Treatment with a 5-day versus a 10-day schedule of decitabine in older patients with newly diagnosed acute myeloid leukaemia: a randomised phase 2 trial

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Summary

Background Hypomethylating agents, such as decitabine, are the standard of care for older patients with newly diagnosed acute myeloid leukaemia. Single-arm studies have suggested that a 10-day schedule of decitabine cycles leads to better outcomes than the usual 5-day schedule. We compared the efficacy and safety of these two schedules.

Methods Eligible patients were aged 60 years or older with acute myeloid leukaemia but unsuitable for intensive chemotherapy (or <60 years if unsuitable for intensive chemotherapy with an anthracycline plus cytarabine). The first 40 patients were allocated equally to the two treatment groups by computer-generated block randomisation (block size 40), after which a response-adaptive randomisation algorithm used all previous patients' treatment and response data to decide the allocation of each following patient favouring the group with superior response. Patients were assigned to receive 20 mg/m² decitabine intravenously for 5 or 10 consecutive days as induction therapy, every 4–8 weeks for up to three cycles. Responding patients received decitabine as consolidation therapy on a 5-day schedule for up to 24 cycles. We assessed a composite primary endpoint of complete remission, complete remission with incomplete platelet recovery (CRp), and complete remission with incomplete haematological recovery (CRi) achieved at any time and assessed by intention to treat. This trial is registered with ClinicalTrials.gov, number NCT01786343.

Findings Between Feb 28, 2013, and April 12, 2018, 71 patients were enrolled. 28 received decitabine for 5 days and 43 for 10 days, and all were assessable for efficacy and safety. The primary endpoint was achieved in similar proportions of patients in the two treatment groups (12 [43%] of 28 in the 5-day schedule group, 95% credible interval 26–60, and 17 [40%] of 43 in the 10-day schedule group, 26–54, p=0·78; difference 3%, –21 to 27). Total follow-up was 38·2 months, during which the median duration of overall survival was 5·5 months (IQR 2·1–11·7) in the 5-day group and 6·0 months (1·9–11·7) in the 10-day group. 1-year overall survival was 25% in both groups. Complete remission, CRp, CRi, and overall survival did not differ between groups when stratified by cytogenetics, de-novo versus secondary or therapy-related acute myeloid leukaemia, or *TP53*^{mut} status. The most common grade 3–4 adverse events were neutropenic fever (seven patients [25%] in the 5-day group and 14 [33%] in the 10-day group) and infection (five [18%] and 16 [37%], respectively). One patient (4%) died from sepsis in the context of neutropenic fever, infection, and haemorrhage in the 5-day group, and in the 10-day group six patients (14%) died from infection. Early mortality was similar in the two groups.

Interpretation In older patients with newly diagnosed acute myeloid leukaemia, efficacy and safety did not differ by the 5-day or the 10-day decitabine schedule.

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Introduction

Older patients with acute myeloid leukaemia have worse outcomes than younger patients because of reduced tolerance to intensive treatment and increased frequency of high-risk cytogenetic and molecular abnormalities.[1–3] The hypomethylating agent decitabine is commonly used to treat older patients with acute myeloid leukaemia who are not candidates for intensive chemotherapy. Complete remission, including that with incomplete haematological recovery (CRi), with standard doses of hypomethylating agents is 20–30%.[4] A randomised trial of 20 mg/m² intravenous decitabine given daily for 5 days every 4 weeks compared with 20 mg/m² (low-dose) cytarabine given daily for 10 days every 4 weeks or supportive care in older patients with acute myeloid leukaemia showed a modest survival benefit for decitabine recipients in an unplanned analysis (hazard ratio 0·82, 95% CI 0·69–0·99, p=0·037).[5]

Research in context

Evidence before this study

We did not do a systematic review before starting this trial, but we searched PubMed , without date or language limitations, with the terms "hypomethylating agents", "decitabine", or "azacitadine" in combination with "AML" or "acute myeloid leukemia" for studies on outcomes in adults with acute myeloid leukaemia treated with hypomethylating agents. 20–30% of patients responded to the standard 5-day schedule of decitabine. Some small, single-arm studies reported greater response with decitabine given for 10 days, especially among patients with *TP53*^{mut} acute myeloid leukaemia. We found no studies that directly compared the 5-day and 10-day schedules of decitabine in patients with acute myeloid leukaemia.

Added value of this study

We directly compared 5-day and 10-day schedules of decitabine in an unselected population of older patients with newly diagnosed acute myeloid leukaemia who were unsuitable for intensive chemotherapy. No differences in efficacy (response and early mortality) and safety were seen. Pretreatment cytogenetics, type of acute myeloid leukaemia (ie, de-novo *vs* secondary or therapy-related disease), and *TP53*^{mut} status did not affect outcomes.

Implications of all the available evidence

Despite modest single-agent activity, decitabine is widely used as a backbone drug for combination approaches in older patients with acute myeloid leukaemia. The similar efficacy we found for 5-day and 10-day schedules might, therefore, inform future studies combining decitabine with novel agents. Given previous evidence suggesting particular efficacy of a 10-day decitabine schedule in patients with *TP53*^{mut} acute myeloid leukaemia, future studies of the two schedules in this population are also warranted.

