



REVIEW

The Clinical Value of Decitabine Monotherapy in Patients with Acute Myeloid Leukemia

Valeria Santini · Michael Lübbert · Agnieszka Wierzbowska · Gert J. Ossenkoppele

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ABSTRACT

Decitabine (5-aza-2'-deoxycytidine) is a hypomethylating agent used in the treatment of acute myeloid leukemia (AML). Decitabine inhibits DNA methyltransferases, causing DNA hypomethylation, and leading amongst others to re-expression of silenced tumor suppressor genes. Decitabine is indicated for the treatment of adult patients with newly diagnosed de novo or secondary AML who are not eligible for standard induction chemotherapy. The initial authorization in 2012 was based on the results of the open-label, randomized, multicenter phase 3 DACO-016 trial, and supported by data from the supportive phase 2 open-label DACO-

017 trial. Compared with standard care, decitabine significantly improved overall survival, event-free survival, progression-free survival, and response rate. Decitabine was generally well tolerated, offering a valuable treatment option in patients with AML irrespective of age, especially for patients achieving a complete response. Several observational “real-life” studies confirmed these results. In contrast to standard chemotherapy, the presence of adverse-risk karyotypes or *TP53* mutations does not negatively impact sensitivity to hypomethylating therapy albeit with lower durability. Data suggest a potential positive effect of decitabine in patients with monosomal karyotype-positive AML. For the time being, decitabine is an appropriate option as monotherapy for patients with AML who are unfit to receive more intensive combination therapies, but emerging data suggest that decitabine-based doublet or triplet combinations may be future treatment options for patients with AML.

V. Santini (✉)
Department of Experimental and Clinical Medicine,
MDS Unit, AOUC- University of Florence, Florence,
Italy
e-mail: valeria.santini@unifi.it

M. Lübbert
Department of Internal Medicine I, Medical Center,
University of Freiburg, Faculty of Medicine,
University of Freiburg Medical Center, Freiburg,
Germany

A. Wierzbowska
Department of Hematology, Medical University of
Lodz, Lodz, Poland

G. J. Ossenkoppele
Department of Haematology, Location VU
University Medical Center, Amsterdam, The
Netherlands

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Key Summary Points

Decitabine (5-aza-2'-deoxycytidine) is a hypomethylating agent (HMA) used in the treatment of acute myeloid leukemia (AML)

Decitabine is indicated in the EU for the treatment of adult patients with newly diagnosed de novo or secondary AML who are not eligible for standard induction chemotherapy

In the registration trial, decitabine significantly improved overall survival, event-free survival, progression-free survival, and response rate compared with standard care, and was generally well tolerated

Similar results were seen in real-world studies

In contrast to standard chemotherapy, the presence of adverse-risk karyotypes or *TP53* mutations does not negatively impact sensitivity to hypomethylating therapy

Future areas of research are necessary to identify biomarkers of response and resistance, and to investigate the use of decitabine-based combination to further improve outcomes in patients with AML

INTRODUCTION

Acute myeloid leukemia (AML) is a clonal disorder characterized by an increase in the number of abnormal myeloid precursor cells in the bone marrow. As a result, the production of normal blood cells is compromised. This shortage results in anemia, thrombocytopenia, and neutropenia. AML is the most common form of acute leukemia in adults, and mostly affects patients over 65 years of age [1]. The incidence of AML is similar in Europe and the USA, with

rates of 3.7 per 100,000 and 4.0 per 100,000, respectively [2, 3].

In the period 2010–2016, the 5-year survival rate for AML was 47.5% for patients younger than 65 years and 8.2% for patients 65 years of age or older [1]. Prognosis is poor in older patients; median survival in this age group is 2.4 months and varies from 3.9 months in patients aged 65–74 years to 1.4 months in patients aged 85 years or older [4–6].

Cytogenetic abnormalities and molecular abnormalities, as defined in the 2017 European LeukemiaNet (ELN) AML recommendations, are important predictors of remission rate, relapse risk, and survival [7]. The 5-year survival rates for patients with favorable, intermediate, and unfavorable risk cytogenetics are 55–65%, 24–41%, and 5–14%, respectively [8–10]. Survival rates are even lower in older patients: 34%, 13%, and 2%, respectively [11].

Standard treatment for patients with AML is intensive induction chemotherapy, usually with a combination of an anthracycline plus cytarabine (cytosine arabinoside) [10]. Despite improvements in outcomes for younger patients in recent decades, there has been little progress in improving prognosis for patients older than 60 years [7]. In those who are not eligible for standard induction chemotherapy, treatment options are low-dose cytarabine (LDAC), LDAC plus glasdegib, the hypomethylating agents decitabine or azacitidine (HMAs), HMAs combined with venetoclax, or best supportive care [10].

HMAs target the epigenetic processes involved in carcinogenesis. Epigenetic modification is an important regulator of gene transcription, and its role in carcinogenesis has been a topic of considerable interest in the past few years. Of all epigenetic modifications, hypermethylation has been the most extensively studied. Hypermethylation inhibits activation of promoters and therefore transcription of tumor suppressor genes, leading to gene silencing [12].

