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ORIGINAL RESEARCH

Clinical efficacy of decitabine in combination with standard-dose cytarabine, aclarubicin hydrochloride, and granulocyte colony-stimulating factor in the treatment of young patients with newly diagnosed acute myeloid leukemia

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Purpose: The chemotherapeutic regimen DCAG (decitabine with cytarabine, aclarubicin hydrochloride, and granulocyte colony-stimulating factor) is effective for elderly patients with acute myeloid leukemia, but recommendations for young patients remain controversial. This study investigated the tolerance and efficacy of DCAG for patients with newly diagnosed acute myeloid leukemia (aged 14–60 years). The clinical features or molecular markers that may predict response to DCAG were identified.

Patients and methods: One-hundred sixty-one consecutive patients with newly diagnosed acute myelogenous leukemia received DCAG or standard (idarubicin plus cytarabine, IA) induction chemotherapy (n=64 and 97, respectively).

Results: The rates of complete remission after the first cycle, overall survival (OS), and event-free survival (EFS) were comparable. After the second cycle, the complete remission rate of the DCAG group (54.7%) was significantly lower than that of the reference (78.35%, P=0.005). The following were associated with significantly worse OS, and EFS, in the DCAG group: Eastern Cooperative Oncology Group (ECOG) score ≥3 and no response after the second induction therapy; and FLT3-ITD. The multivariate analysis showed the DCAG group with significantly shorter OS associated with ECOG ≥3 and FLT3-ITD. In the DCAG group, after the first cycle of induction chemotherapy the median recovery times of neutrophils and platelets were 15.8 and 13 days.

Conclusion: The DCAG and IA groups were similar with regard to complete remission rate after the first cycle, OS, and EFS. The complete remission rate after the second cycle of the DCAG was significantly lower than that of the IA. Grade 4 neutropenia and thrombocytopenia were a major adverse event associated with DCAG.

Keywords: decitabine, acute myeloid leukemia, induction therapy, conventional chemotherapy

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Introduction

Despite recent progress in leukemogenesis and diagnosis of acute myelogenous leukemia (AML), advances in AML induction chemotherapy treatment are limited. Over the past years, combination chemotherapy with anthracycline and standard dose cytarabine (standard 3+7 induction therapy) remains the standard induction

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therapy. IA induction chemotherapy (idarubicin plus cytarabine, or, conventional chemotherapy) for AML, results in an overall response rate of about 70%.[1–7] Prognosis of patients who are resistant to standard induction chemotherapy is dismal.

The heterogeneity of AML suggests that two-drug 3+7 induction chemotherapy is unlikely to cure all patients, and that combinations of traditional chemotherapy with novel agents will be required to achieve this goal. Recent progress in matching clinical and genomic data may assist in selecting the best-individualized induction therapy for each patient. Emerging evidence indicates that a hypomethylating agent such as decitabine may be effective in certain AML subtypes and selected patients, providing further rationale for a personalized medicine approach. However, clinical data about decitabine in combined induction chemotherapy are limited for the treatment of AML.

Randomized trials of the hypomethylating agent decitabine, used solely to treat patients with newly diagnosed AML, have shown complete response rates of 18–28%, and median overall survival (OS) from 8 to 10 months.[7–13] Our research group and others[14–16] have combined the chemotherapy regime CAG (ie, low-dose cytarabine [10 mg/m^2 q12 hrs for 5 days], aclarubicin hydrochloride [10 mg/day for 5 days], and granulocyte colony-stimulating factor) with decitabine (DCAG). This regimen was designed to exploit the synergy among these agents to improve the proportion of patients achieving response. The overall rate of response (ie, hematologic improvement and partial and complete remission) for elderly AML patients to two cycles of DCAG has been 72.4%. In elderly AML patients, the 2-year disease-free survival and OS were, respectively, 36.9% and 59.6%. However, recommendations regarding DCAG chemotherapy for younger patients with AML have remained controversial.[8,17–25]

In this study, we evaluated the efficacy and toxicity of DCAG, relative to the standard dose chemotherapy regimen (IA), for patients with newly diagnosed AML, aged 14–60 years. The modifications included that the dose of cytarabine was increased to 100 mg/m^2 q12 hrs for 5 days and aclarubicin hydrochloride was increased to 20 mg/m^2 for 5 days. (For elderly patients with AML, the standard dosages of cytarabine and aclarubicin hydrochloride are 10 mg/m^2 q12 hrs and 10 mg/day, respectively, each for 5 days.)

The primary objective of this study was to determine whether induction therapy with DCAG resulted in similar remission rates, OS, or event-free survival (EFS) compared the IA regimen. Additionally, clinical features or molecular markers were investigated that may predict the response of patients with AML to DCAG, and may differentiate those patients who are more likely to respond to DCAG.

Materials and methods

The review board of Chinese PLA General Hospital approved all the study procedures, and the informed consent forms, in accordance with the Declaration of Helsinki.

Patients

Between April 2012 and September 2017, 161 consecutive patients with AML were enrolled in this study (Table 1). Among these patients, 97 received IA induction chemotherapy, with idarubicin and cytarabine for 7 days. DCAG was administrated to the other 64 patients.

All these patients received diagnoses of AML (not including acute promyelocytic leukemia) based on criteria of the French-American-British and World Health Organization,[1,5] and all patients provided written informed consent.

At diagnosis, bone marrow was obtained from each patient, and chromosomal analysis and immunophenotyping were conducted. The following molecular markers were analyzed: AML1-ETO, PML (promyelocytic leukemia)/RARA (retinoic acid receptor alpha), NPM1 (nucleophosmin 1), CBFB (core-binding factor beta)/MYH11 (myosin heavy chain 11), and MLL PTD (partial tandem duplication). Patients with AML1-ETO, PML/RARA, CBFB/MYH11, and/or NPM1 without FLT3 (FMS-like tyrosine kinase receptor 3)-ITD (internal tandem duplication) were defined as favorable-risk in accordance with the NCCN (National Comprehensive Cancer Network) AML risk status evaluation. Patients with the following were considered poor-risk: complex karyotypes, unfavorable cytogenetics, FLT3-ITD gene expression, or TP53 mutation. Other patients were classified as intermediate-risk.

