Braess *et al.*[11] [h]Four HDC/IL-2 patients (29%) and 6 control patients (29%) also received low-dose maintenance chemotherapy. FAB: French-American-British.

was approved by ethi cal committees at participating sites (Köln, #12-111, approval date October 22, 2013; Ulm, #207-12, approval date August 8, 2012; Wien, #1384/2013, approval date June 10, 2013), all patients gave written informed consent, and the study was performed according to the Declaration of Helsinki.

The primary endpoint was time from completion of previous chemotherapy to switch from MRD negativity to positivity ("molecular relapse") in HDC/IL-2-treated patients *versus* matched controls. The secondary endpoint was leukemia-free survival (LFS) where events were defined as hematological relapse or death whichever occurred first. Sensitivity analyses included the proportion of patients with molecular relapse among all patients, in patients with *NPM1*-mutated AML and in AML of FAB M4/M5 morphology. Analysis was performed in a predefined group of patients ≤60 years old, aligning with the reported efficacy of HDC/IL-2 in reducing hematological relapse in younger patients.[1] *P* values were calculated using the log-rank test. Patients in the registry received the same HDC/IL-2 regimen as in the phase III trial.[1] Treatment with HDC/IL-2 was started at a median of 71 (range, 26-122) days after the last chemotherapy and nine of 14 patients completed the prescribed regimen of HDC/IL-2 with early termination of maintenance therapy mainly because of early molecular relapse. One patient in the HDC/IL-2 arm and two in the control arm proceeded to allo-SCT after molecular or hematological relapse.

We observed a trend towards reduced incidence of molecular relapse in HDC/IL-2-treated patients *versus* controls (Figure 1A; N=35; *P*=0.08 [primary endpoint]). There were no LFS events in the HDC/IL-2 group (N=14) *versus* four LFS events (2 deaths) among matched controls (N=21; *P*=0.12 [secondary endpoint]). All LFS events in the control arm were preceded by molecular relapse. Molecular relapse tended to be reduced by HDC/IL-2 within the group of *NPM1*-mutated AML (Figure 1B; N=25; *P*=0.07) and in corresponding patients with FAB class M4 or M5 (Figure 1C; N=15; *P*=0.03).

In the previous phase III trial, HDC/IL-2 was reported to prevent late rather than early hematological relapse; this trend was evident in normal karyotype AML, in which few patients relapsed beyond 6-9 months after commencing immunotherapy.[8] The registry data aligned with these previous findings in that HDC/IL-2 tended to prevent late but not early molecular relapse. There were thus no molecular relapses among HDC/IL-2-treated patients with *NPM1*-mutated AML beyond 6 months *versus* 64% late molecular relapses among matched controls (Figure 1D; N=22; *P*=0.009). These results support that HDC/IL-2 may exert anti-leukemic efficacy in younger AML patients with *NPM1*-mutated leukemia, including AML of myelomonocytic/monocytic morphology. The favor-