This review focuses on decitabine (Janssen-Cilag International N.V., B-2340 Beerse, Belgium, <http://www.janssen.com/>), a HMA which is indicated for the treatment of adults with newly diagnosed de novo or secondary AML,

according to the World Health Organization (WHO) classification, who are not eligible for standard induction chemotherapy. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

MECHANISM OF ACTION AND ADMINISTRATION

Decitabine (5-aza-2'-deoxycytidine) is a cytidine deoxynucleo-9 side analogue. It inhibits DNA methyltransferases (DNMTs), leading to DNA hypomethylation including gene promoter regions. This in turn may lead to re-expression of silenced tumor suppressor genes and others involved in cellular proliferation and differentiation. In several leukemia cell lines, decitabine elicited morphological and functional differentiation at concentrations that inhibited cellular DNA methylation. Moreover, decitabine also induces cytotoxicity by incorporation into DNA and the generation of DNA–DNMT adducts that interfere with DNA synthesis during cell replication, resulting in apoptosis. In addition, decitabine affects angiogenesis, decreasing vessel formation in different tumor models [13, 14].

Decitabine is administered at a dose of 20 mg/m² body surface area by intravenous (IV) infusion over 1 h daily for five consecutive days in a treatment cycle. The cycle should be repeated every 4 weeks, depending on the patient's clinical response and toxicity for a minimum of four cycles; however, more may be needed to achieve a complete or partial remission. Therefore, treatment may be continued as long as the patient continues to derive a benefit, such as a response or stable disease.

REGISTRATION TRIAL DACO-016

In 2012, a marketing authorization valid throughout the European Union was issued for decitabine monotherapy for the treatment of patients aged 65 years and older with newly diagnosed or secondary AML who are not eligible for standard induction chemotherapy. The

initial authorization was based on the results of the open-label, randomized, multicenter phase 2 DACO-016 trial, and supported by data from the phase 2 open-label DACO-017 trial in subjects with AML [15, 16]. Patients with newly diagnosed de novo or secondary AML received either decitabine monotherapy or control treatment (the physician's preferred treatment of choice [TC] of cytarabine or supportive care alone).

The pivotal DACO-016 trial enrolled 485 patients from 65 international sites, of whom 242 received decitabine and 243 received TC.

Eligible patients were 65 years or older, had newly diagnosed histologically confirmed de novo or secondary AML, and had a poor or intermediate cytogenetic risk profile, Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and adequate organ function determined by laboratory evaluation. Exclusion criteria were a history of acute promyelocytic leukemia or any other active systemic malignancy, previous chemotherapy (except hydroxyurea) for any myeloid disorder, and being a potential candidate for bone marrow or stem cell transplant within 12 weeks after randomization.

Decitabine 20 mg/m² was administered by 1-h IV infusion once daily for five consecutive days every 4 weeks. Cytarabine 20 mg/m² was administered subcutaneously once daily for 10 consecutive days, and repeated every 4 weeks. Patients also received supportive care and could continue the treatment as long as they had clinical benefit.

The primary endpoint of the study was overall survival (OS). At the clinical data cutoff in October 2009, 396 patients had died: 197 in the decitabine group and 199 in the TC group. Median OS in the intention-to-treat population was 5.0 months (95% confidence interval [CI] 4.3–6.3 months) in the TC arm versus 7.7 months (95% CI 6.2–9.2 months) in the decitabine arm (Fig. 1). This equated to a hazard ratio (HR) of 0.85 (95% CI 0.69–1.04; $p = 0.1079$). Analysis of OS data with an additional year of follow-up (October 2010) enforced earlier data: median OS was 5.0 months in the TC arm and 7.7 months in

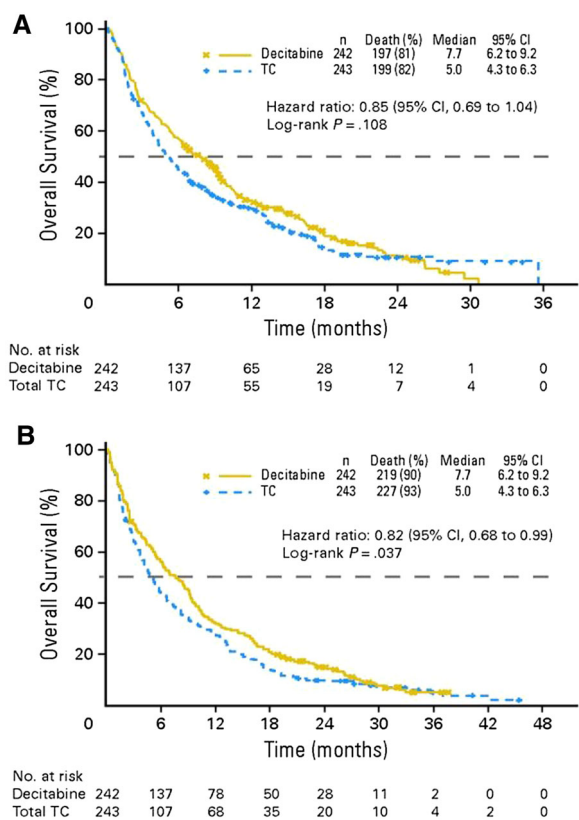


Fig. 1 Kaplan–Meier curve of overall survival with decitabine versus physician’s treatment choice (TC) in the intention-to-treat population of the DACO-106 study **a** at the protocol-specified clinical cutoff date in 2009, and **b** in an ad hoc mature analysis in 2010 [16]

the decitabine arm (HR 0.82; 95% CI 0.68–0.99; $p = 0.0373$).