Therapy

We retrospectively studied 161 patients with AML who received induction chemotherapy (DCAG or IA induction chemotherapy). The treatment choice was based on patient wishes, as policy. Specifically, 97 patients received IA induction chemotherapy, with idarubicin (10–12 mg/m^2) for 3 days and cytarabine (100 mg/m^2, every 12 hrs) for 7 days. Another 64 patients received a DCAG regimen: decitabine 20 mg/m^2, days 1–5; aclarubicin 20 mg/m^2, days 1–5; cytarabine 100 mg/m^2, every 12 hrs, days 1–5; and granulocyte colony-stimulating factor 300 μg/day subcutaneously from day 0 to the time of neutrophil recovery.

Table 1 Clinical characteristics of the patients[a]

		DCAG	IA	*P*
Acute myeloid leukemiapatients, n		64	97	
Male gender		37 (57.8)	58 (59.8)	0.870
Age at diagnosis, year[b]		43 (14–60)	38 (13–60)	0.624
White blood cells at diagnosis, $\times 10^9$/L[b]		24.1 (208.4–0.57)	36.2 (405.1–1.3)	0.321
Cytogenetic risk	Favorable	4 (6.25)	17 (17.50)	0.218
	Intermediate	45 (70.30)	58 (59.80)	
	Poor	11 (17.20)	16 (16.50)	
	No results	4 (6.25)	6 (6.20)	
Molecular abnormalities	Favorable	10 (15.6)	14 (14.40)	0.414
	Intermediate	28 (43.8)	54 (55.70)	
	Poor	24 (37.5)	29 (29.9)	
	Not performed	2 (3.1)	–	
Performance status (ECOG)	PS 0	8 (12.5)	22 (22.70)	0.528
	PS 1	19 (29.7)	24 (24.74)	
	PS 2	19 (29.7)	30 (30.93)	
	PS 3	8 (12.5)	16 (16.49)	
	PS 4	5 (7.8)	5 (5.15)	
Response after the first cycle	CR complex [c]	32 (50.0)	57 (58.76)	0.195
	PR	11 (17.2)	19 (19.59)	
	No response	20 (31.3)	18 (18.56)	
Response after the second cycle	CR complex [c]	35 (54.7)	76 (78.35)	0.005
	PR	10 (15.6)	4 (4.12)	
	No response	13(20.3)	12 (12.37)	
Consolidation after induction therapy	CT	27 (42.2)	31 (31.96)	0.211
	SCT	29 (45.3)	49 (50.52)	

Notes: [a]Reported as n (%), unless noted otherwise; [b]reported as median (range); [c]CR complex, complete remission (CR) + CR with incomplete blood count recovery (CRi).
Abbreviations: PS, performance status; CT, chemotherapy; CR, complete remission; PR, partial remission; SCT, stem cell transplant.

Consolidation chemotherapy was administered to 56 patients in the DCAG group and 89 patients in the IA induction chemotherapy group, consisting of the following: conventional dose of cytarabine and anthracycline, or mitoxantrone; or middle-to-high-dose cytarabine; or hematopoietic stem cell transplantation. Among them, the patients who received >2 cycles of consolidation chemotherapy were used for the survival analysis.

Routine blood count, liver function, and electrolyte and creatinine levels were recorded twice each week. Adverse events, concomitant medications, and clinical laboratory analyses were recorded weekly. The treatment continued until any of the following occurred: disease progression, intolerable toxicity, death, loss to follow-up, abandonment of treatment, or withdrawal of consent to further treatment. All patients received supportive care in accordance with institutional practices, including blood product transfusions and prophylactic or symptomatic use of anti-infective agents and cytokines, and other therapies appropriate for the symptomatic treatment of AML and its complications.

Targeted sequencing

The sequencing panel targets a ~250-kb genomic region, which comprises the entire coding sequences of 126 genes that are recurrently mutated in acute leukemia[8] (Tables S1 and S2). Mononuclear cells were enriched from pretreatment bone marrow by Ficoll density gradient centrifugation. Nimble Design GenSeq Cap EZ Choice was performed in accordance with the manufacturer's protocol. With an Illumina HiSeq 2500, multiplexed libraries were sequenced using 100-bp paired-end runs. Reads were aligned to human genomic reference sequences using the Burrows-Wheeler alignment tool (HG19, NCBI built 37). To identify single nucleotide polymorphisms and short insertions and deletion, MuTect2 was performed with recommended parameters. A subset of somatic mutations

was selected randomly for validation using Sanger sequencing (Table S3).

Efficacy evaluation

Routine blood cell counts were performed twice each week after chemotherapy. Three to four weeks after chemotherapy, bone marrow aspiration was performed and the responses to treatment were evaluated.

OS was measured from the time of diagnosis to death from any cause. EFS was measured from the time of first complete response to leukemia relapse. For secondary endpoints, bone marrow biopsies and aspirates were obtained from patients at the time of screening.

The nature of response was defined in accordance with the criteria of the International Working Group.[14] Specifically, for a complete response the patient demonstrated <5% bone marrow myeloblasts, no myeloblasts with Auer rods, the absence of extramedullary disease, an absolute neutrophil count $>1\times10^9$/L, and a platelet count $\geq100\times10^9$/L. A complete response with incomplete blood count recovery was diagnosed when the patient had <5% bone marrow myeloblasts, no myeloblasts with Auer rods, the absence of extramedullary disease, but with incomplete blood cell recovery. A partial response was defined as a decrease of ≥50% (ie, to 5–25% total) in the myeloblasts detected in bone marrow aspirates, and those with normalized blood counts. No response was the absence of both complete and partial response. Relapse was the reappearance of leukemia cells in the peripheral blood, or >5% myeloblasts in the bone marrow. Induction death was defined as death occurring before response evaluation, unless evidence of resistant disease was provided at least 7 days after the conclusion of the chemotherapy.

Neutrophil and platelet recovery were defined, respectively, as absolute neutrophil count $>0.5\times10^9$/L and platelet count $>30\times10^9$/L, for 3 consecutive days. The time to neutrophil or platelet recovery was the time to the first day of 3 consecutive days of recovery. Toxicities were assessed in accordance with the National Cancer Institute Common Toxicity Criterion Version 3.[14]

Statistical analyses

We studied associations between various gene mutations and patient clinical characteristics, using Fisher's exact test or chi-squared tests for categorical endpoints (eg, response), and the Wilcoxon rank-sum test for continuous variables. Analyses of treatment outcomes were based on commonly accepted definitions of complete remission, OS, and EFS. *P*-values were calculated using the Kaplan–Meier method for survival analyses. A Cox proportional hazard model was used to assess the prognostic significance of the genetic mutations and clinical variables. To investigate clinical features and genetic mutations predicting outcomes after DCAG or IA induction chemotherapy, logistic and Cox multivariable analyses of the entire cohort for EFS and OS were performed, including treatment arm as a covariate. All analyses were performed with GraphPad Prism 5 software. Statistical analyses were conducted with SPSS 19.0. $P<0.05$ was considered statistically significant.