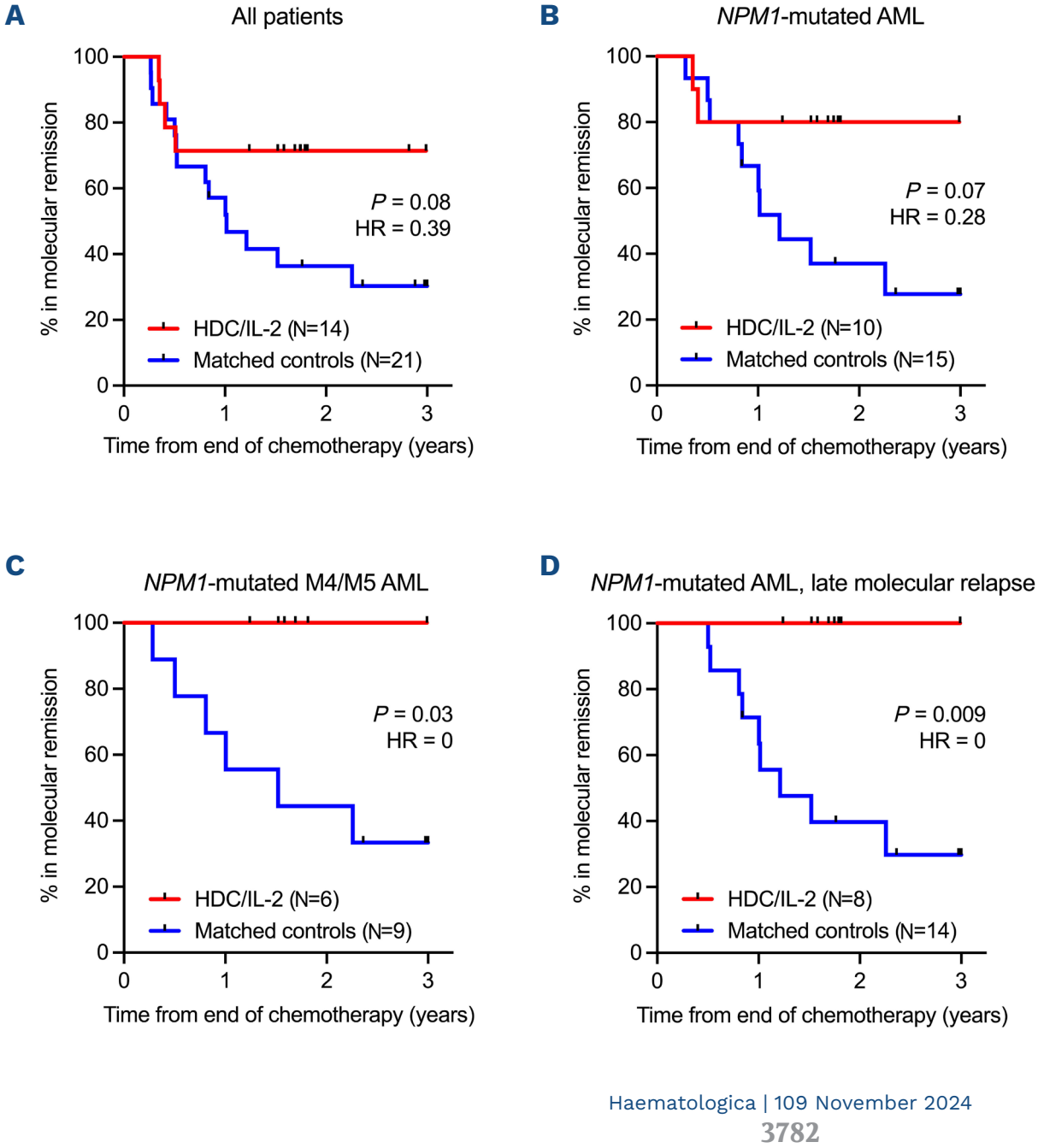

Figure 1. Efficacy of histamine dihydrochloride and low-dose interleukin-2 for prevention of molecular relapse in acute myeloid leukemia in a registry analysis. Analysis of the impact of histamine dihydrochloride and low-dose interleukin-2 (HDC/IL-2) on switch from minimal/measurable residual disease (MRD) negativity to positivity ("molecular relapse") in (A) all patients in the registry, (B) patients with *NPM1*-mutated acute myeloid leukemia (AML), and (C) patients with *NPM1*-mutated myelomonocytic/monocytic (FAB class M4/M5) AML. (D) Impact of HDC/IL-2 on late molecular relapse (occurring later than 6 months after the end of chemotherapy) in patients with *NPM1*-mutated AML. Statistics by the log-rank test. HR: hazard ratio.

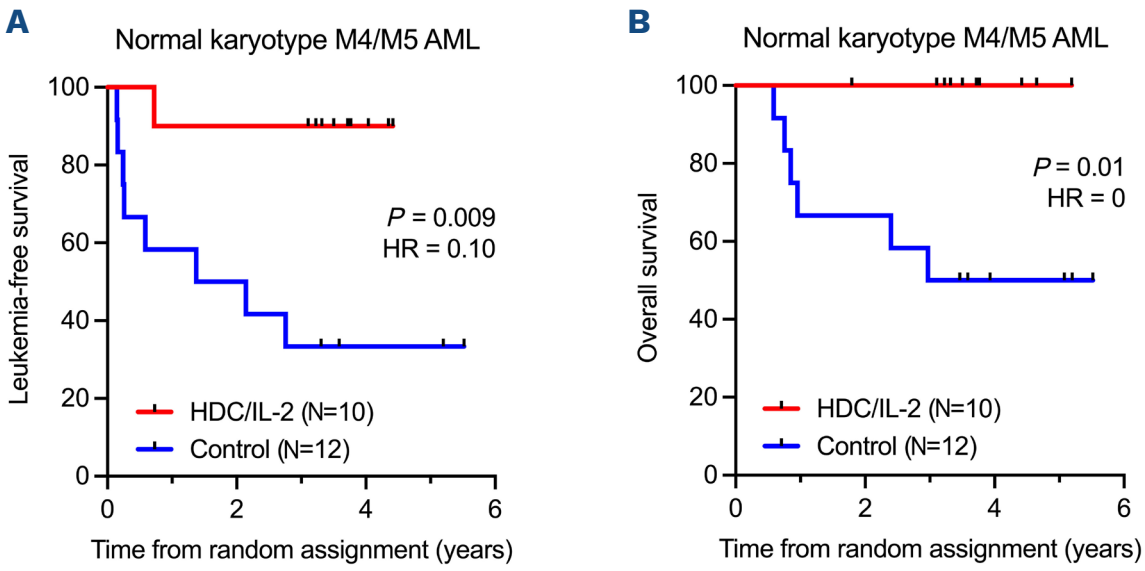

Figure 2. Efficacy of histamine dihydrochloride and low-dose interleukin-2 for relapse prevention in patients with normal karyotype M4/M5 acute myeloid leukemia in a phase III trial. *Post hoc* analysis of the impact of histamine dihydrochloride and low-dose interleukin-2 (HDC/IL-2) on leukemia-free survival and overall survival in patients with normal karyotype (devoid of chromosomal aberrations) myelomonocytic/monocytic (French-American-British class M4/M5) acute myeloid leukemia (AML) in a phase III trial. Patients were in first complete remission and below 60 years of age. Statistics by the log-rank test. HR: hazard ratio.