Secondary endpoints were event-free survival (EFS), progression-free survival (PFS), and clinical response based on morphologic complete remission (CR) or CR with incomplete platelet recovery (CRp). Median EFS was 3.5 months in the decitabine arm compared with 2.1 months in the TC arm (HR 0.75; 95% CI 0.62–0.91; $p = 0.0029$), and median PFS was 3.7 months in the decitabine arm versus 2.1 months in the TC arm (HR 0.75; 95% CI 0.62–0.91; $p = 0.0036$) [17]. In the decitabine arm, 17.8% of patients achieved CR or CRp compared with 7.8% in the TC arm (odds ratio [OR] 2.5; 95% CI 1.40–4.78; $p = 0.0011$). All efficacy results and OS are summarized in Table 1 [17].

Subgroup analyses by age (65–69 vs. ≥ 70 years), ECOG performance status (0–1 vs. 2), cytogenetic risk (poor vs. intermediate), and geographic region are shown in Fig. 2 [16]. Median OS appeared to vary geographically, particularly in western Europe where median OS in the TC arm (14.4 months) was longer than in the decitabine arm.

Results from the supportive DACO-017 trial were consistent with those from the pivotal study [15]. DACO-017 was a multicenter, single-arm, phase 2 study of decitabine as first-line therapy in patients with AML aged older than 60 years. The primary efficacy endpoint (morphologic CR) was achieved in 13 of 55 patients (23.6%) with a median time to CR of 4.1 months. Median OS (the secondary endpoint) was 7.6 months (95% CI 5.7–11.5 months).

Post Hoc and Subgroup Analyses

The results of the DACO-016 trial resulted in approval of decitabine in AML in the European Union, but not in the USA. Although prespecified, the log-rank test could be considered not optimal to assess the observed survival difference because of the non-proportional hazard nature of the survival curves. Therefore, Tomeczkowski et al. conducted a sensitivity analysis of DACO-016 in which they applied the Wilcoxon test [18]. The stratified Wilcoxon test showed a significant improvement in OS with decitabine; median OS was 7.7 months (95% CI 6.2–9.2 months) in the decitabine arm compared with 5.0 months (95% CI 4.3–6.3 months; $p = 0.0458$) in the TC arm. Patients who achieved CR (17.8% of participants) had a median OS of 18.6 months [18].

A subgroup analysis investigated prognostic factors for outcomes in the DACO-016 trials and examined OS and responses in prespecified subgroups based on age, sex, cytogenetic risk, AML type, ECOG performance status, geographic region, bone marrow blasts, platelets, and white blood cells, based on mature data [19]. Patient characteristics that appeared to negatively influence OS were more advanced age (hazard ratio [HR] 1.560 for ≥ 75 vs.

Table 1 Summary of the efficacy results in the DACO-016 study (intention-to-treat population) [17]

	TC (<i>n</i> = 243)	Decitabine (<i>n</i> = 242)	HR (95% CI)	<i>p</i> -value
Overall survival, median (95% CI), months				
Primary analysis	5.0 (4.3–6.3)	7.7 (6.2–9.2)	0.85 (0.69–1.04)	0.1079
Censored for the use of DMT	5.3 (4.3–6.7)	8.5 (6.5–9.5)	0.80 (0.64–0.99)	0.0437
Excluding subjects who received HMA	4.5 (3.8–5.5)	7.9 (6.0–9.3)	0.77 (0.62–0.94)	0.0109
Analysis of mature data	5.0 (4.3–6.3)	7.7 (6.2–9.2)	0.82 (0.68–0.99)	0.0373
Censored for the use of DMT	5.3 (4.3–6.7)	8.6 (6.5–9.5)	0.79 (0.65–0.98)	0.0295
Excluding subjects who received HMA	4.4 (3.7–5.5)	7.9 (6.0–9.3)	0.73 (0.60–0.88)	0.0014
EFS, PFS, RFS, median (95% CI), months				
EFS	2.1 (1.9–2.8)	3.5 (2.5–4.1)	0.75 (0.62–0.91)	0.0029
PFS	2.1 (1.9–3.1)	3.7 (2.7–4.6)	0.75 (0.62–0.91)	0.0036
RFS				
In patients with CR	6.7 (2.9–13.4)	8.3 (4.6–11.4)	–	
In patients with cytogenetic CR	–	–	–	
Clinical response, <i>n</i> (%)				
CR + CRp	19 (7.8)	43 (17.8)	2.5 (1.40–4.78) ^a	0.0011
Cytogenetic CR	3/41 (7.3)	4/40 (10.0)	1.4 (0.22–10.24) ^a	0.712
Time to and duration of response, median (95% CI), months				
Time to best response (CR or CRp)	3.7 (2.8–4.6)	4.3 (3.8–5.1)		
Duration of response (CR or CRp)	12.9 (4.2–NE)	8.3 (6.2–11.4)		