Results

Patient characteristics

This study enrolled 161 individuals with newly diagnosed AML (median age 43.8 years), among whom 64 and 97, respectively, were treated with the DCAG regimen and IA induction chemotherapy (Table 1). The two groups were similar with respect to gender, risk status, white blood cell (WBC), and performance status.

The DCAG group received a median of 2 cycles induction therapy of DCAG; 56 (87.5%) received >2 cycles of consolidation therapy, including consolidation chemotherapy or stem cell transplantation (Figure 1). The IA induction chemotherapy group underwent a median of 2 cycles of IA induction chemotherapy; 80 (82.5%) received >2 cycles of other consolidation chemotherapy or stem cell transplantation. All patients underwent molecular testing at baseline using a next-generation sequencing 126-gene panel, comprising genes that are recurrently known to be mutated in acute leukemia (Tables S1 and S2).

Response and survival

Of the 64 subjects enrolled in the DCAG group, 43 responded to a single induction cycle. Of these, 32 (50%), 11 (17.2%), and 20 (31.3%) experienced, respectively, a complete (with complete or incomplete count recovery), partial, or no response (Table 1). Forty-five responded to 2 induction cycles; of these, 35 (54.7%), 10 (15.6%), and 13 (20.3%) experienced achieved a complete (with complete or incomplete count recovery), partial, or no response. All subjects with a partial response had a complete hematologic response with a median bone marrow myeloblast count of 13.2% at the beginning of the second induction. One patient withdrew from the DCAG group before a bone marrow biopsy could be

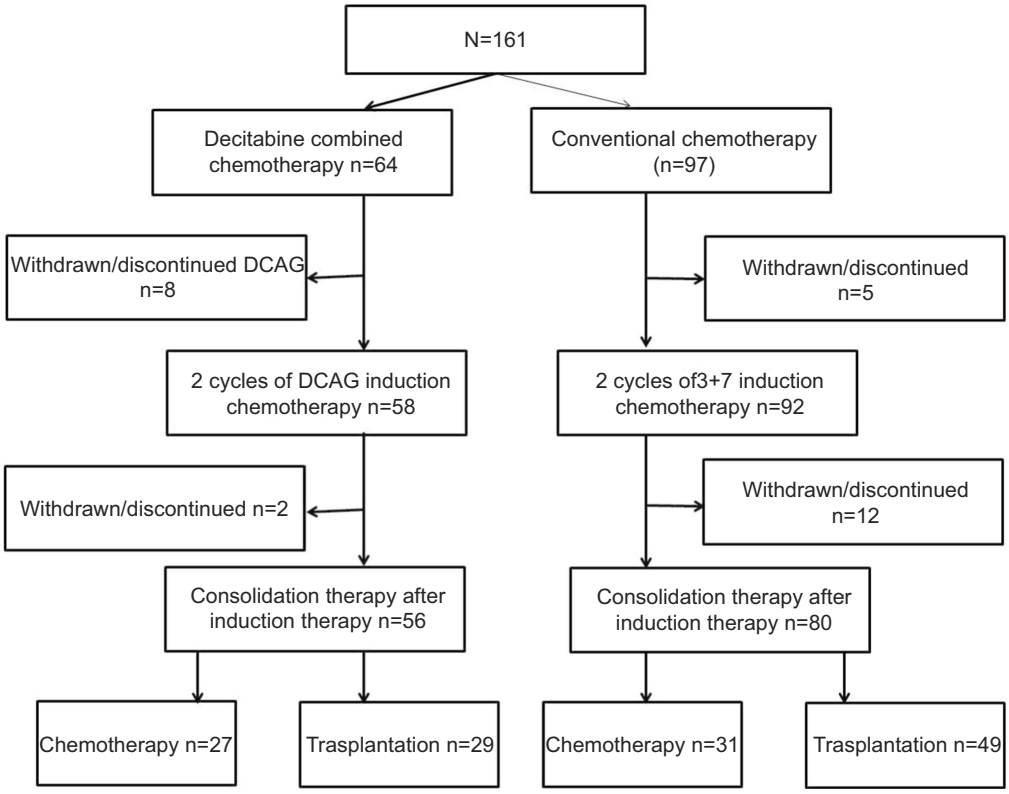

Figure 1 Schematic of patient selection for analysis.
Abbreviation: DCAG, decitabine combined with chemotherapy.

performed at the end of the first cycle and therefore could not be clinically evaluated for a response.

Of the 97 subjects in the IA induction chemotherapy group, 76 responded to a single induction cycle. Of these, 57 (58.7%), 19 (19.6%), and 18 (18.6%) showed, respectively, a complete (with complete or incomplete count recovery), partial, or no response (Table 1). Eighty responded to 2 induction cycles; of these, 76 (78.35%), 4 (4.12%), and 12 (12.37%) experienced, respectively, a complete (with complete or incomplete count recovery), partial, or no response.

The DCAG and IA induction chemotherapy groups were statistically similar with regard to complete remission rate after the first cycle (P=0.195), OS (P=0.271), and EFS (P=0.831). Kaplan–Meier estimates for OS for the DCAG group at 1 and 2 years were 66.2% (95% CI 50.3–78.1%) and 60.2% (95% CI 50.3–78.1%). The estimated EFSs of the DCAG group at 1 and 2 years were 61.8% (95% CI 45.8–73.6%) and 58.4% (95% CI 41.9–71.7%). Kaplan–Meier estimates for OS for the IA group at 1 and 2 years were 80.9% (95% CI 7.09–87.7%) and 64.7% (95% CI 52.8–74.3%). The estimated EFSs for the IA group at 1 and 2 years were 73.1% (95% CI 62.5–81.2%) and 62.6% (95% CI 50.9–72.3%).

