High-Dose Cytarabine in Acute Myeloid Leukemia Treatment: A Systematic Review and Meta-Analysis

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Abstract

The optimal dose, scheme, and clinical setting for Ara-C in acute myeloid leukemia (AML) treatment remain uncertain. In this study, we performed a meta-analysis to systematically assess the impact of high-dose cytarabine (HDAC) on AML therapy during the induction and consolidation stages. Twenty-two trials with a total of 5,945 de novo AML patients were included in the meta-analysis. Only patients less than 60 year-old were included in the study. Using HDAC in induction therapy was beneficial for RFS (HR = 0.57; 95% CI, 0.35–0.93; P = 0.02) but not so for CR rate (HR = 1.01; 95% CI, 0.93–1.09; P = 0.88) and OS (HR = 0.83; 95% CI, 0.66–1.03; P = 0.1). In consolidation therapy, HDAC showed significant RFS benefits (HR = 0.67; 95% CI, 0.49–0.9; P = 0.008) especially for the favorable-risk group (HR = 0.38; 95% CI, 0.21–0.69; P = 0.001) compared with SDAC (standard dose cytarabine), although no OS advantage was observed (HR = 0.84; 95% CI, 0.55–1.27; P = 0.41). HDAC treatment seemed less effective than auto-BMT/allo-BMT treatment (HR = 1.66, 95% CI, 1.3–2.14; P<0.0001) with similar OS. HDAC treatment led to lower relapse rate in induction and consolidation therapy than SDAC treatment, especially for the favorable-risk group. Auto-BMT/allo-BMT was more beneficial in prolonging RFS than HDAC.

Introduction

Cytarabine (Ara-C) has been a major drug for acute myeloid leukemia (AML) treatment for more than three decades. Initially, the drug was used at 100–200 mg/m² for 7–10 days for standard treatment [1]. In recent years, multiple cycles of high-dose cytarabine (HDAC) therapy (at 3.0 g/m² every 12 hours) have been commonly used as the consolidation therapy in multicenter trials; it was observed to maximize Ara-C’s anti-leukemia effect in AML patients, leading to improve disease- free-survival (DFS) [2,3]. After that, HDAC instead of standard-dose cytarabine multiagent chemotherapy has become a common practice in the treatment of AML, especially in patients younger than 60 years of age, either for remission induction or consolidation, based on the guidelines of the National Comprehensive Cancer Network (NCI, 2013). However, recent randomized controlled trials with 781 patients have challenged the benefits of HDAC [4]. HDAC failed to show significant improvement in five-year relapse-free survival and five-year overall survival as compared with SDAC regimen in AML treatments, especially in the consolidation therapy. After these new studies, the dose and effect of HDAC during AML induction and consolidation therapies are open for new evaluation [5]. Therefore, a systematic analysis needs to be performed to clarify these issues, which is the focus of this meta-analytical review. Specifically, this review study compared the effectiveness of HDAC versus SDAC as AML therapy in adult patients during the induction and consolidation phases, in order to shed lights on defining the optimal dose and scheme of Ara-C treatment with minimum possible toxicity. On the other hand, we assessed the effectiveness of HDAC compared with bone marrow transplantation (BMT) in order to explore the best therapy in the consolidation phase.

Methods

Literature Search

Independent reviewers (LW and G. XY) systematically searched PubMed for relevant research papers published in English between January 1990 and March 2013 using the following query terms: acute myeloid leukemia, high-dose, and cytarabine. The titles and abstracts of the identified studies were reviewed to determine potential eligibility for meta-analysis. Relevant review and meta-analysis articles were included to identify additional studies that met the inclusion criteria. Further studies were referred by means of manual search of secondary sources.
sources. Divergences among the reviewers must be resolved to reach a consensus after further discussion.

Inclusion and Exclusion Criteria

Identified articles were independently appraised according to the inclusion criteria by the same two reviewers (L.W and G. XY). All patients were required to have untreated acute myeloid leukemia, de novo AML, and patients with acute promyelocytic leukemia and translocation t(15;17) did not included this study. The included trials described the comparison of HDAC (2.0–3.0 g/m²) and standard-dose cytarabine (SDAC, ≤200 mg/m²) in induction and consolidation therapy, or bone marrow transplantation (BMT) in consolidation therapy. New medicine research and phase II/III clinical trials were excluded. Studies reported hazard ratios (HRs) and 95% confidence intervals (CIs) for overall survival (OS)/relapse free survival (RFS) benefit, or those provided data to estimate HR by the method of Parmar et al [6]. If multiple articles were identified to report on the same study, the most recent one was analyzed. Only randomized controlled trials (RCT) were included in the comparison of HDAC and SDAC, but observed study met the inclusion criteria for the study of BMT vs HDAC, because it was difficult to ensure that each patient had donor and even more difficult to complete RCT.

