Benefit of intermediate-dose cytarabine-containing induction in molecular subgroups of acute myeloid leukemia

The outcome of acute myeloid leukemia (AML) is affected by disease characteristics as well as treatment regimens.[1-3] In the CALGB8525 trial, patients with core binding factor (CBF)-positive leukemia benefited from consolidation with a high dose of cytarabine.[4] More recently, high-dose daunorubicin (60-90 mg/m^2) has become widely used.[5,6] High-dose daunorubicin confers a favorable prognosis for patients with *NPM1* mutations.[1,7,8]

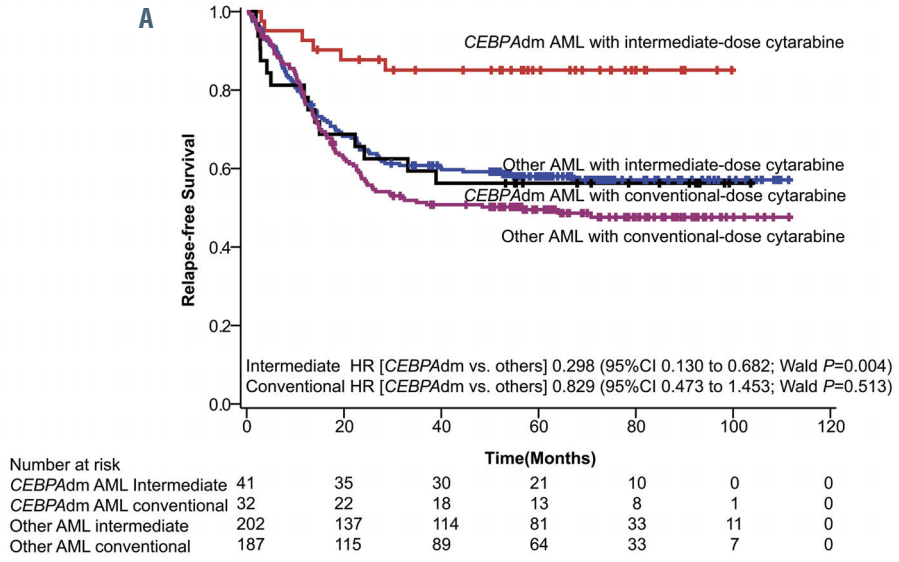
Higher-dose cytarabine was also introduced into AML induction therapy.[3,9] Recently, we investigated the role of intermediate-dose cytarabine in induction therapy of AML and found that the introduction of intermediate-dose cytarabine, combined with daunorubicin and omacetaxine mepesuccinate, improved outcomes in patients with new-diagnosed AML.[2] Overall, 591 patients aged 15 to <55 years with *de novo* newly-diagnosed AML were enrolled in our study, registered at *www.chictr.org.cn* (trial identifier: ChiCTR-TRC-10001202), as described in detail in our previous report.[2] The characteristics of the patients at study entry were included in that report.[2] The distribution of the cytogenetic and mutation subgroups is shown in *Online Supplementary Table S1*. Eligible patients were randomly-assigned to conventional-dose cytarabine (100 mg/m^2/day on days 1-7 as a 12-h intravenous infusion) or intermediate-dose cytarabine (100 mg/m^2/day on days 1-4 as a 12-h intravenous infusion and 1 g/m^2 every 12 h as a 3-h intravenous infusion on days 5-7). Patients also received daunorubicin (40 mg/m^2/day on days 1-3) and omacetaxine mepesuccinate (2 mg/m^2/day on days 1-7) (see the *Online Supplementary Materials and Methods* for details). Here we updated results with longer follow-up and focused on the benefit of intermediate-dose cytarabine induction in molecular subgroups of AML. The median follow-up time of survivors in the current report was 70 months (range, 5-115 months).

In total, 107 of 591 patients underwent allogeneic transplantation in first complete remission (CR1). With longer follow-up, the induction regimen with intermediate-dose cytarabine improved relapse-free survival (RFS), event-free survival (EFS), and overall survival (OS) in the entire cohort compared with outcomes achieved with conventional-dose cytarabine (*Online Supplementary Figure S1*), as before.[2] The intermediate-dose cytarabine still improved RFS, EFS, and OS in patients with intermediate-risk cytogenetics (*Online Supplementary Table S2*). Intermediate-dose cytarabine produced better RFS and EFS in patients with favorable cytogenetics in univariate and multivariable analyses, as shown in *Online Supplementary Table S2* and *Online Supplementary Figure S2*. However, intermediate-dose cytarabine was not associated with better OS, despite the longer follow-up, in patients with favorable cytogenetics. We were unable to determine the benefit of intermediate-dose cytarabine in the adverse cytogenetic cohorts due to small sample sizes.