Among the 15 patients with FLT3-ITD mutation in the DCAG group, after 2 induction cycles, 5 (33.33%), 4 (26.67%), and 6 (40.00%) achieved, respectively, a complete (with complete or incomplete count recovery), partial, or no response. Among 11 patients with FLT3-ITD mutation in the IA induction chemotherapy group, after 2 induction cycles, 8 (72.70%), 1 (9.09%), and 2 (18.17%) achieved, respectively, a complete (with complete or incomplete count recovery), partial, or no response. When considering only the patients with FLT3-ITD mutation, the response of those receiving DCAG was similar to that of the patients who received IA induction chemotherapy (P=0.395). In the present study, 9 FLT3-ITD-positive patients in the DCAG group and 9 FLT3-ITD-positive patients in the DCAG group received allogeneic peripheral blood stem cell transplantation.

Prognostic significance of clinical features and gene mutations in patients receiving DCAG

Several disease and patient characteristics are known to affect survival in AML. These include Eastern Cooperative Oncology Group (ECOG) score ≥3;

cytogenetics poor-risk status; NCCN poor-risk status; with or without hematopoietic stem cell transplantation; number of DNA methylation-related mutations; number of overall mutations; lack of response (i.e, no response, NR) after the first induction therapy; NR after the second induction therapy; and without extramedullary infiltration.

The associations of these variables with OS and EFS were investigated. For the patients who underwent DCAG, the following factors were associated with poor OS according to the log-rank test: ECOG score ≥3 (P=0.001); NCCN high-risk status (P=0.004); NR after the second induction therapy (P=0.040); and FLT3-ITD (P=0.0001; Figure 2A). In addition, the following factors

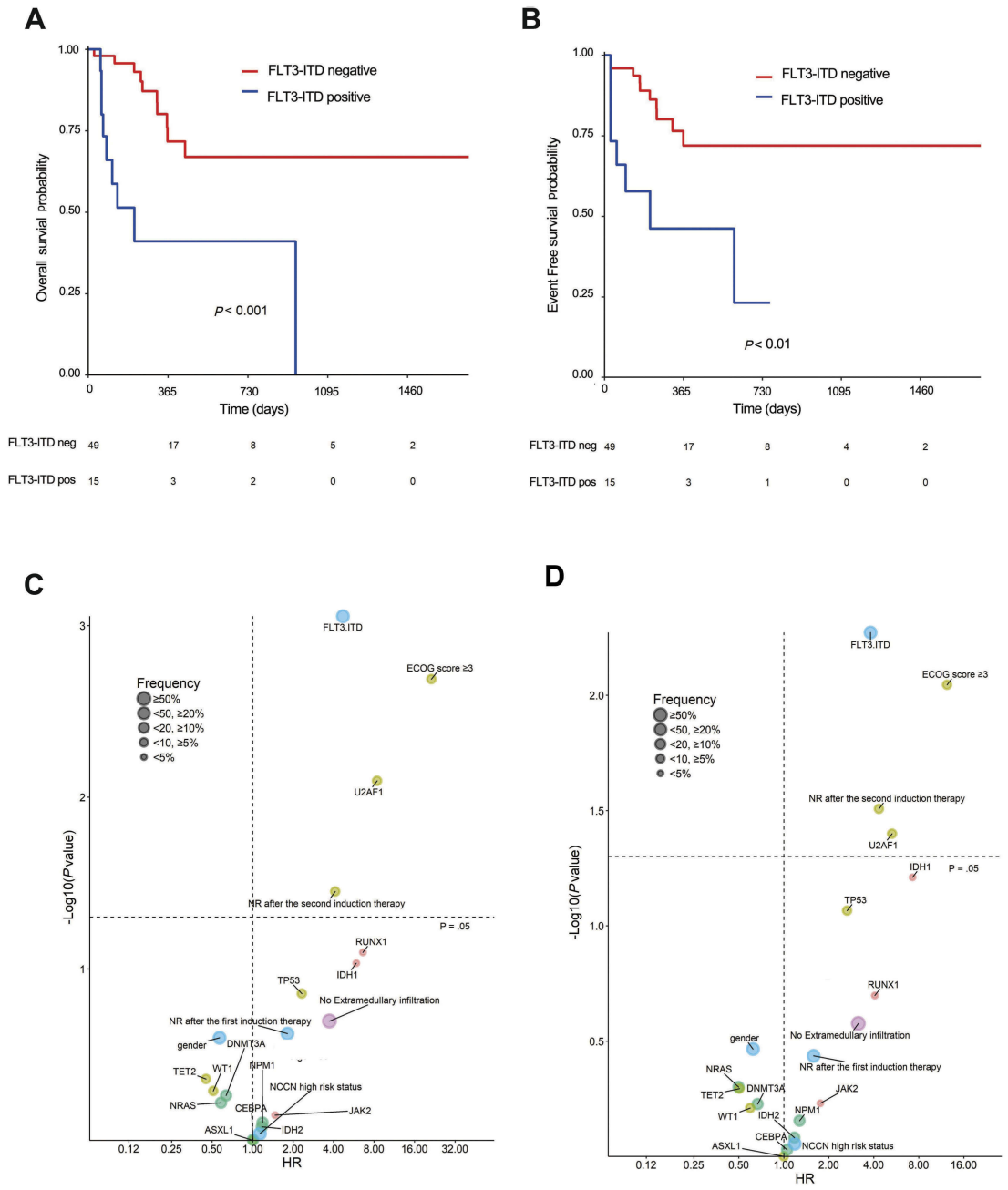

Figure 2 Effects of genetic risk on survival in patients receiving DCAG therapy. (**A–D**) Kaplan–Meier curves for patients with and without mutations are depicted for patients who received DCAG. (**A**) FLT3-ITD for OS. (**B**) FLT3-ITD for EFS. (**C** and **D**) Volcano plots of hazard ratios (in horizontal axis) and corresponding *P*-values (in vertical axis) according to the univariate analysis of the effect of individual genetic and clinical features on (**C**) OS and (**D**) EFS. Size of circles corresponds to the fraction of patients carrying indicated factors. Those that are significant (P<0.05) are annotated.

Abbreviations: DCAG, decitabine combined with chemotherapy; OS, overall survival; EFS, event free survival; FLT3-ITD, FMS-like tyrosine kinase receptor 3 internal tandem duplication; HR, hazard ratios; NR, no response; NCCN, National Comprehensive Cancer Network; ECOG, Eastern Cooperative Oncology Group.

were associated with poor EFS for patients who underwent DCAG: ECOG score ≥3 (P=0.0004); NCCN poor-risk status (P=0.008); cytogenetics poor-risk status (P=0.025); NR after the second induction therapy (P=0.046); or FLT3-ITD (P=0.002; Figure 2B).