Data Extraction. Data were extracted in the standardized format by two independent reviewers. Data collected for each study included study name, name of first author, year of publication, period of enrollment, total number of subjects allocated to therapies, median patient age (years), chemotherapy regimens, number of events (death, relapse) in each group, and study end points of overall survival benefit, RFS benefit, or both. We used overall survival (OS) and relapse free survival (RFS) of individual studies. Discrepancies in data extraction were resolved by identifying consensus, referring back to the original article, or contacting study authors if necessary. When missing data were encountered, the authors were contacted to complete data analysis.

Figure 1. Flow chart explaining the selection of eligible studies included in the meta-analysis.
doi:10.1371/journal.pone.0110153.g001
Table 1. Characteristics of included Studies for induction therapy.

| Source | Study ID | Enrollment Period | Multi-center | No. of patients | RCTs | Study entry criteria | Induction therapy |
|--------|----------|-------------------|--------------|-----------------|------|---------------------|-------------------|
| J.P. Matthews et al, 2001 [7] | ALSG | 1987–1991 | Yes | 248 | Yes | de novo AML, median age: 42 years | DEA (DNR+VP-16+Ara-c 100 mg/m²/d x 7d) DA (DNR+VP-16+Ara-c 3 g/m² q12 h d1,3,5,7d) |
| J.K. Weick et al, 1996 [8] | SWOG | 1986–1991 | Yes | 723 | Yes | de novo or secondary AML, M/F: 397/326, age range: 15–64 years, WBC range: 0.4–416 x 10⁹/L | DA (DNR+Ara-c 200 mg/m²/d x 7d) DA (DNR+Ara-c 3 g/m² q12 h x6d) |
| T. Büchner et al, 2006 [9] | CAMLCG | 1999–2005 | Yes | 1770 | Yes | de novo or secondary AML, age range: 16–85 years | Double induction TAD (6-TG+DNR+Ara-c 100 mg/m²/d x 8d) + HAM (MTZ+Ara-c 3 g/m² q12 h x3d) HAM+HAM (MTZ+Ara-c 3 g/m² q12 h x3d) |
| T. Büchner et al, 1999 [10] | CAMLCG | 1985–1992 | Yes | 725 | Yes | de novo AML, M/F: 336/389, median age: 44 (16–60) years, WBC range: 0.1–405 x 10⁹/L | Double induction TAD (6-TG+DNR+Ara-c 100 mg/m²/d x8d) TAD (6-TG+DNR+Ara-c 100 mg/m²/d x8d) + HAM (MTZ+Ara-c 3 g/m² q12 h x3d) |
| T. Büchner et al, 2009 [11] | CAMLCG | 1993–2005 | Yes | 1284 | Yes | de novo AML, years range: 16–85 years, WBC range: 0.05–1017 x 10⁹/L | Double induction TAD (6-TG+DNR+Ara-c 100 mg/m²/d x8d) + HAM (MTZ+Ara-c 3 g/m² q12 h x3d) HAM+HAM (MTZ+Ara-c 3 g/m² q12 h x3d) |

Note: ▲ T. Büchner et al. 2009 repeated the same trial of T. Büchner et al. 2006.

Abbreviations: NR, not reported; IDA, idarubicin; Ara-c, cytarabine; VP-16, etoposide; DNR, daunorubicin.
doi:10.1371/journal.pone.0110153.t001
Assessment of methodological quality. Two reviewers assessed the methodological quality of each trial. The risk of bias in each trial was assessed according to the Cochrane methodology by using the following criteria: considering random sequence generation, allocation concealment, the blinding of patients and personnel, incomplete outcome data, selective reporting, and Begg’s funnel plots and Egger’s test were used to reveal possible publication bias. Heterogeneity was assessed by forest plots and with a standard Chi2 test and an inconsistency (I²) statistic. Both the fixed-effect model and the random-effect model were initially used to calculate total HRs, and finally selected with regards to heterogeneity in the survival analyses. If the heterogeneity (I² > 75%) was too great for a summary estimate to be calculated, subgroup analysis was needed.

Data synthesis. Data were synthesized using the Cochrane Statistics package RevMan (version 4.0.4). The threshold of significance was P=0.05. A forest plot with combined HRs (with 95% CIs) for OS and RFS benefit of HDAC in induction therapy. We also performed additional analysis that stratified treatment options by cytogenetic characteristics. In such analysis, patients were stratified into poor-, intermediate-, and favorable-risk groups by cytogenetic characteristics. OS and RFS benefits of HDAC for different cytogenetic risk groups were analyzed.