CI confidence interval, CR complete remission, CRp CR with incomplete platelet recovery, DMT disease-modifying therapy, EFS event-free survival, HMA hypomethylating agent, HR hazard ratio, NE not estimable, OR odds ratio, PFS progression-free survival, RFS relapse-free survival, TC patient's choice of treatment with physician's advice

^aValues are odds ratios (95% CI)

< 70 years; *p* = 0.0010), poorer performance status at baseline (HR 0.771 for 0 or 1 vs. 2; *p* = 0.0321), poor cytogenetics (HR 0.699 for intermediate vs. poor; *p* = 0.0010), higher bone marrow blast counts (HR 1.355 for > 50% vs. ≤ 50%; *p* = 0.0045), low baseline platelet counts (HR 0.775 for each additional 100 × 10⁹/L; *p* = 0.0015), and high white blood cell counts (HR 1.256 for each additional 25 × 10⁹/L; *p* = 0.0151). Response rates were higher in the decitabine group than the TC group in all subgroups, including patients ≥ 75 years old (OR 5.94; *p* = 0.0006).

Another post hoc analysis of the DACO-106 study demonstrated that decitabine significantly reduced transfusion dependence in older patients with AML compared with TC [20]. Of the patients who were red blood cell (RBC) transfusion dependent at baseline (168 patients in the decitabine arm and 162 in the TC arm) significantly more patients in the decitabine arm (*n* = 44 [26%]) than the TC arm (*n* = 21 [13%], *p* = 0.0026) became RBC transfusion independent, which was defined as no transfusions for at least eight consecutive weeks. The duration of RBC transfusion independence was also significantly longer in the decitabine arm

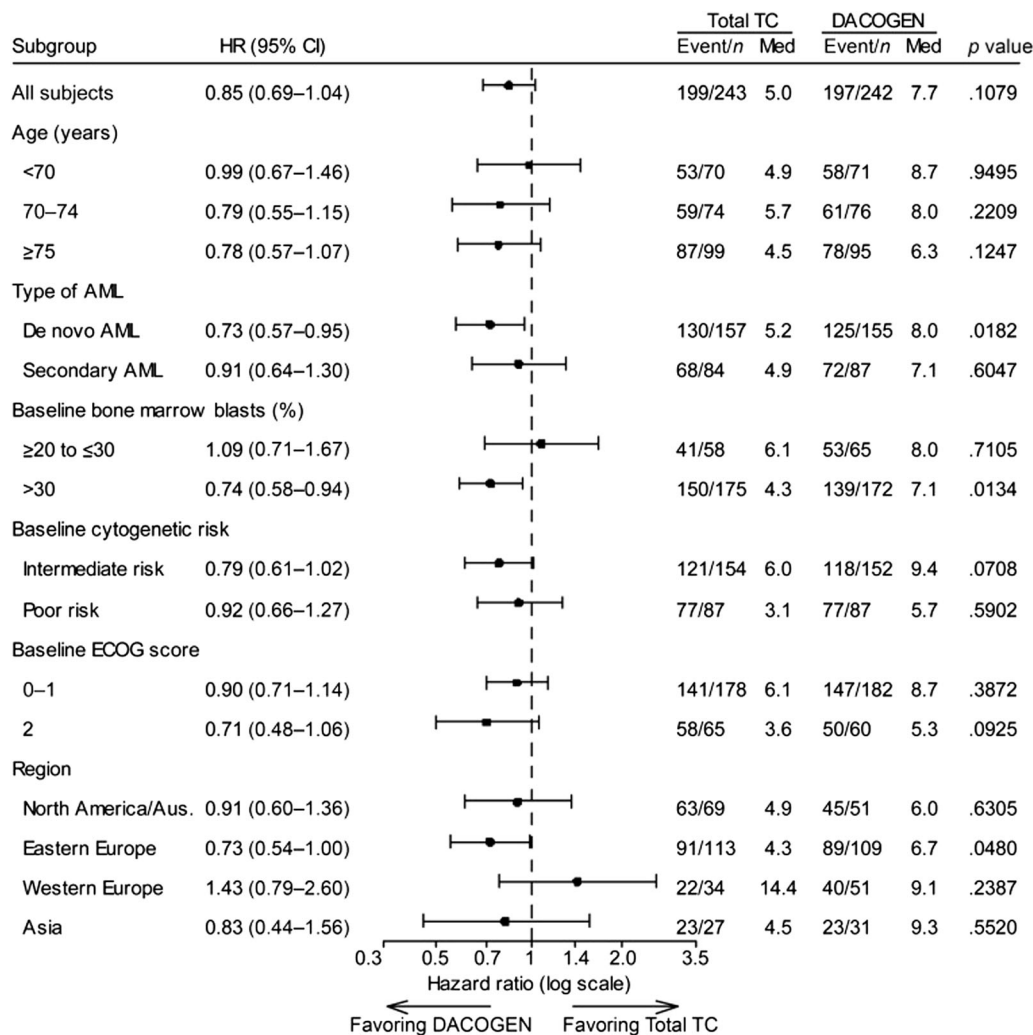


Fig. 2 Overall survival subgroup analysis in the DACO-16 intent-to-treat population (clinical cutoff date, 2019). The *p* value is based on a two-sided log-rank test stratified by age, cytogenetic risk, and ECOG performance status. AML