Next examined was the HR for death associated with mutations in the 10 genes mutated in >5% of patients in this DCAG cohort (Table 2, Figure 2C and D). In the univariable analysis, FLT3-ITD mutations were also associated with shorter OS (HR 4.69, 95% CI 1.89–11.69, P<0.05) and shorter EFS (HR 3.80, 95% CI 1.49–9.73, P<0.05). No genetic mutations were associated with longer EFS or OS.

In the DCAG group, the univariate analysis determined that the following were associated with OS and EFS (Table 2, Figure 2C and D): ECOG score ≥3 (OS, HR 7.66, 95% CI 1.39–42.16, P=0.019; EFS, HR 6.04, 95% CI 1.08–33.89, P=0.041); NR after the second induction therapy (OS, HR 4.12, 95% CI 1.10–15.40, P=0.035; EFS, HR 4.34, 95% CI 1.14–16.45, P=0.031); and FLT3-ITD (OS, HR 4.69, 95% CI 1.89–11.69, P=0.001; EFS, HR 3.80, 95% CI 1.49–9.73, P=0.005). Also in the DCAG group, the multivariate analysis (Table 2) showed that the following were significantly associated with shorter OS: ECOG score ≥3 (HR 66.75, 95% CI 1.49–298.53, P=0.030), and FLT3-ITD (HR 20.08, 95% CI 1.18–-342.10, P=0.038).

Prognostic significance of clinical features and gene mutations in patients receiving IA induction chemotherapy

In patients receiving IA induction chemotherapy, the log-rank test indicated that NR after the first (OS, P=0.0004; EFS, P=0.001) or second induction therapy (OS, P=0.0003; EFS, P=0.005) defined a set of subgroups with poor OS and EFS (Figure S1). The univariate analysis showed that the following were significantly associated with OS and EFS (Table 3, Figure 3A and B): NR after the first induction therapy (OS, HR 4.53, 95% CI 2.03–10.10, P=0.0004; EFS, HR 3.97, 95% CI 1.79–8.80, P=0.012) or second induction therapy (OS, HR 11.33, 95% CI 5.13–25.03, P=0.0003; EFS, HR 13.02, 95% CI 5.87–28.86, P=0.001); NCCN poor-risk status (OS, HR 3.37, 95% CI 1.35–8.41, P=0.009; EFS, HR 3.53, 95% CI 1.41–8.82, P=0.007); and no extramedullary infiltration (OS, HR 0.35, 95% CI 0.17–0.71, P=0.004; EFS, HR 0.32, 95% CI 0.16–0.65, P=0.002). In these patients, the univariate analysis also showed that a ECOG score ≥3 (HR 3.99, 95% CI 1.00–15.84, P=0.050) was significantly associated with OS. The multivariate analysis showed that no extramedullary infiltration (HR 0.07, 95% CI 0.02–0.32, P=0.001) and an ECOG score ≥3 (HR 14.49, 95% CI 2.59–83.33, P=0.009) were significantly associated with shorter OS. Significantly associated with EFS were no extramedullary infiltration (HR 0.15, 95% CI 0.05–0.05, P=0.009).

Table 2 Univariate and multivariate analyses for the risk factors of OS and EFS in 64 AML patients receiving DCAG therapy (with statistical significance)*

	Univariate				Multivariate	
	OS (HR 95% CI)	***P***	**EFS (HR 95% CI)**	***P***	**OS (HR 95% CI)**	***P***
NR post-first induction	1.82 (0.67–4.89)	0.239	0.50 (0.11–2.331)	0.377	–	–
NR post-second induction	4.12 (1.10–15.40)	0.035	4.34 (1.14–16.45)	0.031	–	–
No extramedullary infiltration	3.73 (0.50–28.15)	0.201	3.16 (0.42–3.81)	0.265	–	–
NCCN poor-risk status	5.25 (0.69–40.16)	0.110	5.07 (0.66–38.84)	0.119	–	–
Cytogenetic poor-risk status	69.16 (0–94.15)	0.998	72.24 (0–98.56)	0.998	–	–
Stem cell transplant	0.60 (0.17–2.09)	0.421	0.66 (0.19–2.34)	0.523	–	–
ECOG score ≥3	7.66 (1.39–42.16)	0.019	6.04 (1.08–33.89)	0.041	66.75 (1.49–298.53)	0.030
ASXL1	1.00 (0.13–7.56)	0.998	1.00 (0.13–7.59)	0.999	–	–
U2AF1	8.45(1.74–40.92)	0.005	5.31 (1.08–26.08)	0.040	–	–
FLT3-ITD	4.69 (1.89–11.69)	0.001	3.80 (1.49–9.73)	0.005	20.08 (1.18–342.10)	0.038
IDH2	1.17 (0.27–5.11)	0.833	–	–	–	–

Note: *reported as OS or EFS (HR 95% CI).

Abbreviations: OS, overall survival; EFS, event free survival; AML, acute myeloid leukemia; DCAG, decitabine combined with chemotherapy; HR, hazard ratios; CI, cumulative incidence; NR, no response; NCCN, National Comprehensive Cancer Network; ECOG, Eastern Cooperative Oncology Group; ASXL1, additional sex combs like transcriptional regulator 1; U2AF1, U2 small nuclear RNA auxiliary factor 1; FLT3-ITD, FMS-like tyrosine kinase receptor 3 internal tandem duplication; IDH2, isocitrate dehydrogenase 2.