Single-arm studies have suggested that a 10-day decitabine schedule improves response compared with standard 5-day dosing.[6–9] Blum and colleagues[6] reported complete remission in 25 (47%) of 53 patients who received 20 mg/m² decitabine for 10 days, and that 34 (64%) patients overall achieved a morphological leukaemia-free state (with or without count recovery). Longer exposure to decitabine than standard might be beneficial in patients with *TP53*^{mut} acute myeloid leukaemia,[9] which is highly associated with complex karyotypes, resistance to cytotoxic chemotherapy, and poor prognosis.[10,11] In one study of a 10-day schedule of decitabine in older patients with acute myeloid leukaemia, all 21 patients with *TP53*^{mut} had clearance of bone-marrow myeloblasts to less than 5% compared with 32 (41%) of 78 with *TP53*^{WT}.[9] Despite this difference in response, the duration of remission for those with *TP53*^{mut} was generally short and overall survival was similar in the two groups.

Although evidence suggests that 10-day schedules of decitabine are superior to 5-day schedules, no randomised head-to-head comparison between these regimens has been reported. We therefore designed a randomised phase 2 trial to assess the relative safety and efficacy of 20 mg/m² decitabine given intravenously for 5 or 10 consecutive days in older patients with acute myeloid leukaemia unsuitable for intensive chemotherapy.

Methods

Study design and participants

This was a single-centre, open-label, randomised phase 2 trial done at the University of Texas MD Anderson Cancer Center, Houston, TX, USA. Eligible patients were adults aged 60 years or older with newly diagnosed acute myeloid leukaemia (≥20% myeloblasts) except acute promyelocytic leukaemia, who were deemed unsuitable for intensive chemotherapy.[12] Patients younger than 60 years of age could be enrolled if deemed unsuitable by the treating physician for intensive chemotherapy with an anthracycline plus cytarabine. Inclusion criteria were an Eastern Cooperative Oncology Group performance status score of 0–3 and adequate renal and hepatic function, including a creatinine concentration less than 2·5 mg/dL (221 µmol/L) and total bilirubin concentration less than 2·0 mg/dL (34·2 µmol/L). Patients with antecedent haematological disorders (eg, myelodysplastic syndrome or myeloproliferative neoplasm) who had not received any previous treatment with a hypomethylating agent were also eligible. We excluded patients with uncontrolled infections or with uncontrolled intercurrent illness.

This study was approved by the institutional review board of the University of Texas MD Anderson Cancer Center. All patients provided informed consent according to institutional guidelines and the Declaration of Helsinki.

Randomisation and masking

The randomisation schedule was generated by computer with code designed by the Biostatistics Department, University of Texas MD Anderson Cancer Center. The first 40 patients enrolled were randomly assigned equally to the 5-day and 10-day schedule groups by block randomisation (block size 40). After each of these patients had received three cycles of treatment, a research nurse entered the treatment and response data into a database. These data were not masked from the research nurse or computer programmers, but were masked from the study clinicians and statisticians. From patient 41 onwards, all the previous patients' treatment and response data were used by a Bayesian response-adaptive randomisation algorithm at enrolment to determine the randomisation probability of the new patient. In this way, we unbalanced the randomisation probabilities in favour of the group with superior response. All group assignments were open label. The study was monitored by the data safety monitoring board of the University of Texas MD

Anderson Cancer Center for safety and efficacy, with the data masked from the principal investigator.

Procedures

Patients received 20 mg/m² decitabine as induction therapy intravenously over 1 h on 5 or 10 consecutive days every 4–8 weeks dependent on toxicity and recovery of neutrophil and platelet counts, for up to three cycles. If complete remission, complete remission with incomplete platelet recovery (CRp), or CRi was achieved (or earlier at the discretion of the treating physician) patients could receive consolidation therapy of 20 mg/m² decitabine intravenously for 5 consecutive days every 4–8 weeks for up to 24 total cycles dependent on toxicity and the recovery of neutrophil and platelet counts.

Outcomes

The primary endpoint was the composite of complete remission, CRp, and CRi, defined according to the revised International Working Group guidelines for response assessment in acute myeloid leukaemia.[13] Secondary endpoints were remission duration calculated from the time of achieving the primary endpoint to relapse (censored for death in remission), relapse-free survival calculated from the time of remission to relapse or death, overall survival calculated from the time of the start of treatment to death, and the safety profiles of the two schedules. Safety was assessed according to the Common Terminology Criteria for Adverse Events version 4.0.

As standard for trials of acute myeloid leukamia, we assessed partial remission, defined according to the revised International Working Group guidelines,[13] and minimal residual disease, for which multiparameter flow cytometry was done on bone marrow at the time of remission, as previously described.[14] A positive result for minimal residual disease was defined as a cluster of at least 20 cells showing altered expression of two or more antigens. The sensitivity of the assay was at least 0·01%.

Mutation status was assessed with a 28-gene panel, as previously described.[15–17] Genomic DNA was extracted from bone-marrow aspirates or peripheral blood. Amplicon-based next-generation sequencing targeting the entire coding regions of a panel of 28 genes associated with myeloid neoplasms was done with a MiSeq platform (Illumina, San Diego, CA, USA). The genes analysed were *ABL1, ASXL1, BRAF, DNMT3A, EGFR, EZH2, FLT3, GATA1, GATA2, HRAS, IDH1, IDH2, IKZF2, JAK2, KIT, KRAS, MDM2, MLL, MPL, MYD88, NOTCH1, NPM1, NRAS, PTPN11, RUNX1, TET2, TP53*, and *WT1*. For clinical reporting, a minimum of 250 times sequencing coverage (bi-directional true paired-end sequencing) was required. The analytical sensitivity was established at 5% mutant reads in a background of wild-type reads. We also did single-gene sequencing of *TP53* covering the entire coding region in available post-treatment samples from patients with $TP53^{mut}$ disease at baseline, as previously described.[18]