acute myeloid leukemia, Aus Australia, CI confidence interval, ECOG Eastern Cooperative Oncology Group, HR hazard ratio, Med median, TC patient’s choice of treatment with physician’s advice

than the TC arm: 17% of patients in the decitabine arm compared with 9% patients in the TC arm were RBC transfusion independent for at least 24 weeks (*p* = 0.0138). Results were similar in patients who were platelet transfusion dependent at baseline, with 31% of those on decitabine becoming transfusion independent compared with 13% of those on TC (*p* = 0.0069). Moreover, transfusion independence appeared to be associated with an increase in OS, even in the absence of CR,

suggesting that CR is not required for patients to gain benefit from decitabine treatment.

In another unplanned post hoc analysis, the DACO-016 investigators selected a subset of patients with BM blasts ≥ 30% and WBC < 15 × 10⁹/L, and compared the clinical characteristics and outcomes of these patients with data from the AML-001 trial [21]. Of the 485 patients in the DACO-016 trial, 271 (56%) met the criteria for this analysis, of whom 127 (47%) were randomized to decitabine and 144 (53%) to TC. Within this subgroup, the CR/CRi rate was 27%

in patients receiving decitabine and 11% in patients receiving TC. This was associated with a significant improvement in OS in the decitabine group (median 8.6 vs. 4.7 months, HR 0.67; $p = 0.0033$), a relative improvement of 83% over TC. These results compare favorably with the outcomes reported in the AML-001 trial. Although comparison of two different trials is problematic, the results indicate that decitabine is another viable treatment option for older patients with higher-blast-count AML.

REAL-WORLD DATA

Since September 2012, when decitabine received marketing authorization [22], several observational “real-life” studies confirmed the effectiveness of decitabine for the treatment of older patients with AML unfit for intensive chemotherapy. A retrospective, non-interventional, multicenter registration study in Belgium assessed data from 45 patients, of whom 30 (67%) had secondary AML. Median OS and PFS were 7.3 months (95% CI 2.2–11.1) and 4.1 months (95% CI 2.1–7.6), respectively. Patients who received at least four cycles ($n = 21$) seemed to have significantly better outcomes than patients receiving fewer than four cycles of decitabine (median OS 17.5 vs. 1.6 months, and median PFS 17.5 vs. 1.4 months, respectively). Overall, 25% of the patients who were RBC infusion dependent and 58% of those who were platelet transfusion dependent at baseline became transfusion independent [23].

Investigators pooled data from three multicenter observational studies conducted in Italy that included 306 older patients with AML (median age 75 years) who were not candidates for intensive chemotherapy [24]. These patients received a total of 1940 cycles of decitabine, a median of 5 cycles per patient. The objective response rate (ORR) was 48.4%, with 23.2% of patients achieving CR, 10.5% partial remission [PR], and 4.7% hematologic improvement. Median OS was 10.0 months in the overall patient cohort, but 11.6 months for patients with favorable-intermediate cytogenetic risk and 7.9 months for patients with adverse

cytogenetic risk. Patients who achieved CR had a median OS of 22.1 months.

Using the Surveillance, Epidemiology, and End Results-Medicare linked database, US investigators examined outcomes in older patients with AML who received a first-line hypomethylating agent [25]. They identified 2263 patients (aged ≥ 66 years) diagnosed with AML during 2005–2015 who received azacitidine ($n = 1154$; 51%) or decitabine ($n = 1109$; 49%). Median OS was 7.1 and 8.2 months ($p < 0.01$) for azacitidine- and decitabine-treated patients, respectively. The mortality risk was higher with azacitidine than with decitabine (HR 1.11; 95% CI 1.01–1.21; $p = 0.02$). The findings were similar when evaluating only patients completing at least four cycles (42% of patients treated with either azacitidine or decitabine), but they were no longer significant when evaluating those who completed a standard 7-day schedule of azacitidine (34%) versus a 5-day schedule of decitabine (66%; HR 0.95; 95% CI 0.83–1.08; $p = 0.43$). RBC transfusion independence was achieved in one-third of patients, with no difference between the two HMAs. Overall, there were no clinically meaningful differences between azacitidine- and decitabine-treated patients in their OS or achievement of RBC transfusion independence. However, the majority of older patients with AML did not receive the minimum of four cycles of HMA recommended to elicit clinical benefit.