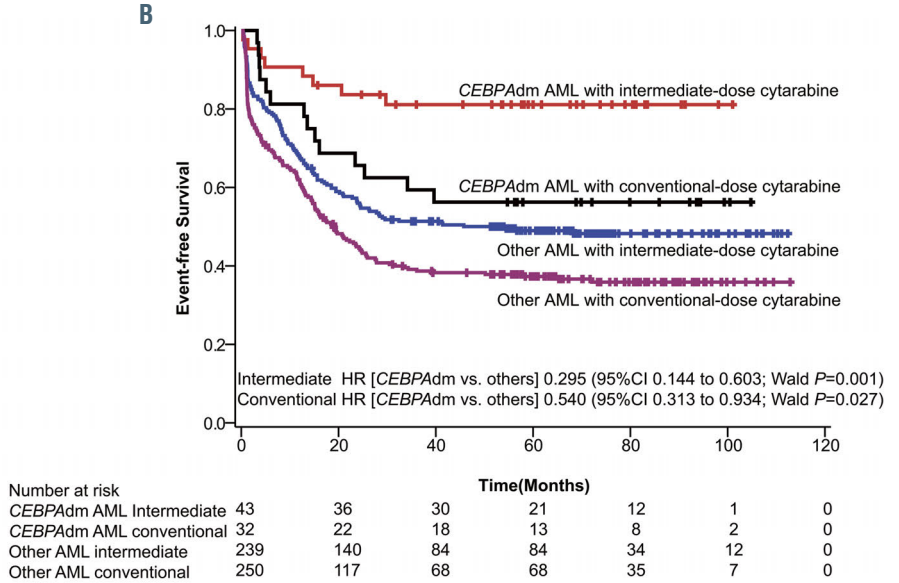
Overall, there were 75 patients with *CEBPA* double mutations (*CEBPA*dm) in our cohort, including 32 in the conventional-dose group and 43 in the intermediate-dose group. Intermediate-dose cytarabine did not increase the complete remission rate in patients with *CEBPA*dm (95% and 100% in the intermediate-dose and conventional-dose cytarabine groups, respectively; *P*=0.504). Intermediate-dose cytarabine did, however, produce better RFS and EFS rates and showed a marked tendency to improve the OS of patients with *CEBPA*dm in both univariate and multivariable analyses, as shown in *Online Supplementary Table S2*. Five-year RFS, EFS, and OS rates were 85%, 81%, and 88% in the intermediate-dose compared with 56%, 56%, and 68% in the conventional-dose group, respectively (Figure 1). In total, 13 of 75 (17%) patients with *CEBPA*dm AML underwent allogeneic transplantation in CR1, including five of 32 (16%) in the conventional-dose group and eight of 43 (19%) in the intermediate-dose group. To analyze results in the absence of any possible contributory effect of transplantation, patients were censored at the time of transplantation in CR1. Patients with *CEBPA*dm AML exposed to intermediate-dose cytarabine achieved an increase in 5-year RFS, censored at the date of transplantation, from 56% to 83% (hazard ratio [HR], 0.313; 95% confidence interval [95% CI]: 0.119-0.824; Wald *P*=0.019) (*Online Supplementary Figure S3*). Intermediate-dose cytarabine showed a tendency to increase EFS and OS rates, censored at the date of transplantation, from 58% to 79% (HR, 0.420; 95% CI: 0.174-1.013; Wald *P*=0.053), and from 74% to 89% (HR, 0.398; 95% CI: 0.133-1.187; Wald *P*=0.099), respectively (*Online Supplementary Figure S3*). We found a significant interaction between treatment assignment and *CEBPA*dm status in RFS (*P*=0.042), but not EFS (P=0.184) or OS (P=0.119). The hazard ratios for relapse or death of *CEBPA*dm AML compared with other types of AML were 0.298 (95% CI: 0.130-0.682; Wald *P*=0.004) in the intermediate-dose cytarabine group and 0.829 (95% CI: 0.473-1.453; Wald *P*=0.513) in the conventional-dose cytarabine group (Figure 1). The data indicated that the favorable RFS of patients with *CEBPA*dm AML depended on treatment assignment. After adjusting for the presence of *FLT3*-ITD and transplantation in CR1, the interaction between treatment assignment and *CEBPA*dm status still existed for RFS (*P*=0.042), but not for EFS (*P*=0.215) or OS (*P*=0.148).