Results

Studies selected for meta-analysis

The initial search on MEDLINE (PubMed) database and the abstract review identified 643 articles. After the screening of titles and abstracts (by two reviewers LW and G. XY), 160 non-relevant articles were excluded, which were those that were published in languages other than English, case reports, reviews, and studies on pediatric AML. For the secondary search, the reference lists of review articles were manually examined to identify additional studies. The 483 selected articles were retrieved for further reviews in a structured format. As a result, 160 more articles were excluded, because those studies involved relapsed/refractory AML, APL, high-risk MDS, CML, therapy-related AML, myeloid sarcoma, or other concurrent diseases (including status of other concurrent tumors, definite MDS history) of AML that conflicted with the inclusion criteria. For the remaining 323
### Table 2. Characteristics of Included Studies for consolidation therapy.

| Source                          | Study ID | Enrollment Period | RCT | Multicenter | No. of patients | Median age/age range (years) | Consolidation therapy                                                                 | follow-up (years) |
|---------------------------------|----------|-------------------|-----|-------------|-----------------|-----------------------------|---------------------------------------------------------------------------------------|-------------------|
| JK. Weick et al., 1996 [8]      | SWOG     | 1986–1991         | Yes | Yes         | 287             | 45 (15–64)                  | DA (DNR+Ara-C 200 mg/m²/d×7) continuous 2 courses DA (DNR+Ara-C 3 g/m² q12 h×1 course)| 4.3               |
| K.F. Bradstock et al, 2005 [12] | ALLG     | 1995–2000         | Yes | Yes         | 202             | 43 (15–60)                  | ICE (IDA+VP-16+Ara-c 100 mg/m²/d×5) continuous 2 courses ICE (IDA+VP-16+Ara-c 3 g/m² q12 h d1,3,5,7) 1 course| 4                |
| M. Fopp et al, 1997 [13]        | SAKK     | 1985–1992         | Yes | Yes         | 137             | 16–64                       | DA (DNR+Ara-C 100 mg/m²/d×7) 1 courses DA (DNR+Ara-C 3 g/m² q12 h×3) 1 course           | 6                |
| PA. Cassileth et al, 1992 [14]  | ECOG     | 1984–1988         | Yes | Yes         | 170             | 15–65                       | TA (6-TG+Ara-c 60 mg/m²/d×5) AA (Amsa+Ara-c 3 g/m² q12 h×3) (no courses in detail)      | 4                |
| R.J. Mayer et al, 1994 [15]     | CALGB    | 1985–1990         | Yes | Yes         | 389             | 16–86★                      | SDAC ( Ara-c 100 mg/m²/d×5) continuous 4 courses HDAC (Ara-c 3 g/m² q12 h×3) continuous 4 courses (no detail therapy) | 4.3               |
| S. Miyawaki et al, 2011b [4]    | JALSG    | 2001–2005         | Yes | Yes         | 781             | 15–64                       | DA, MA, VEA (DNR, MTZ, Acl-a, VP-16, VCR+Ara-C200 mg/m²/d×5) continuous 4 courses HDAC (2 g/m² q12 h×3) continuous 3 courses| 4                |
| S. Ohtake et al, 2011a [16]     | JALSG    | 2001–2005         | Yes | Yes         | 781             | 15–64                       | DA, MA, VEA (DNR, MTZ, Acl-a, VP-16, VCR+Ara-C200 mg/m²/d×5) continuous 4 courses HDAC (2 g/m² q12 h×3) continuous 3 courses| 4                |
| T. Büchner et al, 2003 [17]     | CAMLGC   | 1992–1999         | Yes | Yes         | 576             | 16–82★                      | TAD (6-TG, DNR, Ara-C200 mg/m²/d×5) continuous several courses HAM (MTZ+Ara-c 2 g/m² q12 h d1,2,8,9) | NR               |
| CD. Bloomfield et al, 1998 [19] | CALGB    | 1985–1990         | Yes | No          | 186             | >16                         | Ara-c 100 mg/m²/d×5 continuous 4 courses Ara-c 3.0 g/m² q12 h d1,3,5 continuous 4 courses (no detail therapy) | 5                |
| X. Thomas et al, 2011 [20]      | ALFA     | 1999–2006         | Yes | Yes         | 237             | 15–50                       | AA (Amsa+Ara-C), TSC (MTZ+VP-16+Ara-c 500 mg/m²/d×10) continuous 2 courses Ara-c 3.0 g/m² q12 h d1,3,5 continuous 4 courses | 10               |
| AM. Tsimberidu et al, 2003 [21] | HCG      | 1996–2000         | No  | No          | 120             | 15–60                       | Ara-c 3.0 g/m² q12 h d1,3,5 continuous 2 courses preparative regimen: BU+VP-16+CTX Allo-BMT/auto-SCT | 5.3              |
| J.L. Harousseau et al, 1997 [22]| GOELAM   | 1987–1994         | No  | No          | 517             | 15–60                       | ICC (IDR+Ara-c 3.0 g/m² q12 h d1–4) continuous 2 courses preparative regimen: BU+CTX/TBI+CTX Allo-BMT/auto-SCT | 8.5              |
| PA. Cassileth et al, 1998 [23]  | No       | 1990–1997         | Yes | Ye翻 | 808             | 16–55                       | Ara-c 3.0 g/m² q12 h d1–3 preparative regimen: CTX+BU Allo-BMT/auto-SCT                  | 4                |
| RA. Zittoun et al, 1995 [24]    | GMEMMA   | 1986–1993         | Yes | Ye翻 | 623             | 33 (10–59)                  | AA (Amsa+Ara-c 2.0 g/m² d1–6) continuous 2 courses preparative regimen: CTX+TBI−/− BU Allo-BMT/auto-SCT | 8                |
| S. Brunet et al, 2004 [25]      | Spain    | 1994–1999         | No  | No          | 200             | 15–60                       | Ara-c 3.0 g/m² q12 h d1−3 continuous 2 courses preparative regimen: CTX+TBI−/− BU Allo-BMT/auto-SCT | 7                |
| R. Bassan et al, 1998 [26]      | Italy    | 1987–1993         | No  | No          | 108             | 15–60                       | Ara-c 2.0 g/m²/d d1–6 preparative regimen: Dox+TBI Allo-BMT/auto-SCT                     | >5               |
| PA. Cassileth et al, 1992 [14]  | ECOG     | --               | No  | Ye翻 | 534             | 44 (15–65)                  | Ara-c 3.0 g/m² q12 h d1–6 1 course preparative regimen: CTX+TBI Allo-BMT                  | 6                |
articles, full texts were further reviewed. A total of 256 articles were further excluded: 114 articles did not report data comparing the efficacy of HDAC on the OS and RFS of adult AML patients; 121 articles did not provide prospective data on OS and RFS outcome; and 21 articles used non-traditional chemotherapy regimens. The remaining 67 articles met the inclusion criteria. However, 22 articles were further excluded by experts, because the induction or consolidation therapy used in these studies were not consistent, along with confusing risk groups in some articles; 10 more articles were also excluded because only HDAC was used thus no comparison data available; and 4 articles were reporting the same trials [2, 9, 18, 4]. As a result, a total of 22 articles passed through all examinations and were finally used for the meta-analysis in this study [Figure 1].