Table 3 Univariate and multivariate analyses for the risk factors of OS and EFS in 97 AML patients receiving IA chemotherapy (with statistical significance)*

	Univariate				Multivariate			
	OS (HR 95% CI)	*P*	EFS (HR 95% CI)	*P*	OS (HR 95% CI)	*P*	EFS (HR 95% CI)	*P*
NR post-first induction	4.53 (2.03–10.10)	0.0004	3.97 (1.79–8.80)	0.012	–	–	–	–
NR post-second induction	11.33 (5.13–25.03)	0.0003	13.02 (5.87–28.86)	0.001	–	–	–	–
No Extramedullary infiltration	0.35 (0.17–0.71)	0.004	0.32 (0.16–0.65)	0.002	0.07 (0.02–0.32)	0.001	0.15 (0.05–0.05)	0.009
NCCN poor-risk status	3.37 (1.35–8.41)	0.009	3.53 (1.41–8.82)	0.007	–	–	–	–
Cytogenetic poor-risk status	2.09 (0.38–11.45)	0.394	2.33 (0.43–12.75)	0.328	–	–	–	–
Stem cell transplant	0.13 (0.06–0.28)	0.0004	0.14 (0.07–0.29)	0.0005	0.08 (0.02–0.33)	0.0003	0.12 (0.03–0.39)	0.0004
ECOG score ≥3	3.99 (1.00–15.84)	0.050	3.54 (0.91–13.81)	0.069	14.49 (2.59–83.33)	0.009	–	–
U2AF1	1.64 (0.22–12.03)	0.628	1.65 (0.23–12.07)	0.624	–	–	–	–
FLT3-ITD	0.48 (0.12–2.03)	0.320	0.75 (0.23–2.45)	0.632	–	–	–	–
ASXL1	2.63 (1.13–6.11)	0.025	3.08 (1.33–7.11)	0.009	–	–	–	–
IDH2	2.71 (0.95–7.75)	0.064	3.03 (1.05–8.68)	0.040	–	–	–	–

Note: *reported as OS or EFS (HR 95% CI).

Abbreviations: OS, overall survival; EFS, event free survival; AML, acute myeloid leukemia; DCAG, decitabine combined with chemotherapy; HR, hazard ratios; CI, cumulative incidence; NR, no response; NCCN, National Comprehensive Cancer Network; ECOG, Eastern Cooperative Oncology Group; ASXL1, additional sex combs like transcriptional regulator 1; U2AF1, U2 small nuclear RNA auxiliary factor 1; FLT3-ITD, FMS-like tyrosine kinase receptor 3 internal tandem duplication; IDH2, isocitrate dehydrogenase 2.

Clinical features of patients with mutations in DNA methylation

In this study, the highest rate of mutations were class I (62/161, 38.5%), such as FLT3-ITD, KIT, NRAS, KRAS, and PTPN11 (protein tyrosine phosphatase, non-receptor type 11). The next most frequent were epigenetic modification mutations (60/161, 37.3%) including DNMT3A, TET2, IDH1/2, ASXL1, DOT1L. The third and fourth most frequent mutations were class II (42/161, 26.1%; NPM1, CEBPA, RUNX1, GATA2, and ETV6) and tumor suppressor mutations (26/161, 16.1%; WT1, PHF6, and TP53). Also, spliceosome genes, cohesion complex genes, and NOTCH family mutations were identified in 12 (7.5%), 17 (10.6%) and 1 (0.6%) patient, respectively (Figure S2). Spliceosome genes included U2AF1, SRSF2, and SF3B1/2, and cohesion complex genes were STAG2, RAD21, SMC1A, and SMC3.

Gene mutations related to DNA methylation (TET2, DNMT3A, and IDH1/IDH2) are among the most frequently identified in AML, and demethylating agents are effectively used in treating AML (6–8). Therefore, we analyzed the clinical features of the patients harboring these mutations. Of the 161 patients with follow-up data, 37 carried altogether 43 mutations in TET2 or DNMT3A/DNMT3B, with or without IDH1/IDH2. DNMT3A/IDH1 co-mutations were found in 4 patients, DNMT3A/TET2 co-mutations in one patient, and DNMT3A/IDH2 co-mutation in one patient. Thirty-one patients carried only one DNA methylation-related mutation. Compared with the 124 patients without DNA methylation-related mutations (age 40.9 years, favorable risk status 19.83%, ≥2 mutations 59.7%), the 37 patients with these mutations were significantly older with progressive diseases (53.5 years; P=0.01), with lower favorable risk status (2.86%; P=0.027), and were more likely to have ≥2 mutations (91.9%; P=0.0004). However, the 2 groups were similar in EFS and OS (P=0.36 and P=0.47, respectively).

In the DCAG group specifically, there were no significant differences in OS or EFS (P=0.57 and P=0.48, respectively) between patients without DNA methylation-related mutations and those with such mutations (TET2, DNMT3A, and IDH1/IDH2).

Hematopoietic toxicity and treatment-related death

Grade 4 neutropenia and thrombocytopenia were universal in the study population (Table S4).

In the DCAG group specifically, after the first cycle of induction chemotherapy the median recovery times of