SAFETY AND TOXICITY

Most patients ($\geq 97\%$) in the registration trial had experienced a treatment-emergent adverse event (TEAE) at the time of the October 2009 data cutoff, and most reported at least one serious TEAE (decitabine, 80%; cytarabine, 72%; SC, 41%). The most common serious TEAEs included febrile neutropenia (decitabine, 24%; cytarabine, 16%; SC, 0%), pneumonia (decitabine, 20%; cytarabine, 16%; SC, 10%), and progressive disease (PD) (decitabine, 11%; cytarabine, 14%; SC, 7%), and the most common grade 3 or 4 TEAEs were thrombocytopenia (decitabine, 40%; cytarabine, 35%; SC, 14%)

Table 2 Treatment-emergent adverse events of grade 3 or 4 in at least 10% of patients in any group (2009 clinical cutoff) [16]

Adverse event, <i>n</i> (%)	TC (control)			Decitabine (<i>n</i> = 238)
	Supportive care (<i>n</i> = 29)	Cytarabine (<i>n</i> = 208)	Total TC (<i>n</i> = 237)	
Any grade 3 or 4 adverse event	16 (55)	188 (90)	204 (86)	221 (93)
Thrombocytopenia	4 (14)	73 (35)	77 (32)	95 (40)
Anemia	4 (14)	56 (27)	60 (25)	80 (34)
Febrile neutropenia	0	51 (25)	51 (22)	76 (32)
Neutropenia	1 (3)	41 (20)	42 (18)	76 (32)
Leukopenia	0	20 (10)	20 (8)	47 (20)
Pneumonia	4 (14)	39 (19)	43 (18)	51 (21)
Bronchopneumonia	3 (10)	9 (4)	12 (5)	10 (4)
Disease progression	2 (7)	46 (22)	48 (20)	43 (18)
General physical health deterioration	5 (17)	33 (16)	38 (16)	30 (13)
Pyrexia	3 (10)	17 (8)	20 (8)	24 (10)
Hypokalemia	5 (17)	19 (9)	24 (10)	27 (11)
Dyspnea	3 (10)	11 (5)	14 (6)	16 (7)

TC patient’s choice of treatment with physician’s advice

and anemia (decitabine, 34%; cytarabine, 27%; SC, 14%). Similar results were seen in the mature analysis, when data cutoff was 2010 (Table 2) [16].

Fourteen patients (6%) who received decitabine recipients and 17 (8%) who received cytarabine stopped therapy because of TEAEs. In the first 30 days in treatment, 21 patients (9%) in the decitabine arm and 17 (8%) in the cytarabine arm died. Mortality after 60 days was 19.7% in the decitabine arm and 24.9% in the TC arm (cytarabine, 23%; SC, 34.5%). Overall, 77 decitabine-treated patients (32%) and 59 cytarabine-treated patients (28%) died during treatment or at least 30 days after the last study drug dose; the cause of death was a TEAE in 58 patients (24%) in the decitabine arm and 39 patients (19%) in the cytarabine arm, and PD in 16 (7%) and 17 (8%) patients, respectively. The overall death rate was lower in the decitabine arm (0.57 per patient-year; 95% CI 0.45–0.72)

than in the cytarabine arm (0.73 per patient-year, 95% CI 0.56–0.94), but the rate of death due to AEs was similar in the decitabine (0.43 per patient-year; 95% CI 0.33–0.56) and cytarabine (0.48 per patient-year; 95% CI 0.34–0.66) groups.

When interpreting the safety data, it is important to note that exposure to medication was 83% longer in the decitabine arm (median 4.4 months) than the TC arm (median 2.4 months with cytarabine), in both the initial (2009) and mature (2010) analyses. Data from the two DACO trials and real-world studies show that the toxicity associated with decitabine is relatively mild and AEs are manageable [23–26], with no additional AEs report in real-world studies compared with those reported in the DACO-016 and DACO-017 trials [15, 16].

GENETICS

AML has been extensively studied using whole genome sequencing technologies [27], starting with the publication of the first genome in 2008. Since then, numerous novel recurrent somatic disease alleles have been identified, including mutations of the DNA methyltransferase 3A gene (*DNMT3A*) and the isocitrate dehydrogenase genes (*IDH1* and *IDH2*) [28–31]. Most of the mutations in genes encoding epigenetic modifiers are acquired early and are present in the leukemic clone. Mutations in these genes are common in older people and, together with clonal expansion of hematopoiesis, lead to an increased risk of developing hematologic cancers [32].

Genetic data are currently used for disease classification, risk stratification, and planning the clinical care of patients. The WHO added two new entities—AML with mutated *RUNX1* and AML with *BCR-ABL1*—to its current update of the classification of myeloid neoplasms and AML, and the 2017 European LeukemiaNet recommendations added mutations in *RUNX1*, *ASXL1*, and *TP53* to their risk stratification guidelines for AML [7].