The OS and RFS rates of AML patients with *CEBPA*dm are approximately 54%-63% and 44-48%, respectively.[10-13] However, relapsed patients with *CEBPA*dm have a favorable outcome after reinduction followed by allogeneic transplantation. Schlenk *et al*. proposed both strategies, allogeneic or autologous transplantation in CR1 *versus* intensive chemotherapy in CR1, and reinduction followed by allogeneic transplantation in the case of relapse.[13] We demonstrated that *CEBPA*dm AML patients receiving intermediate-dose cytarabine had a remarkable increase in RFS as well as in RFS rates censored at the date of allogeneic transplantation. This indicated that more patients would not relapse and did not need transplantation after intermediate-dose cytarabine induction therapy.

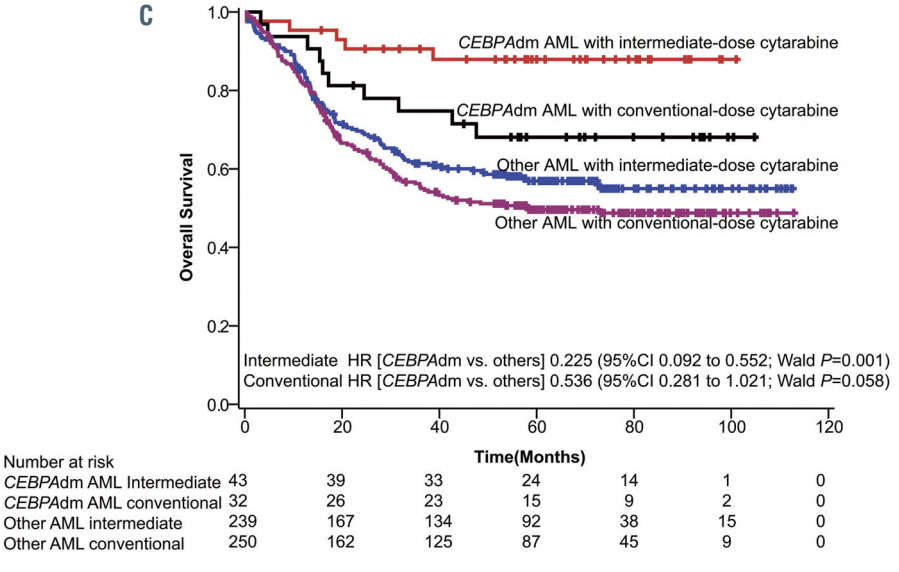
Overall, there were 131 patients with *RUNX1-RUNX1T1* in our cohort, including 60 in the conventional-dose group and 71 in the intermediate-dose group. Intermediate-dose cytarabine did not increase the complete remission rate in patients with *RUNX1-RUNX1T1* compared to that in patients treated with conventional-dose cytarabine (97% and 93%; *P*=0.528). However, intermediate-dose cytarabine produced better RFS and EFS and showed a marked tendency to improve OS in patients with *RUNX1-RUNX1T1* in both univariate and multivariable analyses, as shown in *Online Supplementary Table S2*. The 5-year RFS, EFS, and OS rates in patients with *RUNX1-RUNX1T1* AML were 72%, 70%, and 74% in the intermediate-dose cytarabine group compared to 56%, 52%, and 58% in the conventional-dose group, respectively (Figure 2). There was no interaction between



Number at risk							
CEBPAdm AML Intermediate	41	35	30	21	10	0	0
CEBPAdm AML conventional	32	22	18	13	8	1	0
Other AML intermediate	202	137	114	81	33	11	0
Other AML conventional	187	115	89	64	33	7	0