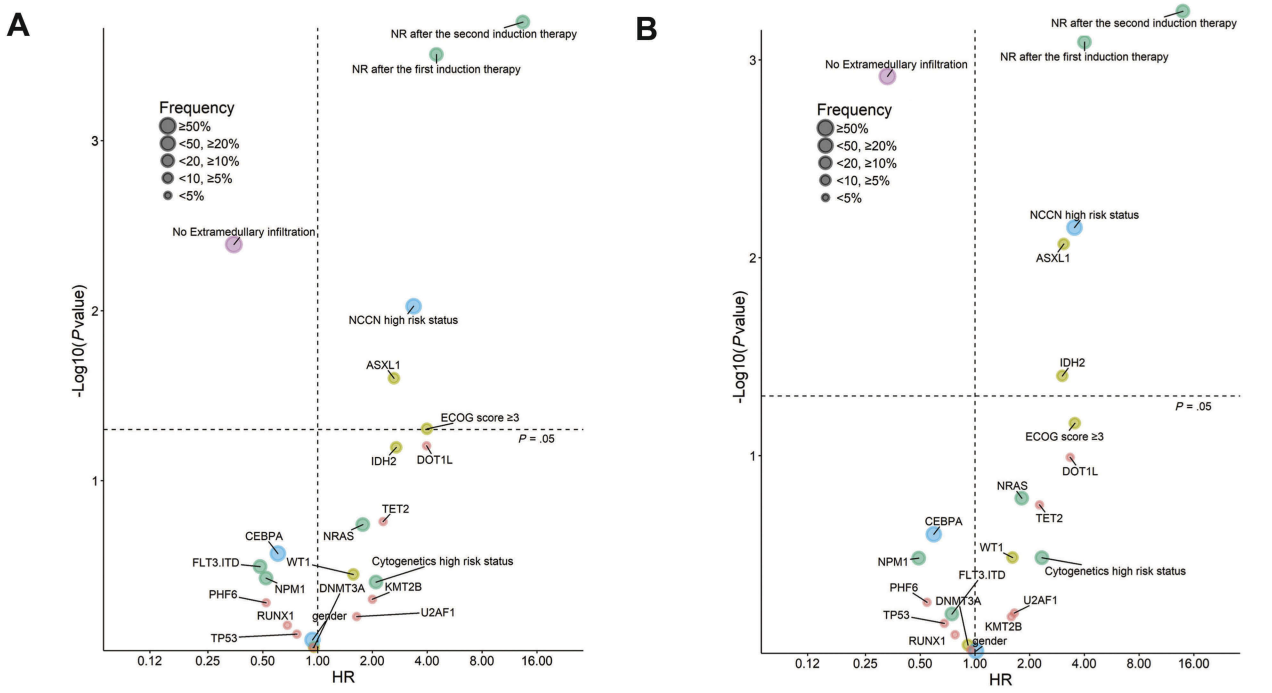

Figure 3 Effects of genetic risk on survival in patients receiving IA chemotherapy. (**A** and **B**) Volcano plots of hazard ratios (in horizontal axis) and corresponding *P*-values (in vertical axis) according to univariate analysis of the effect of individual genetic and clinical features on (**A**) OS and (**B**) EFS. Size of circles corresponds to the fraction of patients carrying indicated factors. Those that are significant ($P<0.05$) are annotated.

Abbreviations: IA: idarubicin plus cytarabine; HR; hazard ratios; NR: No response; NCCN: National Comprehensive Cancer Network; ECOG: Eastern Cooperative Oncology Group. OS: overall survival; EFS: event free survival.

neutrophils and platelets were 15.8 and 13 days, respectively. Platelet recovery ($\geq 20\times10^9$/L) typically preceded WBC count recovery after induction chemotherapy for AML. The pace of platelet recovery was generally brisk, with a median of 13 days for the platelet level to rise higher than 20×10^9/L. The most common grade-3 or grade-4 adverse events were related to myelosuppression. No patients in the DCAG group died during the induction therapy and no subject required to transfer to intensive care.

Discussion

This study evaluated the efficacy and toxicities of DCAG (standard dose cytarabine [100 mg/m^2 q12 hrs for 5 days] and increased dose of aclarubicin hydrochloride [20 mg/d for 5 days]) relative to standard dose chemotherapy regimens (control) for non-elderly patients with newly diagnosed AML (aged 14–60 years). Pretreatment genetic testing was also conducted. The DCAG and IA induction chemotherapy groups were statistically similar with regard to complete remission rate after the first cycle, OS, and EFS.

Previous reports[6,26] showed that AML patients treated with decitabine only responded poorly. Most patients required at least two monthly cycles to achieve a clinical response, and many needed three or more cycles. The present study showed that induction therapy combining decitabine with a modified CAG regimen was safe, but with a complete remission rate of 54.7% after two cycles of induction chemotherapy. The DCAG regimen was well tolerated, with a low early-death rate and short duration of pancytopenia. The clinical response toward DCAG motivated us to investigate further for biomarkers of response and prognosis.

The spectrum of frequent mutations in the AML patients of our study is similar to that reported for other large AML populations.[27] Of the 72 identified genes, 10 genes in our study were mutated in >5% of the patients. The two most commonly identified mutations in the present study were in CEBPA (19.9%) and FLT3-ITD (16.1%), and then mutations in NRAS (13.0%), NPM1 (12.4%), DNMT3A (11.2%), ASXL1 (8.7%), and IDH2 (8.1%). The genes with a mutation frequency of >10% (CEBPA, FLT3-ITD, NPM1, and DNMT3A) were similarly reported in studies by Lin et al[27] and Mccurdy and Levis.[28] Despite the prevalence of dozens of these recurrent mutations in AML, only NPM1, CEBPA, FLT3-ITD, and TP53 have been used in widely accepted risk-stratification schemas, such as the NCCN guidelines.

The Fms-like tyrosine kinase 3 (FLT3) gene has been an important marker in acute myeloid leukemia, where FLT3

mutations have been associated with clinical prognosis and treatment.[29–31] Studies have found that survival for FLT3-ITD AML is improved by allogeneic stem cell transplantation in CR1.[29–32] In the present study, 9 FLT3-ITD-positive patients in the DCAG group, and 9 FLT3-ITD-positive patients in the IA group, received allogeneic peripheral blood stem cell transplantation. In the DCAG group, mutations of FLT3-ITD were identified as significantly associated with poor OS and poor EFS. The outcomes associated with FLT3-ITD mutations in the DCAG group contrast with those of patients who received IA chemotherapy, in whom the presence of FLT3-ITD mutations had no effect on OS or EFS. As there are 15 patients with a FLT3-ITD mutation in the DCAG group and 11 in the IA group, more studies are warranted to verify our results.