Molecular alterations are presumably correlated with the therapeutic effect of HMA treatment. Mutations in *DNMT3A*, *IDH2*, *ASXL1*, *TET2*, *RUNX1*, *CEBPA* single (*CEBPA^{single-mut}*), and *TP53* are common in older patients with AML. In younger patients with AML treated with standard therapy, *DNMT3A*, *FLT3-ITD*, and *TP53* mutations were associated with poor OS and EFS. On the other hand, in older patients receiving decitabine-based treatment, harboring *FLT3-ITD* and *ASXL1* mutations, but not *DNMT3A* or *TP53* mutations, was associated with worse outcomes (OS and EFS). *CEBPA^{single-mut}* was also identified as an unfavorable prognostic factor, whereas *CEBPA* double mutation (*CEBPA^{double-mut}*) was not [33].

In various studies, patients with AML with an unfavorable-risk cytogenetic profile and *TP53* mutations have poorer outcomes when treated with standard chemotherapy [34, 35]. Although clinical responses vary, recent studies have demonstrated that the presence of

adverse-risk karyotypes or *TP53* mutations does not have a negative effect on sensitivity to HMA treatment [15, 36, 37].

TP53 Mutations

TP53 aberrations seem to predict favorable responses to hypomethylating treatment with decitabine. Becker et al. analyzed outcomes associated with chromosome 17p loss or *TP53* gene mutations in unfit elderly patients with AML [38]. In that study, 25 of 178 patients had loss of 17p, of whom 24 had a complex (CK+) and 21 a monosomal karyotype (MK+). Patients with 17p loss showed a better CR, PR, and antileukemic effect (ALE). However, there was no significant difference in OS between patients with or without loss of 17p, and this was also true in the subgroups of patients with CK+ or MK+. Eight of 45 patients had *TP53* mutations, and five of these patients also had 17p loss. CR, PR, and ALE rates were similar in patients with *TP53* mutations and those with wild-type *TP53*, but OS appeared to be worse in patients with mutated *TP53* ($p = 0.036$) [38].

HMA treatment can probably partly overcome the adverse prognostic impact of a *TP53* mutation. A registry-based analysis by Middeke et al. also found that outcomes were not negatively influenced by a *TP53* mutation [39]. These researchers analyzed data from 311 patients with AML treated with decitabine, and found that ORR was 30% and median OS was 4.7 months. Patients on first-line decitabine treatment showed better responses than patients who had relapsed/refractory AML (ORR 38% versus 21%), and a slightly longer OS (median 5.8 months versus 3.9 months). Next generation sequencing was undertaken in 180 patients, and 20 (11%) had a *TP53* mutation. Response rates and in this real-world data also the survival were comparable between patients with or without *TP53* mutations [39].

Monosomal Karyotype

Monosomal karyotype-positive (MK+) AML (i.e., the presence of at least two autosomal monosomies or one autosomal monosomy with

at least one structural chromosomal abnormality) is associated with poor prognosis, but several studies suggest a potential positive effect of decitabine in patients with MK+ AML. The DACO-016 investigators conducted a post hoc analysis to determine the effects of decitabine versus TC on outcomes in MK+ patients [40, 41]. A phase 2 trial of decitabine as first-line treatment for older patients with AML showed a higher ORR, and comparable OS, in MK+ patients than MK– patients with abnormal cytogenetics [42].

In DACO-016, 64 of 485 patients were MK+ (decitabine, $n = 33$; TC, $n = 31$) and 99 were MK– with poor-risk cytogenetics (decitabine, $n = 49$; TC, $n = 50$). In MK+ patients, CR/CRi was 27% in the decitabine arm and 3% in the TC arm ($p = 0.0132$), ORR was 36% and 3%, respectively ($p = 0.0013$), and PFS was 2.6 and 1.3 months, respectively (HR 0.53 [95% CI 0.31–0.90]; $p = 0.0181$). There was a trend toward longer OS in the decitabine arm compared with the TC arm (median OS 6.3 vs. 2.6 months; HR 0.67 [95% CI 0.39–1.15]; $p = 0.141$), which was significant when the analysis was censored for the initiation of follow-up treatment (median OS 6.3 vs. 2.6 months; HR 0.52; 95% CI 0.29–0.93; $p = 0.0253$).

In MK– patients with poor-risk cytogenetics, DACO-016 investigators found no difference between patients who received decitabine or TC in CR/CRi rate (decitabine arm 18% vs. TC 0%; $p = 0.262$), ORR (decitabine 20% vs. TC 12%; $p = 0.287$), or median PFS (decitabine 2.4 months vs. TC 2.2 months; $p = 0.576$). Even after censoring at the time of subsequent therapy, there was also no difference in median OS between the decitabine and TC groups in this subpopulation (5.0 vs. 5.0 months; $p = 0.789$).

These results are similar to data from a subgroup analysis of a phase 3 study of decitabine treatment in patients with high-risk myelodysplastic syndrome (MDS) [43]. Investigators of that study concluded that decitabine treatment may overcome the poor prognosis of patients with MK+ AML and offer a new therapeutic option for these patients who are difficult to treat.