Statistical analysis

The planned maximum sample size was 100. Based on previous studies, we expected the composite primary endpoint to be achieved in about 30% of patients in each group. Therefore, we assumed that response had a previous β distribution of 0·6–1·4, with mean 0·3 for each arm. The distribution was updated after every patient's response outcome was recorded. For either group, the posterior distribution for the composite response outcome was updated as β (0·6+x, 1·4+y), where x=responders and y=non-responders. Beginning with the 21st patient enrolled in each group and for all subsequent patients, we compared the primary endpoint between groups, incorporating data from all patients with assessable responses. To avoid favouring one arm earlier in a large trial, we used the following randomisation formula to assign patients:

$$Aa=\frac{\sqrt{Pa}}{\sqrt{Pa}+\sqrt{Pb}},\ Ab=1-Aa,$$

where Aa is the probability of assigning patients to arm A, Ab is the probability of assigning patients to arm B, Pa is the posterior probability that arm A is superior to arm B, and Pb is the posterior probability that arm B is superior to arm A. If at any time the probability that one treatment schedule would be superior to the other became greater than 0·95, superiority was declared. If the maximum of 100 patients were to be enrolled and the probability of superiority for one treatment schedule became greater than 0·90, it was declared to be superior. Otherwise, the trial would be inconclusive. Detailed operating characteristics and the data safety monitoring board are provided in the appendix.

See Online for appendix

Responses in the two treatment groups were compared with the χ^2 tests. Remission duration, relapse-free

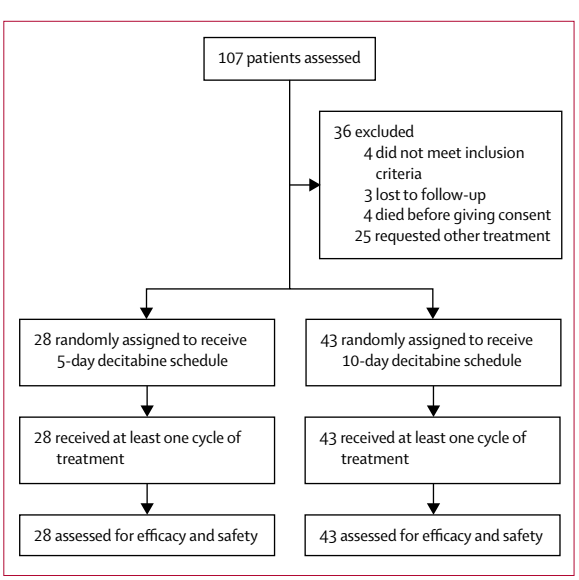

Figure 1: **Trial profile**

	Decitabine for 5 days (n=28)	Decitabine for 10 days (n=43)
Age (years)	77 (70–80)	78 (69–82)
WBC count (10⁹/L)	2·0 (1·5–3·9)	3·2 (1·9–10·6)
Haemoglobin concentration (g/dL)	9·4 (8·7–9·8)	9·2 (9·0–9·7)
Platelet count (10⁹/L)	25 (14–60)	37 (24–83)
Bone-marrow myeloblasts (%)	40 (29–68)	46 (25–64)
ECOG performance status score		
0–1	18 (64%)	30 (70%)
2–3	10 (36%)	13 (30%)
s-AML/t-AML	13 (46%)	18 (42%)
Cytogenetics		
Diploid	8 (29%)	10 (23%)
–5, –7, complex	13 (46%)	24 (56%)
Others	5 (18%)	6 (14%)
Insufficient metaphases or not done	2 (7%)	3 (7%)
Mutated genes		
ASXL1	3/19 (16%)	2/34 (6%)
DNMT3A	3/24 (13%)	6/42 (14%)
EZH2	1/22 (5%)	1/39 (3%)
FLT3-ITD	2/25 (8%)	2/42 (5%)
NPM1	1/24 (4%)	8/42 (19%)
IDH1/2	6/25 (24%)	14/42 (33%)
JAK2	3/24 (13%)	1/42 (2%)
KRAS/NRAS	2/24 (8%)	4/42 (10%)
PTPN11	0/21 (0%)	4/38 (6%)
RUNX1	5/19 (26%)	2/35 (6%)
TET2	1/19 (5%)	8/36 (22%)
TP53	7/24 (29%)	17/41 (41%)

Continuous variables are shown as median (IQR) and categorical variables as n (%). WBC=white blood cell. ECOG=Eastern Cooperative Oncology Group. s-AML/t-AML=secondary or therapy-related acute myeloid leukaemia. ITD=internal tandem duplication.

Table 1: **Baseline characteristics**

	Decitabine for 5 days (%)	Decitabine for 10 days (%)	p value
Complete remission	8 (29%)	13 (30%)	0·88
CRp	3 (11%)	2 (5%)	..
CRi	1 (4%)	2 (5%)	..
Complete remission +CRp+CRi*	12 (43%)	17 (40%)	0·78
Partial remission	0	1 (2%)	..
No response	15 (54%)	22 (51%)	..
Early death	1 (4%)	3 (7%)	..

CRp=complete remission without platelet recovery. CRi=complete remission with inadequate haematological recovery. *95% credible intervals are 26–60 for the 5-day schedule and 26–54 for the 10-day schedule.