able outcome in the HDC/IL-2 arm was achieved despite that HDC/IL-2-treated patients had received fewer cycles of intensive chemotherapy than their matched controls.

NPM1 mutations accumulate in patients with normal karyotype AML. Within the group of registry patients with *NPM1*-mutated AML receiving HDC/IL-2, eight of nine evaluable patients had AML of normal karyotype, four of eight were classified as low risk, and four of eight as intermediate risk (co-mutated *FLT3*-ITD, N=3; aberrant karyotype, N=1). In contrast to other prevalent mutations such as mutated *FLT3* or *CEBPA*, *NPM1* mutations also accumulate in FAB class M4/M5 AML.[12-14] In order to capture the best available comparator to the registry results, we mined the previous phase III database for patients with normal karyotype FAB class M4/M5 AML randomly assigned to receive HDC/IL-2 or standard of care (control). In the phase III trial, there was a significant benefit of HDC/IL-2 in terms of LFS and OS in this subgroup (Figure 2).

The small sample size and the inherently exploratory nature of these results should be emphasized, and further studies are required to define the benefit of HDC/IL-2 in distinct subgroups of AML. With these reservations, the results point towards anti-leukemic efficacy of HDC/IL-2 in *NPM1*-mutated AML and in AML of myelomonocytic/monocytic morphology.

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Contributions

MF, WH and WRS participated in designing and performing the study. MSN, FBT, AM and KH compiled and analyzed results and drafted a first manuscript version. All authors participated in writing the final manuscript.

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Data-sharing statement

Data used to support the findings of the registry study are available from the corresponding author upon request.

References

1. Brune M, Castaigne S, Catalano J, et al. Improved leukemia-free survival after postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: results of a randomized phase 3 trial. Blood. 2006;108(1):88-96.
2. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.
3. Forman SJ, Rowe JM. The myth of the second remission of acute leukemia in the adult. Blood. 2013;121(7):1077-1082.
4. DeWolf S, Tallman MS, Rowe JM, Salman MY. What influences the decision to proceed to transplant for patients with AML in first remission? J Clin Oncol. 2023;41(29):4693-4703.
5. European Medicines Agency. Ceplene. https://www.ema.europa.eu/en/medicines/human/EPAR/ceplene. Accessed August 18, 2023.
6. Aurelius J, Martner A, Brune M, et al. Remission maintenance in acute myeloid leukemia: impact of functional histamine H2 receptors expressed by leukemic cells. Haematologica. 2012;97(12):1904-1908.
7. Nilsson MS, Hallner A, Brune M, et al. Complete remission after the first cycle of induction chemotherapy determines the clinical efficacy of relapse-preventive immunotherapy in acute myeloid leukaemia. Br J Haematol. 2020;188(4):e49-e53.
8. Nilsson MS, Hallner A, Brune M, et al. Immunotherapy with HDC/IL-2 may be clinically efficacious in acute myeloid leukemia of normal karyotype. Hum Vaccines Immunother. 2020;16(1):109-111.
9. Nilsson MS, Martner A, Thorén FB, Hellstrand K. Preferential efficacy of remission maintenance with HDC/IL-2 in age groups of patients with chemoresponsive or normal karyotype AML. Blood. 2023;142(Suppl 1):732.
10. Braess J, Spiekermann K, Staib P, et al. Dose-dense induction with sequential high-dose cytarabine and mitoxantone (S-HAM) and pegfilgrastim results in a high efficacy and a short duration of critical neutropenia in de novo acute myeloid leukemia: a pilot study of the AMLCG. Blood. 2009;113(17):3903-3910.
11. Braess J, Amler S, Kreuzer KA, et al. Sequential high-dose cytarabine and mitoxantrone (S-HAM) versus standard double induction in acute myeloid leukemia-a phase 3 study. Leukemia. 2018;32(12):2558-2571.
12. Pasqualucci L, Liso A, Martelli MP, et al. Mutated nucleophosmin detects clonal multilineage involvement in acute myeloid leukemia: impact on WHO classification. Blood. 2006;108(13):4146-4155.
13. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood. 2001;98(6):1752-1759.
14. Preudhomme C, Sagot C, Boissel N, et al. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). Blood. 2002;100(8):2717-2723.