Quality Assessment
According to Cochrane methodology, the risk of bias of total RCT articles were assessed by Cochrane factors. The studies at low risk of bias had values (a quantitative index of the risk of bias, range 0–100%) of 64.3%, 64.3%, 0, 92.9%, 50%, 42.9%. (Figure S1).

HDAC versus SDAC in induction therapy
Four randomized controlled trials compared HDAC with SDAC in induction therapy [Table 1]. In all 4 trials, the end points of CR, OS, and RFS were reported. Initial baseline characteristics between the treatment group and the control group were quite balanced. A total of 2,980 de novo AML patients enrolled from 1985 to 2005 were included. In the CAMLCG2009 and CAMLCG 2006 trials, the inclusion criteria for patient age were different from those of the rest of trials. Patients younger than 60 year-old were analyzed in the majority of trials. No significant differences in CR between patients received HDAC and those received SDAC [Figure 2] (HR = 1.01; 95% CI, 0.93–1.09; \( P = 0.88 \)). The OS and RFS results were overall heterogeneous. In the trial ALSG 1996, OS and RFS were much longer in the HDAC group than those in the SDAC group. On the other hand, a larger number of patients receiving HDAC treatment showed shorter OS in the CAMLCG trial. Overall, no significant differences in OS were observed between HDAC and SDAC in the induction phase (HR = 0.83; 95% CI, 0.66–1.03; \( P = 0.10 \)) [Figure 2]. Patients in the HDAC group showed similar OS as that of the SDAC group. However, a statistically significant difference in RFS was observed between HDAC and SDAC in the induction phase (HR = 0.57; 95% CI, 0.35–0.93; \( P = 0.02 \)) [Figure 2]. Therefore, HDAC used in the induction therapy clearly improved RFS but not OS in AML patients.

HDAC versus SDAC in consolidation therapy
Nine trials were identified to contain the comparison of HDAC and SDAC in consolidation therapy [Table 2]. All 9 trials were randomized controlled studies, and 7 of them were multicenter trials. A total of 2,965 de novo AML patients enrolled from 1978 to 2005 were included, and the longest follow-up period of each trial was 10 years. Only patients younger than 60 year-old were analyzed. The initial baseline characteristics (age, sex, race, FAB classification, and cytogenetics) between two groups were similar, although detailed information about initial baseline characteristics in the ECOG1992 trial was not shown. In addition, only 1 course of HDAC was used in the SWOG1996 and SAKK1997 trials, different from all other trials. In 5 trials, HDAC was used concomitantly with other drugs, while 4 other trials only used single dose of Ara-C (2–3 g/m²), which may lead to heterogeneity among different trials. All the 9 trials reported end points of 4-year
Figure 3. Overall survival benefit of HDAC in consolidation therapy. A: Total overall survival benefit of HDAC in consolidation therapy. B: Overall survival benefit of different subgroups of HDAC in consolidation therapy.
doi:10.1371/journal.pone.0110153.g003
OS and RFS. The 4-year OS rate in the HDAC group ranged from 32%–71%. No significant differences in OS were observed between the HDAC and SDAC groups (HR = 0.84; 95% CI, 0.55–1.27; P = 0.41) [Figure 3]. However, patients that used HDAC in consolidation therapy showed longer RFS than those used SDAC (HR = 0.67; 95% CI, 0.49–0.9; P = 0.008) [Figure 4]. Therefore, HDAC improved RFS but did not affect OS in consolidation therapy.