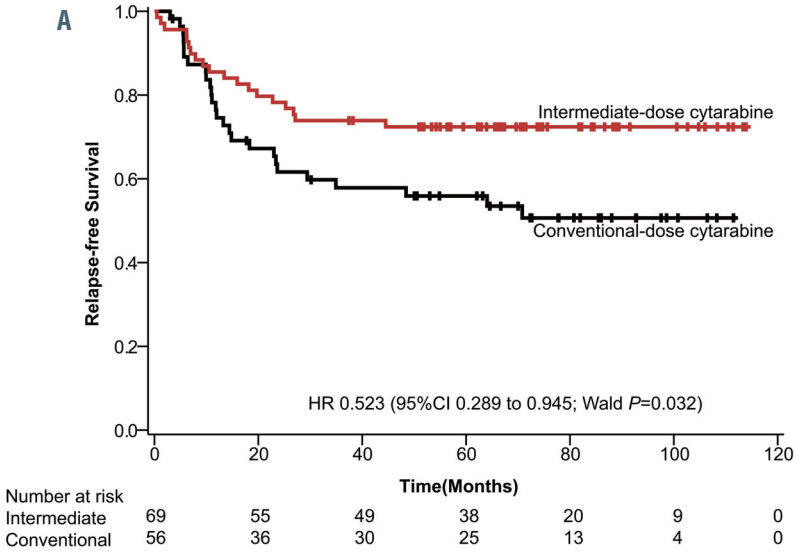


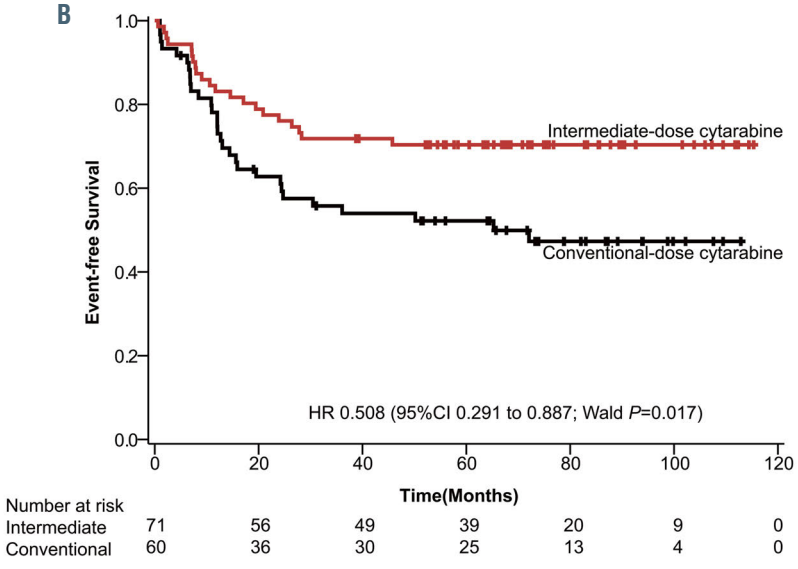
Number at risk							
CEBPAdm AML Intermediate	43	36	30	21	12	1	0
CEBPAdm AML conventional	32	22	18	13	8	2	0
Other AML intermediate	239	140	84	84	34	12	0
Other AML conventional	250	117	68	68	35	7	0



Number at risk							
CEBPAdm AML Intermediate	43	39	33	24	14	1	0
CEBPAdm AML conventional	32	26	23	15	9	2	0
Other AML intermediate	239	167	134	92	38	15	0
Other AML conventional	250	162	125	87	45	9	0

Figure 1. Patients with acute myeloid leukemia with *CEBPA* double mutations had more favorable outcomes only when treated with intermediate-dose cytarabine induction. (A) Relapse-free survival, (B) event-free survival, and (C) overall survival are shown for patients with *CEBPA* double mutations and other types of acute myeloid leukemia by receipt of intermediate-dose or conventional-dose cytarabine induction. AML: acute myeloid leukemia: HR: hazard ratio; 95%CI: 95% confidence interval.