Table 2: **Responses**

survival, and overall survival were calculated with Kaplan-Meier estimates, and survival estimates were compared with the log-rank test. A paired *t* test was used to compare *TP53*^{mut} variant allele frequencies in bone-marrow samples taken before treatment and after the first cycle. The data cutoff for this analysis was May 15, 2018. The data analyses were done with GraphPad Prism version 6. This study is registered with ClinicalTrials.gov, number NCT01786343.

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Feb 28, 2013, and April 12, 2018, 71 patients were enrolled and randomly assigned to receive decitabine for 5 days (n=28) or 10 days (n=43, figure 1). The study groups were imbalanced because of superior performance with the 10-day schedule of decitabine during the initial enrolment period. Baseline characteristics were well balanced across treatment groups (table 1). One patient younger than 60 years was enrolled and was included in the 5-day schedule group. In both groups, substantial proportions of patients had features associated with risk of poor outcomes, including Eastern Cooperative Oncology Group performance status score of 2–3 and cytogenetic features (table 1). Seven (29%) of 24 patients in the 5-day group and 7 (41%) of 41 in the 10-day group had *TP53*^{mut} at baseline. The total duration of follow-up was 38·2 months (median 5·3 months, IQR 2·0–11·0).

The composite primary endpoint of complete remission, CRp, and CRi was achieved in similar proportions of patients in the two treatment groups (12 [43%] of 28 in the 5-day schedule group, 95% credible interval 26–60, and 17 [40%] of 43 in the 10-day schedule group, 26–54, p=0·78; table 2). Thus, the difference in overall response between groups was 3% (95% credible interval –21 to 27). We calculated that with full enrolment (n=100), the probability that the 5-day treatment or 10-day treatment group would be declared superior was 11% and 0·1%, respectively. Due to both the similar proportions of responders in the two groups and the introduction of more effective treatments for older people with acute myeloid leukaemia outside of the study, we decided to close enrolment, which was endorsed by the institutional data safety monitoring board.

Complete remission was seen in eight (29%) of 28 patients in the 5-day schedule group and in 13 (30%) of 43 in the 10-day schedule group (table 2). One patient in the 10-day schedule group achieved partial response. Four patients in the 10-day group and three patients in the 5-day group did not meet formal criteria for response but received more than three courses of decitabine. Among patients who achieved response, there was no difference in the proportions with minimal residual disease negativity between the two groups (five [42%] of 12 patients in the 5-day schedule group *vs* six [40%] of 15 in the 10-day schedule group). We noted slightly earlier responses in

the 10-day schedule group than in the 5-day schedule group, but the difference was not significant. The median number of courses to achieve best response was two (IQR 1–2) with the 5-day schedule and two (1–3) with the 10-day schedule (p=0·09). Two patients in the 5-day schedule group and one in the 10-day schedule group achieved best response after more than three courses, which was stable disease in two patients (one in each group) and CRi in one patient (5-day group); these three patients eventually achieved complete remission. The patient in the 10-day schedule group achieved complete remission after four courses of decitabine (two courses for 10 days and two for 5 days). Only one patient (4%) in the 5-day schedule group compared with six (14%) in the 10-day schedule group achieved complete remission after one cycle of induction (p=0·15). The primary endpoint did not differ between the 5-day and 10-day schedules in disease subgroups: diploid cytogenetics (four [50%] of eight *vs* three [30%] of ten, p=0·39), adverse-risk cytogenetics (four [31%] of 13 *vs* 20 [46%] of 43, p=0·37), de-novo acute myeloid leukaemia (seven [47%] of 15 *vs* nine [36%] of 25, p=0·50), secondary or therapy-related acute myeloid leukaemia (five [38%] of 13 *vs* eight [44%] of 18, p=0·74), and *TP53*^{mut} acute myeloid leukaemia (two [29%] of seven *vs* eight [47%] of 17, p=0·40).

The median duration of remission was 9·4 months (IQR 5·6–17·9) in the 5-day schedule group and 6·4 months (2·8–12·4) in the 10-day schedule group, and at 1 year 26% and 33% of patients, respectively, had been in continuous complete remission (figure 2).

Among the 29 patients who met the primary endpoint, 19 relapsed (eight in the 5-day group and 11 in the 10-day group). No patients underwent haemopoietic stem-cell transplantation during first remission. The median duration of relapse-free survival was 5·7 months (IQR 1·6–10·4) in the 5-day schedule group and 4·6 months (2·7–12·4) in the 10-day schedule group, and at 1 year the proportions of patients with relapse-free survival were 21% and 27%, respectively (figure 2). At last follow-up, nine patients were still alive (five in the 5-day group and four in the 10-day group).

Median overall survival was 5·5 months in the 5-day schedule group (IQR 2·1–11·7) and 6·0 months (1·9–11·7) in the 6-day schedule group . Overall survival at 1 year was 25% in both treatment groups (figure 2). Overall survival did not differ significantly between the 5-day and 10-day schedules when stratified by disease subgroups: diploid cytogenetics median 4·7 months (IQR 1·6–18·1) versus 7·2 months (2·6–9·5, p=0·98); adverse–risk cytogenetics 5·5 months (2·7–11·7) versus 5·4 months (1·9–15·5, p=0·76); de-novo acute myeloid leukaemia 7·3 months (3·4–28·5) versus 7·1 months (1·9–14·2, p=0·19); secondary or therapy-related acute myeloid leukaemia 4·4 months (1·9–8·5) versus 5·4 months (2·2–9·3, p=0·19); and *TP53*^{mut} acute myeloid leukaemia 5·5 months (1·9–8·5) *vs* 4·9 months (1·9–9·5, p=0·55). Among patients in the 5-day arm, there was no difference in median overall survival between patients with and without *TP53*^{mut} disease (5·5 months, IQR 1·9–8·5, *vs* 4·9 months, 3·1–10·6), but in patients who received the 10-day schedule of decitabine, overall survival was slightly but