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Figure 4. Relapse free survival benefit of HDAC in consolidation therapy. A: Total relapse free survival benefit of HDAC in consolidation therapy. B: Relapse free survival benefit of different subgroups of HDAC in consolidation therapy. doi:10.1371/journal.pone.0110153.g004
We further performed stratified analysis for different subgroups. We restricted the stratification for cytogenetic risk (SWOG/ECOG, NCCN, and MRC) (Table S1). Five trials were included in the stratified analysis. A significant RFS benefit was observed with HDAC treatment (HR = 0.38; 95% CI, 0.21–0.69; \(P = 0.001\)) in the favorable-risk group [Figure 4]. However, no significant RFS benefit was shown with HDAC treatment in the immediate-risk and poor-risk groups (HR = 0.68; 95% CI, 0.4–1.16; \(P = 0.16\); HR = 1.04; 95% CI, 0.36–2.95; \(P = 0.95\)). On the contrary, HDAC did not show any significant effects on OS as compared to SDAC. The OS with HDAC was not significantly different from that with SDAC treatment in all 3 stratified risk groups (HR = 0.81; 95% CI, 0.49–1.33; \(P = 0.43\); HR = 1.09; 95% CI, 0.79–1.49; \(P = 0.6\); HR = 1.01; 95% CI, 0.47–2.14; \(P = 0.99\)) [Figure 3].

**HDAC versus BMT in consolidation therapy**

Nine trials containing the comparison of the effect of HDAC treatment with that of auto-SCT/all-BMT were included in the analysis. They included 5 randomized trials and 4 observational trials. Randomized trials were defined as those in which patients who did not have donors would be randomly allocated into the HDAC and auto-SCT groups. Only 2 trials were multicentre trials. End points of OS and RFS were reported across all cytogenetic risk groups in all 9 trials, so we were not able to perform stratified analysis for different cytogenetic risk groups when evaluating OS and RFS outcomes. A total of 3,128 de novo AML patients enrolled from 1986 to 2000 were included. The longest follow-up period of each trial was 8.5 years [Table 2]. Of them, 29.8% patients received auto-SCT; 30.8% received allo-BMT; and 39.4% received HDAC. No imbalance in preparative regimen was observed between trials. The data were highly homogeneous in different studies concerning RFS endpoint (\(I^2 = 0\%\)). Only patients younger than 65 year-old were enrolled in the analysis considering the risk of transplantation.

This analysis revealed that the combined HR was 0.89 (95% CI, 0.67–1.19; \(P = 0.43\), 1.01 (95% CI, 0.79–1.3; \(P = 0.92\), and that patients received HDAC had an OS similar to that of patients received auto-SCT/allo-BMT in consolidation therapy [Figure 5].

![Effect of HDAC versus BMT on overall survival.](https://doi.org/10.1371/journal.pone.0110153.g005)

**Figure 5. Effect of HDAC versus BMT on overall survival.**

\(P = 0.43\)
On the other hand, the RFS was significantly different between the auto-SCT/allo-BMT group and the HDAC group [Figure 6]. Auto-SCT had a combined HR of 1.41 (95% CI, 1.06–1.87; \(P = 0.02\)), while allo-BMT had a combined HR of 1.95 (95% CI, 1.35–2.81; \(P = 0.0004\)), indicating a significant RFS benefit of auto-SCT/allo-BMT over HDAC. Overall, the results indicated that auto-SCT/allo-BMT significantly reduced the hazard rate of relapse but failed to improve overall survival.

Discussion

In the past 20 years, Ara-C has been widely used in the induction and consolidation therapy for AML. Multiple prospective studies on Ara-C have been reported, and the application of HDAC has been tested extensively beyond first-line therapy and is considered a standard therapy. However, HDAC started to be questioned in recent studies with larger patient numbers. In this study, we performed a meta-analysis to address whether HDAC application in the induction and consolidation therapy prolongs RFS and decreases AML recurrence comparing with SDAC.