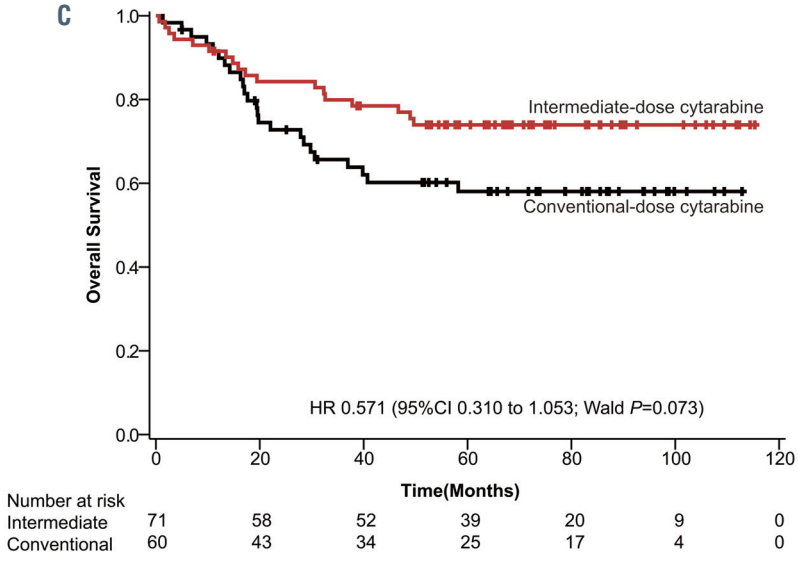


Figure 2. Outcomes of patients with *RUNX1-RUNX1T1* acute myeloid leukemia by treatment assignment. (A) Relapse-free survival, (B) event-free survival, and (C) overall survival. HR: hazard ratio; 95%CI: 95% confidence interval.

the treatment assignment and *RUNX1-RUNX1T1* status (RFS: *P*=0.300; EFS: *P*=0.383; OS: *P*=0.391). All patients with *CBFB-MYH11* AML achieved complete remission after both intermediate-dose and conventional-dose cytarabine. We were unable to determine the impact of intermediate-dose cytarabine in patients with *CBFB-MYH11* AML since there were only 33 patients with *CBFB-MYH11* in our cohort.

In this subgroup analysis of our trial, our data suggested that AML patients with *RUNX1-RUNX1T1* benefited from intermediate-dose cytarabine induction. Previous reports also indicated that a higher dose of cytarabine improved the outcome in patients with *RUNX1-RUNX1T1* AML.[14,15] Hence, all these data suggest that an induction regimen with an intensified dose of cytarabine benefits patients with *RUNX1-RUNX1T1* AML.

There were a total of 89 patients with *NPM1* mutations, regardless of *FLT3*-ITD mutation status, in our cohort,