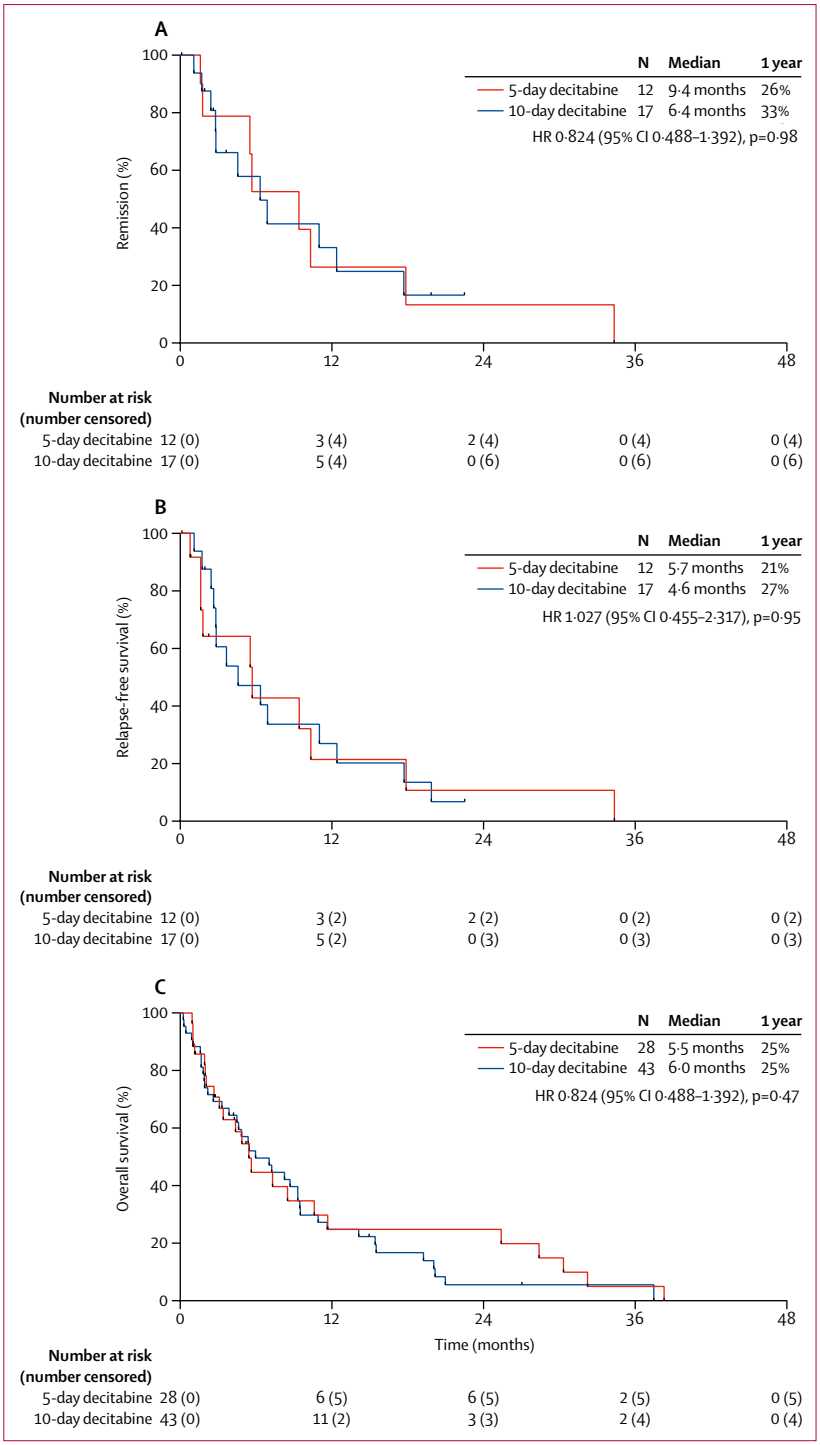

Figure 2: **Kaplan-Meier curves for outcomes by decitabine schedule group**
(A) Continuous remission. (B) Relapse-free survival. (C) Overall survival. HR=hazard ratio.