In a recent meta-analysis, 3 trials were analyzed, which discovered no differences in CR rates between HDAC and SDAC treatments. HDAC in induction therapy improved long-term disease control and OS in adults \(<60\) years of age with de novo AML [29]. However, the effect of HDAC remains unclear in consolidation therapy, especially that for patients younger than 60 years. Therefore, we systematically collected all trials that used HDAC in both induction therapy and consolidation therapy from Jan. 1990 to Mar. 2013. The regimen of induction and consolidation therapy was restricted, which led to the exclusion of 20 articles containing different regimens of induction and consolidation therapy in HDAC and SDAC groups. All trials we identified were reported on an intent-to-treat basis and included a complete description of withdrawals and drop-outs. Some degrees

| Study or Subgroup | log(Risk Ratio) | SE | Weight | IV, Random, 95% CI | IV, Random, 95% CI |
|-------------------|----------------|----|--------|------------------|------------------|
| 5.2.1 HDAC/auto-BMT |               |    |        |                  |                  |
| A.M. Tsimberidu2003 | 0.37           | 0.72 | 2.7%   | 1.45 [0.35, 5.94] |                  |
| J.L. Harousseau 1997 | 0.18           | 0.32 | 8.8%   | 1.20 [0.64, 2.24] |                  |
| P.A. Cassileth 1998 | 0.53           | 0.27 | 10.5%  | 1.65 [0.97, 2.80] |                  |
| R. Bassan 1998     | -0.02          | 0.51 | 4.8%   | 0.98 [0.36, 2.66] |                  |
| R.A. Zitoun (GIMEMA) 1995 | 0.59     | 0.26 | 10.9%  | 1.80 [1.08, 3.00] |                  |
| S. Brunet 2004     | -0.29          | 0.47 | 5.4%   | 0.75 [0.30, 1.88] |                  |
| Subtotal (95% CI)  |               |    |        | 43.1%            | 1.41 [1.06, 1.87] |
| Heterogeneity: Tau_2 = 0.00; Chi^2 = 3.82, df = 5 (P = 0.58); I^2 = 0% |
| Test for overall effect: Z = 2.39 (P = 0.02) |

| Study or Subgroup | log(Risk Ratio) | SE | Weight | IV, Random, 95% CI | IV, Random, 95% CI |
|-------------------|----------------|----|--------|------------------|------------------|
| 5.2.2 HDAC/allo-BMT |               |    |        |                  |                  |
| A.M. Tsimberidu2003 | 1.73           | 0.8  | 2.2%   | 5.47 [1.14, 26.26] |                  |
| G.J. Schiller 1992 | 0.31           | 0.47 | 5.4%   | 1.36 [0.54, 3.43] |                  |
| J.L. Harousseau 1991 | 0.03           | 0.57 | 4.0%   | 1.03 [0.34, 3.15] |                  |
| J.L. Harousseau 1997 | 0.22           | 0.28 | 10.2%  | 1.25 [0.72, 2.16] |                  |
| P.A. Cassileth 1998 | 1.32           | 0.28 | 10.2%  | 3.74 [2.16, 6.48] |                  |
| P.A. Cassileth (ECOG) 1992 | 0.55     | 0.36 | 7.7%   | 1.73 [0.86, 3.51] |                  |
| R. Bassan 1998     | 1.63           | 1.15 | 1.2%   | 5.10 [0.54, 48.62] |                  |
| R.A. Zitoun (GIMEMA) 1995 | 0.92     | 0.24 | 11.7%  | 2.51 [1.57, 4.02] |                  |
| S. Brunet 2004     | 0.03           | 0.54 | 4.4%   | 1.03 [0.36, 2.97] |                  |
| Subtotal (95% CI)  |               |    |        | 56.9%            | 1.95 [1.35, 2.81] |
| Heterogeneity: Tau_2 = 0.13; Chi^2 = 14.68, df = 8 (P = 0.07); I^2 = 45% |
| Test for overall effect: Z = 3.56 (P = 0.0004) |

| Total (95% CI) | 100.0% | 1.66 [1.30, 2.14] |
| Heterogeneity: Tau_2 = 0.08; Chi^2 = 22.20, df = 14 (P = 0.07); I^2 = 37% |
| Test for overall effect: Z = 4.00 (P < 0.0001) |
| Test for subarous differences: Chi^2 = 3.70, df = 1 (P = 0.05). P = 73.0% |

Figure 6. Effect of HDAC versus BMT on relapse free survival.
doi:10.1371/journal.pone.0110153.g006
of heterogeneity still existed in the age inclusion criterion. In one article, patients older than 60 years of age were not analyzed separately from patients younger than 60 years. However, this article was still included because the proportion of patients older than 60 years was very low. Based on the current data, we cannot conclude whether HDAC has the same effects on older patients.

The dose of HDAC has also been questioned. In HOVON/SAKK study [30], Ara-C was used at 1.0 g/m² q12 h x 6 days. In this meta-analysis, we limited HDAC at the dose level of 2.0–3.0 g/m² q12 h x 3–5 days for the majority of the trials.

Overall, endpoint heterogeneity within trials was limited. No evidence was found to support the notion that HDAC improves CR rate as compared to SDAC in induction therapy. However, our analysis revealed that HDAC had a clear benefit on RFS in induction therapy, consistent to the findings from ALSG and CAML/CG [8,10]. A retrospective analysis of CALGB and ECOG trials [14,15,31] discovered a survival advantage of HDAC in consolidation therapy over SDAC. However, our analysis failed to reach this conclusion. Data from the risk group stratified analysis demonstrated that HDAC significantly improved RFS in the favorable-risk group but no significant benefits in the intermediate and poor-risk groups. We also discussed the advantage of using BMT in consolidation therapy and discovered that auto-BMT/allo-BMT improved RFS, but not OS, as compared to HDAC.