In conclusion, the DCAG and IA induction chemotherapy groups were statistically similar with regard to complete remission rate after the first cycle, OS, and EFS. However, after the second cycle, the complete remission rate of the DCAG group was significantly lower than that of the IA reference. The DCAG regimen was well tolerated, with a low early-death rate and short duration of pancytopenia. Clinical sequencing provides important information for accurate prognostication in patients. Recommendations for chemotherapy should be based on both molecular mutations and clinical features.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Dou L, Zheng D, Li J, et al. Methylation-mediated repression of microRNA-143 enhances MLL-AF4 oncogene expression. *Oncogene*. 2012;31:507–517. doi:10.1038/onc.2011.248
2. Dou L, Li J, Zheng D, et al. MicroRNA-142-3p inhibits cell proliferation in human acute lymphoblastic leukemia by targeting the MLL-AF4 oncogene. *Mol Biol Rep*. 2013;40:6811–6819. doi:10.1007/s11033-013-2798-6
3. Dou L, Li J, Zheng D, et al. MicroRNA-205 downregulates mixed-lineage-AF4 oncogene expression in acute lymphoblastic leukemia. *Onco Targets Ther*. 2013;2013:1153–1160.
4. Attar EC, Johnson JL, Amrein PC, et al. Bortezomib added to daunorubicin and cytarabine during induction therapy and to intermediate-dose cytarabine for consolidation in patients with previously untreated acute myeloid leukemia age 60 to 75 years: CALGB (Alliance) study 10502. *J Clin Oncol*. 2013;31:923. doi:10.1200/JCO.2013.49.0219
5. Bowen DT. Etiology of acute myeloid leukemia in the elderly. *Semin Hematol*. 2006;43:82–88. doi:10.1053/j.seminhematol.2006.01.005
6. Cashen AF, Schiller GJ, O'Donnell MR, Dipersio JF. Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia. *J Clin Oncol*. 2010;28:556–561. doi:10.1200/JCO.2009.23.9178
7. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. *J Clin Oncol*. 2010;28:562–569. doi:10.1200/JCO.2009.23.8329
8. Welch JS, Petti AA, Miller CA, et al. TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. *N Engl J Med*. 2016;375:2023–2036. doi:10.1056/NEJMoa1605949
9. Estey EH. Epigenetics in clinical practice: the examples of azacitidine and decitabine in myelodysplasia and acute myeloid leukemia. *Leukemia*. 2013;27:1803–1812. doi:10.1038/leu.2013.173
10. Ferrara F, Musto P. Hypomethylating agents for the treatment of acute myeloid leukemia in the elderly. *Cancer*. 2011;117:3879–3881. doi:10.1002/cncr.25934
11. Goodyear O, Agathanggelou A, Novitzkybasso I, et al. Induction of a CD8+ T-cell response to the MAGE cancer testis antigen by combined treatment with azacitidine and sodium valproate in patients with acute myeloid leukemia and myelodysplasia. *Blood*. 2010;116:1908. doi:10.1182/blood-2009-11-249474
12. Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. *J Clin Oncol*. 2012;30:2670–2677. doi:10.1200/JCO.2011.38.9429
13. Craddock C, Houlton AE, Quek LS, et al. Outcome of azacitidine therapy in acute myeloid leukemia is not improved by concurrent vorinostat therapy but is predicted by a diagnostic molecular signature. *Clin Cancer Res*. 2017;23:6430–6440. doi:10.1158/1078-0432.CCR-17-1423
14. Yu J, Jin X, Wang L, et al. Decitabine-based chemotherapy followed by haploidentical lymphocyte infusion improves the effectiveness in elderly patients with acute myeloid leukemia. *Oncotarget*. 2017;8:53654.
15. Zheng R, Xie B, Wang C, Yu B, Xu J, Tan H. D-CAG (decitabine followed by cytarabine, aclarubicin, and G-CSF) for relapsed acute myeloid leukemia after hematopoietic cell transplantation. *Ann Hematol*. 2016;95:157–159. doi:10.1007/s00277-015-2505-x
16. Song LX, Xu L, Li X, et al. Clinical outcome of treatment with a combined regimen of decitabine and aclacinomycin/cytarabine for patients with refractory acute myeloid leukemia. *Ann Hematol*. 2012;91:1879. doi:10.1007/s00277-012-1550-y
17. Chang CK, Zhao YS, Xu F, et al. TP53 mutations predict decitabine-induced complete responses in patients with myelodysplastic syndromes. *Br J Haematol*. 2017;176:600. doi:10.1111/bjh.14455
18. Takahashi K, Patel K, Buesoramos C, et al. Clinical implications of TP53 mutations in myelodysplastic syndromes treated with hypomethylating agents. *Oncotarget*. 2016;7:14172. doi:10.18632/oncotarget.7290
19. Metzeler KH, Walker A, Geyer S, et al. DNMT3A mutations and response to the hypomethylating agent decitabine in acute myeloid leukemia. *Leukemia*. 2012;26:1106. doi:10.1038/leu.2011.342
20. Bejar R, Lord A, Stevenson K, et al. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. *Blood*. 2014;124:2705–2712. doi:10.1182/blood-2014-06-582809
21. Jung SH, Kim YJ, Yim SH, et al. Somatic mutations predict outcomes of hypomethylating therapy in patients with myelodysplastic syndrome. *Oncotarget*. 2016;7:55264–55275. doi:10.18632/oncotarget.10526

22. Lh VDH, Berger G, Diepstra A, Huls G, Vellenga E. Overexpression of TP53 is associated with poor survival, but not with reduced response to hypomethylating agents in older patients with acute myeloid leukaemia. *Br J Haematol*. 2017;178:810–812.
23. Dinardo CD, Patel KP, Garciamanero G, et al. Lack of association of IDH1, IDH2, and DNMT3A mutations with outcome in older patients with AML treated with hypomethylating agents. *Leuk Lymphoma*. 2014;55:1925–1929. doi:10.3109/10428194.2013.855309
24. Yan P, Frankhouser D, Murphy M, et al. Genome-wide methylation profiling in decitabine-treated patients with acute myeloid leukemia. *Blood*. 2012;120:2466–2474. doi:10.1182/blood-2012-05-429175
25. Hiller JK, Schmoor C, Gaidzik VI, et al. Evaluating the impact of genetic and epigenetic aberrations on survival and response in acute myeloid leukemia patients receiving epigenetic therapy. *Ann Hematol*. 2017;96:559. doi:10.1007/s00277-017-3116-5
26. Ritchie EK, Feldman EJ, Christos PJ, et al. Decitabine in patients with newly diagnosed and relapsed acute myeloid leukemia. *Leuk Lymphoma*. 2013;54:2003–2007. doi:10.3109/10428194.2012.762093
27. Lin PH, Li HY, Fan SC, et al. A targeted next-generation sequencing in the molecular risk stratification of adult acute myeloid leukemia: implications for clinical practice. *Cancer Med*. 2017;6. doi:10.1002/cam4.969
28. Mccurdy SR, Levis MJ. Emerging molecular predictive and prognostic factors in acute myeloid leukemia. *Leuk Lymphoma*. 2017;59:1–19.
29. Kar SA, Jankowska A, Makishima H, et al. Spliceosomal gene mutations are frequent events in the diverse mutational spectrum of chronic myelomonocytic leukemia but largely absent in juvenile myelomonocytic leukemia. *Haematologica*. 2013;98:107–113. doi:10.3324/haematol.2012.064048
30. Makishima H, Visconte V, Sakaguchi H, et al. Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. *Blood*. 2012;119:3203. doi:10.1182/blood-2011-10-388512
31. Kihara R, Nagata Y, Kiyoi H, et al. Comprehensive analysis of genetic alterations and their prognostic impacts in adult acute myeloid leukemia patients. *Leukemia*. 2014;28:1586–1595. doi:10.1038/leu.2014.55
32. Ohgami RS, Ma L, Merker JD, et al. Next-generation sequencing of acute myeloid leukemia identifies the significance of TP53, U2AF1, ASXL1, and TET2 mutations. *Mod Pathol*. 2015;28:706–714. doi:10.1038/modpathol.2014.160