Gene Derepression

Gene derepression and reactivation probably explains the superiority of HMA over cytarabine in patients with AML with chromosomal deletions. Investigators discovered that decitabine induced transcriptome changes in AML cell lines with or without a deletion of chromosomes 7q, 5q, or 17p. Treating these cell lines with HMA caused an upregulation of several hemizygous tumor suppressor genes, derepressing endogenous retrovirus (ERV)3–1 and increasing H3K4me3 levels. Decitabine reactivated multiple transposable elements, such as double-strand RNA sensor RIG-I and interferon regulatory factor 7. Similar changes were observed in experiments on peripheral blood blasts from patients with AML receiving in vivo treatment with decitabine [44].

RESPONSE AND RESISTANCE TO DECITABINE

Although decitabine monotherapy has shown clear efficacy in patients with AML, many patients will not respond (showing primary resistance) or will lose response (showing adaptive or secondary resistance). The mechanisms underlying development of resistance to decitabine have been widely investigated but not completely clarified [45, 46]. There are also no confirmed biomarkers that can predict response to decitabine, although there are indications that fetal hemoglobin induction during decitabine treatment may be associated with prognosis [47, 48]. On the other hand, clinical resistance to decitabine results from the complex interaction of cellular, disease-related and individual patient characteristics [49].

The supposed mechanism of action of decitabine is DNA hypomethylation of leukemic cells and the subsequent re-expression of silenced genes. DNA hypermethylation is a feature of leukemic cells and its downregulation may be a sign of decitabine activity. In fact, response to HMAs has been correlated generically with hypomethylation. Studies have demonstrated that the modulation of methylation induced by decitabine is regional and

involves non-promoter as well promoter regions, but it is hard to identify specific baseline methylation biomarkers predictive of response. The expression of nucleotide uptake and activating enzymes like cytidine deaminase (CDA) and deoxycytidine kinase (DCK) is very heterogeneous in AML and has been correlated with *in vitro* and *in vivo* response to decitabine. More recently, baseline levels of triphosphohydrolase SAMHD1 have been correlated with clinical response to decitabine in patients with AML. A possible approach to overcoming decitabine resistance could thus be to target pathways and molecules other than those involved in DNA methylation [50, 51].

COMBINATION THERAPY

To increase the likelihood and duration of response, several novel agents are being combined with a backbone of decitabine therapy in AML. The mechanisms of action of these novel agents are quite heterogeneous, and although some have been used empirically, in other cases, there is preclinical evidence suggesting possible clinical synergy with decitabine.

Decitabine has been combined with histone deacetylase inhibitors of various types. In addition to DNA methylation, the epigenetic alterations that occur in AML may be caused by histone modifications. Chromatin rearrangements are driven by the cooperative function of enzymes regulating histones; therefore, the first agents thought to increase the efficacy of HMAs were histone deacetylase inhibitors. Despite a solid scientific rationale and promising preclinical evidence, the combination of decitabine with valproic acid or vorinostat did not show any advantage in terms of responses or survival when used clinically in patients with AML [52–54].

Decitabine has been demonstrated to increase in the expression of programmed cell death protein 1 (PD1), and programmed cell death ligand 1 and 2 (PD-L1 and PD-L2) in a dose-dependent manner, suggesting that decitabine plus an immune checkpoint inhibitor may be an effective combination. Such combinations have been evaluated in clinical trials in

patients with refractory/relapsed AML, including decitabine with the anti-PD-1 antibody PDR001 (NCT03066648), ipilimumab (NCT02890329), and pembrolizumab (NCT02996474), but the results were inconclusive. In addition, the anti-Tim3 antibody sabatolimab has been evaluated in combination with decitabine in *de novo* patients with AML in a phase 1b study with encouraging results [55].

Venetoclax is an orally bioavailable small-molecule inhibitor that selectively targets Bcl-2 and has shown great efficacy in AML, in combination with low-dose cytarabine and HMAs [52, 56]. Larger clinical trials evaluating decitabine and venetoclax combination therapy are ongoing, and HMAs plus venetoclax are at present standard of care for elderly patients with AML not eligible for intensive chemotherapy.

Although only preliminary data are available for the combination of decitabine with other molecules like magrolimab (anti-CD47), APR246, and the IDH inhibitors ivosidenib and enasidenib, the concept of HMA-based doublet and triplet regimens as first-line therapy of AML is quite clearly gaining ground, and we expect to see more combinations of decitabine to optimize treatment of patients who are not candidates for intensive chemotherapy. Besides possible gains in efficacy, it is important that the side effects of such combinations should remain manageable in a population prone to multiple comorbidities.

CONCLUSION

Decitabine offers an important treatment option for the many patients with AML who are unfit to receive more intensive combination therapies. In this context, decitabine offers a meaningful improvement in survival, with manageable toxicity. Whether patients with *TP53* mutations have more benefit from HMAs is a matter of debate since existing data are inconclusive. Alternative schemes, such as 10-day administration, are currently being investigated. So far, the 5-day scheme is still the recommended dosage to apply in the EU [10].

Further research is needed to identify patients who are most likely to benefit from decitabine treatment, as well as predictors of treatment resistance, and to identify potentially effective combinations with decitabine to further enhance the benefits of this treatment.

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