	Decitabine for 5 days (n=28)				Decitabine for 10 days (n=43)			
	Grade 1–2	Grade 3	Grade 4	Grade 5	Grade 1–2	Grade 3	Grade 4	Grade 5
Acute kidney injury	5 (18%)	0	0	0	1 (2%)	2 (5%)	1 (2%)	0
Altered mental status	1 (4%)	0	0	0	1 (2%)	2 (5%)	0	0
Anorexia	0	1 (4%)	0	0	2 (5%)	0	0	0
Cholecystitis	0	0	0	0	0	1 (2%)	0	0
Constipation	2 (7%)	0	0	0	0	1 (2%)	0	0
Diarrhoea	2 (7%)	1 (4%)	0	0	1 (2%)	0	0	0
Dysphagia	0	1 (4%)	0	0	0	1 (2%)	0	0
Fatigue	3 (11%)	2 (7%)	0	0	4 (9%)	2 (5%)	0	0
Neutropenic fever	0	7 (25%)	0	1 (4%)	0	12 (28%)	2 (5%)	0
Hearing loss	0	0	0	0	0	0	1 (2%)	0
Haemorrhage	1 (4%)	0	1 (4%)	1 (4%)	0	1 (2%)	0	0
Hyperglycaemia	0	0	0	0	0	1 (2%)	0	0
Hypoglycaemia	0	0	0	0	0	1 (2%)	0	0
Hyponatraemia	0	0	0	0	0	2 (5%)	0	0
Hypotension	0	1 (4%)	0	0	1 (2%)	2 (5%)	0	0
Increased bilirubin	1 (4%)	1 (4%)	0	0	0	0	0	0
Infection	1 (4%)	4 (14%)	1 (4%)	1 (4%)	3 (7%)	16 (37%)	0	6 (14%)
Myocardial infarction	0	0	0	0	0	1 (2%)	0	0
Nausea	1 (4%)	1 (4%)	0	0	4 (9%)	0	0	0
Pain	1 (4%)	1 (4%)	0	0	8 (19%)	2 (5%)	0	0
Pleural effusion	0	2 (7%)	0	0	0	0	0	0
Syncope	0	1 (4%)	0	0	0	1 (2%)	0	0
Transient ischaemic attack	0	0	0	0	0	1 (2%)	0	0
Venous thromboembolism	0	1 (4%)	0	0	1 (2%)	0	0	0

Adverse events of any grade occurring in ≥10% of patients and all grade 3, 4, and 5 adverse events are listed. Adverse events are defined according to the Common Terminology Criteria for Adverse Events version 4.0.

Table 3: **Non-haematological adverse events**

non-significantly worse in patients with *TP53*^{mut} than in those with *TP53*^{WT} acute myeloid leukaemia (median 4·7 months, 1·9–9·5, *vs* 8·3 months, 3·0–15·5; p=0·16).

The median number of cycles received was 2·0 (IQR 2·0–5·8) in the 5-day decitabine group and 3·0 (2·0–6·0) in 10-day group. Among responders, the median numbers of cycles of decitabine received were 5·0 (IQR 2·5–5·0) and 6·0 (4·0–9·5), respectively. Of 33 patients in the 10-day schedule group who received two or more cycles of treatment, 23 (70%) received the 10-day schedule only for the first cycle. Among these 23 patients, the decitabine schedule was decreased to 5 days from the second cycle because of achievement of complete remission or CRp in seven and because the treating physician was concerned about sustained myelosuppression in the other 16 patients.

Adverse events are reported in table 3. Most adverse events were grade 1–2. The most common grade 3–4 adverse events across both groups were neutropenic fever and infection. Nine patients died, three in the 5-day schedule group (one from sepsis in the context of neutropenic fever, one from haemorrhage, and one from infection) and six in the 10-day schedule group (all from infection). No death in either group was judged to be related to treatment. Early mortality was similar in the two treatment groups. 30-day mortality was 4% (one death among 28 patients) in the 5-day schedule group and 9% (four among 43) in the 10-day schedule group, and 60-day mortality was 21% (six among 28) and 25% (11 among 43), respectively.

In the exploratory post-hoc analysis of the effect of decitabine on *TP53*^{mut} variant allele frequencies after treatment, we found 31 mutations (including 29 unique mutations) in 24 (37%) of 65 patients in whom sequencing was performed at baseline (figure 3). Six (9%) of 65 patients had more than one *TP53*^{mut} detected. Before treatment, the median variant allele frequency of *TP53*^{mut} was higher among patients in the 10-day schedule group than in the 5-day schedule group (50·3% IQR 33·3–78·8 *vs* 23·1%, 11·7–50·9).

14 patients had *TP53* sequencing done at least once after treatment started (appendix). Nine responders had *TP53*

sequencing done at the time of remission, at which time the median variant allele frequency was 8·4% (IQR 4·1–47·9), and four had frequencies of 20% or greater. *TP53*^{mut} variant allele frequencies differed between patients who responded to decitabine and those who did not respond (mean 56·1% [SE 5·9%] *vs* 36·4% [7·4%]), but not significantly so (p=0·07). Among responders, the mean *TP53*^{mut} variant allele frequency had declined significantly at the end of cycle one (from 53·2% [SD 19·7%] at baseline to 27·5% [23·9%], p<0·001; figure 3), whereas no significant change was seen among non-responders (from 52·0% [SD 33·8%] at baseline to 51·2% [35·8%], figure 3). Patients who received the 10-day schedule of decitabine had significant decline in mean variant allele frequency after one cycle (from 43·0% [SD 27·1%] at baseline to 31·3% [28·9%], p<0·001). Too few patients in the 5-day schedule group underwent *TP53* sequencing after cycle one to assess the effect of this decitabine schedule or allow a comparison with the 10-day schedule.

Three patients had *TP53* sequencing done at the time of relapse, and in all three the previously detected *TP53* clone (ie, same variant) was detected. Two patients had no *TP53*^{mut} detected after treatment (both in the 10-day schedule group and first undetectable at the end of cycle four). Among patients with *TP53*^{mut}, these two patients had the longest duration of remission. One patient died while in complete remission after 19·9 months of continuous remission and the other eventually relapsed after complete remission for 12·4 months.