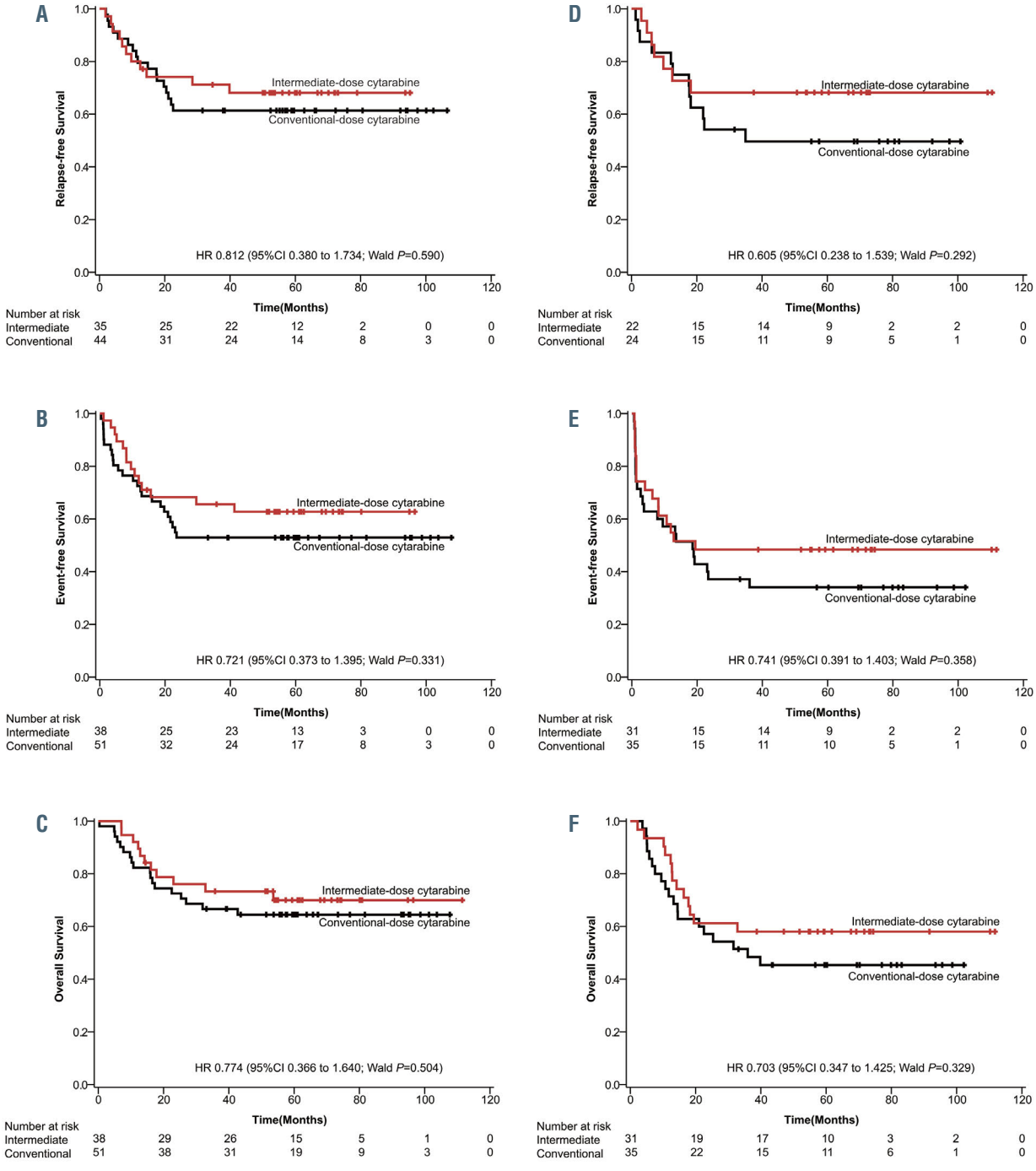


Figure 3. Outcomes of *NPM1* and *FLT3*-ITD mutant acute myeloid leukemia by treatment assignment. (A) Relapse-free survival, (B) event-free survival, and (C) overall survival of patients with *NPM1* mutations. (D) Relapse-free survival, (E) event-free survival, and (F) overall survival of patients with *FLT3*-ITD mutation. HR: hazard ratio; 95%CI: 95% confidence interval.

including 51 in the conventional-dose group and 38 in the intermediate-dose group. There were 66 patients with *FLT3*-ITD mutations, regardless of *NPM1* mutations, including 35 in the conventional-dose group and 31 in the intermediate-dose group. Intermediate-dose cytarabine did not increase the complete remission rate or improve RFS, EFS, or OS compared to conventional-dose cytarabine in patients with *NPM1* or *FLT3*-ITD mutations, as shown in *Online Supplementary Table S2*. In patients with *NPM1* mutations, the 5-year RFS, EFS, and OS rates were 68%, 63%, and 70% in the intermediate-dose cytarabine group compared to 61%, 53%, and 65% (Figure 3A-C), respectively, in the conventional-dose group. In patients with *FLT3*-ITD mutations, the 5-year RFS, EFS, and OS rates were 68%, 48%, and 58% in the intermediate-dose cytarabine group compared to 50%, 34%, and 45% (Figure 3D-F), respectively, in the conventional-dose group. We then investigated the impact of intermediate-dose cytarabine in *NPM1*$^{+}$/*FLT3*-ITD^{-}, *NPM1*$^{+}$/*FLT3*$^{-}$ITD^{+}, and *NPM1*–/*FLT3*-ITD^{+} subgroups. Intermediate-dose cytarabine did not increase complete remission rate or improve RFS, EFS, or OS compared to conventional-dose cytarabine in all these subgroups, as shown in *Online Supplementary Table S3*.

Death rates within 30 days were similar in the intermediate- and conventional-dose cytarabine induction cohorts.[2] There were no significant differences in RFS, OS, cumulative incidence of relapse or cumulative incidence of death in complete remission between the consolidation regimens even with longer follow-up (*data not shown*). With inclusion of the second randomization in multivariable analyses, the conclusions regarding outcomes depending on induction treatment were not modified by the second randomization, as shown in *Online Supplementary Table S4*, except that the OS in the intermediate cytogenetic-risk group was not significantly different, but with a trend, and no difference in EFS in the poor cytogenetic-risk group.