In conclusion, this meta-analysis demonstrated that HDAC improved RFS in induction therapy while reducing the relapse rate in consolidation therapy, as compared with SDAC, especially for the favorable-risk group. Auto-BMT/allo-BMT had a more beneficial effect in prolonging RFS as compared with HDAC. The analysis also posed some challenges to previous trial results. Overall, treatment with HDAC regimen did show some advantages for some outcome endpoints, especially in certain risk groups. However, it failed to show predominant advantages in all cases. Considering its high toxicity, caution should be taken when HDAC treatment regimen is chosen for patients. We also discovered varied degrees of heterogeneity within trials in our meta-analysis, which may interfere with the interpretation of results and limit the validity of the findings. In the future, more comprehensive clinical trials with improved study designs are needed to help elucidate the advantages and drawbacks of each treatment regimen in order to identify the optimal dose and treatment schedule for AML patients.

Supporting Information

Checklist S1 PRISMA Checklist.

Table S1 Risk status based on validated cytogenetics.

Acknowledgments

We thank Professor Taixiang Wu (Head of Chinese Clinical Trial Registry; Head of Research Manager, e-mail: twutsx@hotmail.com) for his technical support and careful reading.

Author Contributions

Conceived and designed the experiments: JW. Performed the experiments: WL. Analyzed the data: MS. Contributed reagents/materials/analysis tools: BG. Contributed to the writing of the manuscript: YM. WU.

References

1. Lowenberg B, Downing JR, Burnett A (1999) Acute myeloid leukemia. N Engl J Med. 341(14): 1051–1062.
2. Bishop JF, Matthews JP, Young GA, Szer J, Gillett A, et al. (1996) A randomized study of high-dose cytarabine in induction in acute myeloid leukemia. Blood. 87: 1170–1171.
3. Moore JO, George SL, Dodge RK, Amrein PC, Powell BL, et al. (2005) Sequential multiagent chemotheraphy is not superior to high-dose cytarabine alone as postremission intensification therapy for acute myeloid leukemia in adults under 60 years of age. Cancer and Leukemia Group B Study 9222. Blood. 105(9): 3420–7.
4. Miyawaki S, Ohtake S, Fujisawa S, Kivos H, Shinaqwak K, et al. (2011) A randomized comparison of 4 courses of standard-dose multiagent chemotherapy versus 3 courses of high-dose cytarabine alone in postremission therapy for acute myeloid leukemia in adults: the JALSG AML201 Study. Blood. 117(8): 2366–72.
5. Lowenberg B (2013) Sense and nonsense of high-dose cytarabine for acute myeloid leukemia. Blood. 121(1): 26–28.
6. Parmar MK, Torri V, Stewart L (1998) Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med. 17(24): 2815–2834.
7. Matthews JP, Bishop JF, Young GA, Jenea SK, Lowenthal RM, et al. (2003) Patterns of failure with increasing intensification of induction chemotherapy for acute myeloid leukemia. Br J Haematol. 113(3): 727–36.
8. Weick JK, Koprey K, Appelbaum FR, Head DR, Kingsbury LL, et al. (1996) A randomized investigation of high-dose versus standard-dose cytosine arabinoside with daunorubicin and 6-thioguanine in patients with previously untreated acute myeloid leukemia: a Southwest Oncology Group study. Blood. 88(3): 2941–51.
9. Bischur T, Berdel WE, Schoch C, Hafeler T, Serve HL, et al. (2006) Double induction containing either two courses or one course of high-dose cytarabine plus mitoxantrone and post remission therapy by either autologous stem-cell transplantation or by prolonged maintenance for acute myeloid leukemia. J Clin Oncol. 24(16): 2409-9.
10. Bischur T, Hiddemann W, Wiesmann B, Loffler H, Gassmann W, et al. (1999) Double induction strategy for acute myeloid leukemia: the effect of high-dose cytarabine with mitoxantrone instead of standard-dose cytarabine with daunorubicin and 6-thioguanine: a randomized trial by the German AML Cooperative Group. Blood. 15; 93(12): 4116–24.
11. Bischur T, Berdel WE, Hafeler C, Hafeler T, Schnitter S, et al. (2009) Age-related risk profile and chemotherapy dose response in acute myeloid leukemia: a study by the German Acute Myeloid Leukemia Cooperative Group. J Clin Oncol. 27(1): 61–9.
12. Bradstock KF, Matthews JP, Lowenthal RM, Baxter H, Catalano J, et al. (2005) A randomized trial of high-versus conventional-dose cytarabine in consolidation chemotherapy for adult de novo acute myeloid leukemia in first remission after induction therapy containing high-dose cytarabine. Blood. 105(2): 481–8.
13. Fopp M, Fery MF, Bacchi M, Cavalli F, Gnuerez J, et al. (1997) Post-remission therapy of adult acute myeloid leukemia: one cycle of high-dose versus standard-dose cytarabine. Leukemia Project Group of the Swiss Group for Clinical Cancer Research (SAKK). Ann Oncol. 8(3): 251–7.
14. Cassileth PA, Lynch E, Hines JD, Oken MM, Mazza JJ, et al. (1992) Varying intensity of post-remission therapy in acute myeloid leukemia. Blood. 79(6): 1924–30.
15. Mayer RJ, Davis RB, Schiller CA, Berq DT, Powell BL, et al. (1994) Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N Engl J Med. 331(14): 896–903.
16. Ohmata S, Miyawaki S, Fujita H, Kivos H, Shinaqwak K, et al. (2011) Randomized study of induction therapy comparing standard-dose idarubicin with high-dose daunorubicin in adult patients with previously untreated acute myeloid leukemia: the JALSG AML201 Study. Blood. 117(7): 2358–63.
17. Bischur T, Hiddemann W, Wiesmann B, Schoch C, et al. (2003) 6-Thioguanine, cytarabine, and daunorubicin (TAD) and high-dose cytarabine and mitoxantrone (HAM) for induction, TAD for consolidation, and either prolonged maintenance by reduced monthly TAD or HAM-TAD and one course of intensive consolidation by sequential HAM in adult patients at all ages with de novo acute myeloid leukemia (AML): a randomized trial of the German AML Cooperative Group. J Clin Oncol. 21(24): 4946–504.
18. Schoch C, Hafeler T, Haase D, Fronatsch C, Loffler H, et al. (2001) Patients with de novo acute myeloid leukemia and complex karyotype aberrations show a poor prognosis despite intensive treatment: a study of 90 patients. Br J Haematol. 112(1): 118–26.
19. Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, et al. (1998) Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res. Sep 15;58(18): 4173–9.
20. Thomas X, Elhammi M, Raffoux E, Renneville A, Pautas C, et al. (2011) Comparison of high-dose cytarabine and timed-sequential chemotherapy as consolidation for younger adults with AML in first remission: the ALFA-9602 study. Blood. 118(7): 1754–1762.
21. Tsimeridou AM, Stavroyianni N, Vinisou N, Papaioannou M, Tiniakou M, et al. (2003) Comparison of Allogeneic Stem Cell Transplantation, High-Dose Cytarabine, and Autologous Peripheral Stem Cell Transplantation as Post-remission Treatment in Patients with De Novo Acute Myelogenous Leukemia. Cancer. 97(7): 1721–1731.