Discussion

We did a study comparing two schedules of decitabine as first-line therapy for older patients with acute myeloid leukaemia. In previous studies, a 10-day schedule of decitabine was associated with superior response (up to 64%) to the standard 5-day schedule.[6–9] Owing to the short half-life of decitabine in vivo, treatment for 10 days rather than 5 days might be expected to capture a larger proportion of leukaemic cells as they asynchronously enter the S phase.[19] However, we saw no difference in response, remission duration, or survival between the 5-day and 10-day schedules of decitabine. Although we noted a trend towards earlier response with the 10-day schedules, response did not differ between the two decitabine schedules overall or when patients were stratified by cytogenetic risk, de-novo versus secondary or therapy-related acute myeloid leukaemia, or *TP53*^{mut} status.

In previous phase 3 randomised studies of hypomethylating agents in older patients with acute myeloid leukaemia, median overall survival was 7·7 months with a 5-day schedule of decitabine and 10·4 months with the standard 7-day schedule of azacitidine.[5,20] By contrast, the median overall survival in our study was 6·4 months, which is probably explained by the high-risk features, such as median age of 78 years, performance status score of 2 or greater in around a third of patients, and adverse cytogenetic abnormalities in more than half of patients. Furthermore, although the frequency of *TP53*^{mut} among adults with newly diagnosed acute myeloid leukaemia is generally reported to be 5–20%,[11,21] in our cohort 37% of patients had *TP53*^{mut} at baseline, which is associated with poor prognosis. Given these high-risk features and overall survival of 25% in both schedule groups, it is notable that response was 43% with the 5-day decitabine regimen, whereas in a large randomised phase 3 trial of the same schedule in a similar population of patients, response was only 17·8%.[5]

Leukaemic clones harbouring *TP53*^{mut} show resistance to chemotherapy and, therefore, have a competitive advantage over healthy haemopoietic stem cells when exposed to cytotoxic agents.[10] As such, non-intensive chemotherapeutic agents, including hypomethylating

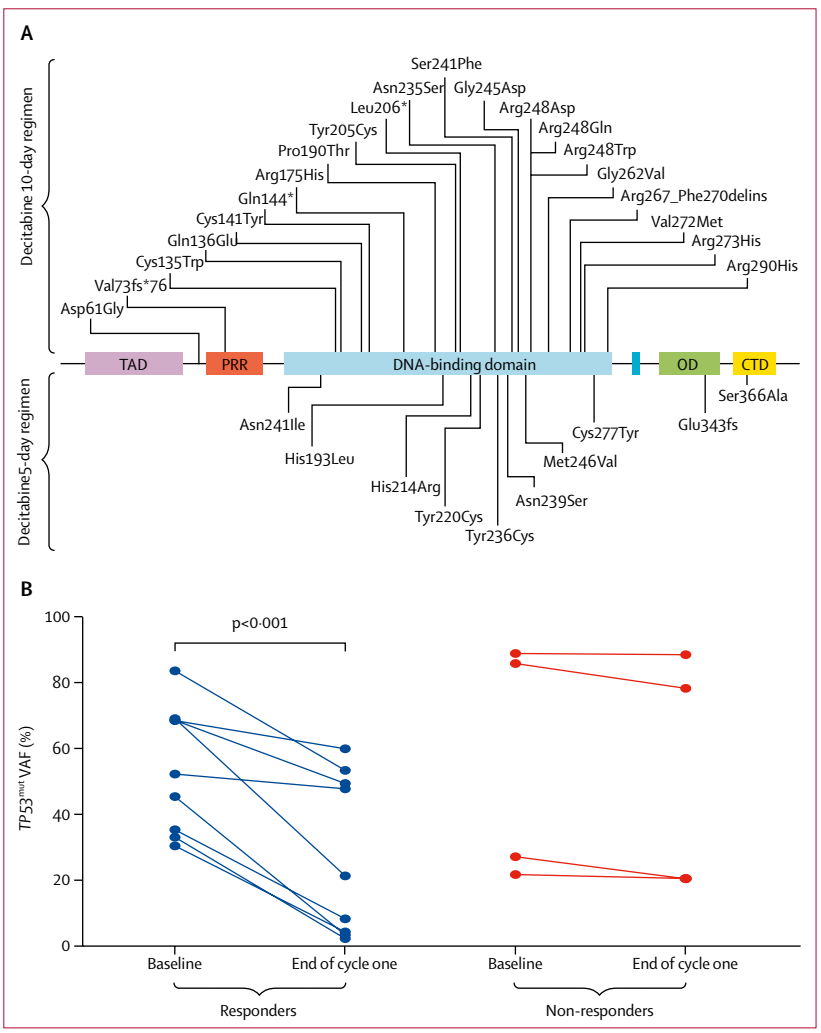

***Figure 3*: *TP53* mutations in 24 patients**

(A) Schematic of TP53 protein showing mutational spectrum in 24 patients. Two patients had His193Leu and two had Val272Met *TP53* mutations. (B) Change in *TP53*^{mut} VAF in nine responders and four non-responders to decitabine with paired samples. CTD=C-terminal regulatory domain (356–393). DBD=DNA-binding domain (101–306). OD=oligomerisation domain (307–355). PRR=proline-rich region (40–92). TAD=transactivation domain (1–42). VAF=variant allele frequency. Panel A adapted by permission of The *TP53* Web Site (http://p53.free.fr).