In this subgroup analysis with updated follow-up, we demonstrated that AML patients with *CEBPA*dm and *RUNX1-RUNX1T1* might benefit from intermediate-dose cytarabine induction. AML patients with *CEBPA*dm had a more favorable RFS than others only when treated with intermediate-dose cytarabine induction. Intermediate-dose cytarabine did not, however, improve outcomes in AML patients with *NPM1* or *FLT3*-ITD mutations. Luskin *et al*. suggested that anthracycline dose intensification induction conferred a favorable prognosis for AML patients with *NPM1* mutations.[7] These data indicate that AML patients with different mutations might benefit from intensified doses of different drugs. Recently, novel drugs, such as gemtuzumab ozogamicin, FLT3 inhibitors and so on, are being used in clinical practice. Prospective trials would be needed to confirm the benefit of induction with intermediate-dose cytarabine, especially when novel drugs are used.

Hui Wei,[1,2,3] Chunlin Zhou,[3] Dong Lin,[3] Bingcheng Liu,[3] Yan Li,[3] Xingli Zhao,[3] Shuning Wei,[3] Benfa Gong,[3] Kaiqi Liu,[3] Xiaoyuan Gong,[3] Yuntao Liu,[3] Guangji Zhang,[3] Jiayuan Chen,[1] Junping Zhang,[3] Jingjing Jin,[3] Shaowei Qiu,[3] Runxia Gu,[3] Ying Wang,[2,3] Yingchang Mi[1,2,3] and Jianxiang Wang[1,2,3]

[1]State Key laboratory of Experimental Hematology; [2]National Clinical Research Center for Blood Diseases and [3]Leukemia Center, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences, Tianjin, China

Correspondence:

JIANXIANG WANG - wangjx@ihcams.ac.cn

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References

1. Fernandez HF, Sun Z, Yao X, et al. Anthracycline dose intensification in acute myeloid leukemia. N Engl J Med. 2009;361(13):1249-1259.
2. Wei H, Wang Y, Gale RP, et al. Randomized trial of intermediate-dose cytarabine in induction and consolidation therapy in adults with acute myeloid leukemia. Clin Cancer Res. 2020;26(13):3154-3161.
3. Willemze R, Suciu S, Meloni G, et al. High-dose cytarabine in induction treatment improves the outcome of adult patients younger than age 46 years with acute myeloid leukemia: results of the EORTC-GIMEMA AML-12 trial. J Clin Oncol. 2014;32(3):219-228.
4. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N Engl J Med. 1994;331(14):896-903.
5. 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.
6. NCCN clinical practice guidelines in oncology acute myeloid leukemia Version 3.2020. http://www.nccn.org. MS28-MS34.
7. Luskin MR, Lee JW, Fernandez HF, et al. Benefit of high-dose daunorubicin in AML induction extends across cytogenetic and molecular groups. Blood. 2016;127(12):1551-1558.
8. Lee JH, Joo YD, Kim H, et al. A randomized trial comparing standard versus high-dose daunorubicin induction in patients with acute myeloid leukemia. Blood. 2011;118(14):3832-3841.
9. Bishop JF, Matthews JP, Young GA, et al. A randomized study of high-dose cytarabine in induction in acute myeloid leukemia. Blood. 1996; 87(5):1710-1717.
10. Green CL, Koo KK, Hills RK, et al. Prognostic significance of CEBPA mutations in a large cohort of younger adult patients with acute myeloid leukemia: impact of double CEBPA mutations and the interaction with FLT3 and NPM1 mutations. J Clin Oncol. 2010; 28(16):2739-2747.
11. Taskesen E, Bullinger L, Corbacioglu A, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. Blood. 2011;117(8):2469-2475.
12. Wouters BJ, Lowenberg B, Erpelinck-Verschueren CA, et al. Double CEBPA mutations, but not single CEBPA mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. Blood. 2009;113(13):3088-3091.
13. Schlenk RF, Taskesen E, van Norden Y, et al. The value of allogeneic and autologous hematopoietic stem cell transplantation in prognostically favorable acute myeloid leukemia with double mutant CEBPA. Blood. 2013;122(9):1576-1582.
14. Burnett AK, Hills RK, Russell N, et al. Reasons for survival improvement in core binding factor AML: a 25 year analysis of the UK MRC/NCRI AML trials. Blood. 2013;122(21):358.
15. Borthakur G, Kantarjian H, Wang X, et al. Treatment of core-binding-factor in acute myelogenous leukemia with fludarabine, cytarabine, and granulocyte colony-stimulating factor results in improved event-free survival. Cancer. 2008;113(11):3181-3185.