22. Harousseau JL, Cahn JY, Pignon B, Witz F, Milpied N, et al. (1997) Chemotherapy as Postremission Therapy in Adult Acute Myeloid Leukemia Comparison of Autologous Bone Marrow Transplant- ation and Intensive. Blood. 90(8): 2978–2986.

23. Cassileth PA, Arington DP, Appelbaum FR, Lazarus HM, Rowe JM, et al. (1998) Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. N Engl J Med. 339: 1649–56.

24. Zittoun RA, Mandelli F, Willems R, de Witte T, Labar B, et al. (1995) autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. N Engl J Med. 332: 217–23.

25. Brunet S, Esteve J, Berlanga J, Ribera JM, Bueno J, et al. (2004) Treatment of primary acute myeloid leukemia: results of a prospective multicenter trial including high-dose cytarabine or stem cell transplantation as post-remission strategy. Haematologica. 89: 940–949.

26. Bassan R, Raimondi R, Lerede T, D’emilio A, Buelli M, et al. (1998) Outcome assessment of age group-specific (≥50 years) post-remission consolidation with high-dose cytarabine or bone marrow autograft for adult acute myelogenous leukemia. Haematologica. 83: 627–635.

27. Schiller GJ, Nimer SD, Territo MC, Ho-WG, Champlin RE, et al. (1992) Bone Marrow Transplant- ation Versus High-Dose Cytarabine-based consolidation chemotherapy for acute myelogenous leukemia in First Remission. J Clin Oncol. 10(1): 41–46.

28. Harousseau JL, Milpied N, Briere J, Desablens B, Leprise PY, et al. (1991) Double Intensive Consolid -ation Chemotherapy in Adult Acute Myeloid Leukemia. J Clin Oncol. 9: 1432–1437.

29. Kern W, Easty EH (2006) High-dose cytosine arabinoside in the treatment of acute myeloid leukemia. Cancer. 107: 116–24.

30. Lowenberg B, Pabst T, Vellenga E, van Putten W, Schouten HC, et al. (2011) Cytarabine dose for acute myeloid leukemia. N Engl J Med. 364: 1027–1036.

31. Farag SS, Ruppert AS, Mrozek K, Mayer RJ, Stone RM, et al. (2005) Outcome of induction and postremission therapy in younger adults with acute myeloid leukemia with normal karyotype: a cancer and leukemia group B study. J Clin Oncol. 23(5): 482–93.