agents, are commonly used for patients with *TP53*^{mut} acute myeloid leukaemia. Thus, the question of the most effective schedule of decitabine is particularly relevant for this subgroup. Although our study was not designed specifically to evaluate the effects of the two decitabine schedules on *TP53*^{mut} acute myeloid leukaemia, with around a third of patients having *TP53*^{mut} disease, we were able to do some comparisons. In a previous single-arm phase 2 study of a 10-day schedule of decitabine, Welch and colleagues[9] reported a 100% response among 21 patients with *TP53*^{mut} acute myeloid leukaemia. Of note, overall survival did not differ between patients with *TP53*^{mut} and *TP53*^{WT} disease. In our study, response among patients with *TP53*^{mut} acute myeloid leukaemia who received the 10-day schedule of decitabine was 47%. Although our findings do not confirm those of Welch and colleagues, they are generally consistent with the findings in several other studies of acute myeloid leukaemia and myelodysplastic syndromes that reported similar responses among patients with *TP53*^{mut} and *TP53*^{WT} acute myeloid leukaemia and myelodysplastic syndromes treated with hypomethylating agents.[22–25]

We did targeted *TP53* sequencing of bone-marrow specimens to explore correlations between clinical response parameters and *TP53*^{mut} variant allele frequency at baseline and in response to decitabine exposure. Although low numbers prevent comparisons between the decitabine schedules, we made several preliminary observations that should be further assessed in larger cohorts. First, we found a slightly greater *TP53*^{mut} variant allele frequency in responders, which is notable because it has been suggested that *TP53*-containing clones are particularly sensitive to decitabine and that increased *TP53* allele burden could increase the likelihood of response.[9] We also found that the median *TP53*^{mut} variant allele frequency at the time of remission was 8·4%, with frequencies being 20% or greater in four patients. This finding suggests *TP53*^{mut} might be present in some preleukaemic clones and not exclusively in myeloblasts.[10] Finally, two patients in whom *TP53*^{mut} could no longer be detected in bone marrow after remission had the longest duration of remission among all patients. This finding supports previous reports of the importance of mutation clearance as a predictor of sustained remission in people with acute myeloid leukaemia.[26–28]

As almost half of patients with acute myeloid leukaemia are older than 70 years at the time of diagnosis and frequently have severe comorbidities and poor performance status, many are considered unfit for intensive chemotherapy.[29,30] Additionally, although fewer patients generally respond to hypomethylating agents than intensive chemotherapy, survival is similar or possibly superior with hypomethylating agents, probably because of lower treatment-related mortality.[4,31,32] As such, the use of hypomethylating agents, such as decitabine, as induction therapy has become a commonly employed strategy for older adults with acute myeloid leukaemia. Combination therapy has been investigated to improve the outcomes achieved with single-agent hypomethylating agents. So far, the combination of the oral Bcl-2 inhibitor venetoclax with either azacitidine or decitabine is emerging as a potential standard of care in this older population. In older patients with relapsed acute myeloid leukaemia, response to single-agent venetoclax is only 19% with a median overall survival of 4·7 months.[33] By contrast, with use of the combination of venetoclax with either azacitidine or decitabine, complete remission or CRi reached 66% in older patients with acute myeloid leukaemia (57% in those with poor cytogenetics) and median overall survival was 17·5 months.[34] Therefore, despite their modest activity when used alone, decitabine and azacitidine are becoming backbone drugs for efficacious strategies suitable for use in older patients with acute myeloid leukaemia. Our study has relevance to the optimum duration of decitabine therapy in future combination regimens and suggests that 5-day and 10-day regimens are equivalent in efficacy. These results also have implications for the delivery of cost-effective care, as they suggest that additional doses of decitabine could raise treatment costs without providing additional benefit.

There are several limitations to this study. Azacitidine is also commonly used as first-line treatment for older patients with acute myeloid leukaemia who are unfit for chemotherapy, and improves outcomes compared with induction chemotherapy, low-dose cytarabine, or supportive care.[20] No large randomised trials have been done to compare decitabine and azacitidine in this setting and, therefore, whether either hypomethylating agent is superior to the other remains unclear. Second, our results might not directly translate to patients with relapsed or refractory acute myeloid leukaemia. A retrospective analysis reported that a 10-day schedule of decitabine was independently associated with increased response compared with a 5-day schedule of decitabine or 7-day schedule of azacitidine when used as salvage therapy, but no survival benefit was seen.[35] Future prospective studies in patients with relapsed or refractory disease will be needed to clarify the outcomes. Finally, despite the adaptive randomisation design, there may have been some imbalance between groups in high-risk features (ie, frequency of adverse cytogenetics and *TP53*^{mut} were greater in the 10-day schedule group), which should be considered when interpreting our findings.

In conclusion, in this randomised trial of 5-day and 10-day schedules of decitabine in older patients with newly diagnosed acute myeloid leukaemia deemed unfit for intensive chemotherapy, both schedules led to similar response and survival. Notably, no subgroup was identified that benefited preferentially from either schedule of decitabine, including patients with *TP53*^{mut} acute myeloid leukaemia. Our results suggest that both schedules of decitabine are safe and effective in the population assessed and could be considered as reasonable non-intensive backbone regimens for future investigational combinations with novel agents.

Contributors

HMK, XH, and FR designed the study. YG and MAR managed the prospective trial. NJS, HMK, GB, TMK, ND, MO, CDD, ZE, CBB, PB, YA, EJ, SMK, NP, NJ, JC, MK, GG-M, and FR treated patients. NJS, SL, RK-S, AM, SP, and FR collected and analysed the data. SL and RK-S did the sequencing. XH provided statistical assistance. NJS, AM, and FR drafted the manuscript, which was reviewed and approved by all authors.

Declaration of interests

We declare no competing interests.

Data sharing

No additional data are available for this Article.

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