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CLINICAL TRIALS AND OBSERVATIONS

Azacitidine maintenance after intensive chemotherapy improves DFS in older AML patients

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KEY POINTS

- **Azacitidine maintenance is feasible in intensively treated older patients with newly diagnosed AML.**
- **Azacitidine maintenance, with adjustment for poor risk cytogenetic risk at diagnosis and platelet count at randomization, improves DFS.**

The prevention of relapse is the major therapeutic challenge in older patients with acute myeloid leukemia (AML) who have obtained a complete remission (CR) on intensive chemotherapy. In this randomized phase 3 study (HOVON97) in older patients (≥60 years) with AML or myelodysplastic syndrome with refractory anemia with excess of blasts, in CR/CR with incomplete hematologic recovery (CRi) after at least 2 cycles of intensive chemotherapy, we assessed the value of azacitidine as postremission therapy with respect to disease-free survival (DFS; primary end point) and overall survival (OS; secondary end point). In total, 116 eligible patients were randomly (1:1) assigned to either observation (N = 60) or azacitidine maintenance (N = 56; 50 mg/m², subcutaneously, days 1-5, every 4 weeks) until relapse, for a maximum of 12 cycles. Fifty-five patients received at least 1 cycle of azacitidine, 46 at least 4 cycles, and 35 at least 12 cycles. The maintenance treatment with azacitidine was feasible. DFS was significantly better for the azacitidine treatment group (logrank; *P* = .04), as well as after adjustment for poor-risk cytogenetic abnormalities at diagnosis and platelet count at randomization (as surrogate for CR vs CRi; Cox regression; hazard ratio, 0.62; 95% confidence interval, 0.41-0.95; *P* = .026). The 12-month DFS was estimated at 64% for the azacitidine group and 42% for the control group. OS did not differ between treatment groups, with and without censoring for allogeneic hematopoietic cell transplantation. Rescue treatment was used more often in the observation group (n = 32) than in the azacitidine maintenance group (n = 9). We conclude that azacitidine maintenance after CR/CRi after intensive chemotherapy is feasible and significantly improves DFS. The study is registered with The Netherlands Trial Registry (NTR1810) and EudraCT (2008-001290-15). (*Blood*. 2019;133(13):1457-1464)

Introduction

About 75% of patients with acute myeloid leukemia (AML) are 60 years of age or older.[1] After intensive chemotherapy, complete remission (CR) rates in the range of 40% to 55% are generally attained, resulting in median disease-free survival (DFS) of between 6 and 12 months.[2-8] The prevention of relapse is the major therapeutic challenge in older patients with AML who are in CR after intensive chemotherapy. No postremission treatment to prevent relapse has been established and generally accepted in this setting, except for the use of allogeneic hematopoietic cell transplantation (allo-HCT) for a selected group of relatively fit patients.[9-11] Although there is a long-standing interest in maintenance therapies such as interleukin 2,[12-14] low-dose cytarabine,[15] and gemtuzumab ozogamicin[16] after intensive induction treatment, the clinical benefits of such maintenance therapy have remained controversial.[17]

Potential candidates for maintenance treatment include hypomethylating agents such as decitabine and azacitidine, which have proven efficacy and limited extra medullary toxicity in older

individuals.[18-20] A small randomized study comparing decitabine (20 mg/m^2 for 5 days every 4-8 weeks) with conventional care (observation, low-dose cytarabine or intensive chemotherapy) was prematurely discontinued without showing a lower relapse rate for the 20 patients in the decitabine group.[21] A phase 2 study exploring decitabine maintenance (20 mg/m^2 for 4-5 days every 6 weeks for 8 cycles) in 134 younger patients with AML in CR1 did not show better DFS compared with historical controls (1- and 3-year DFS, 79% and 54%, respectively).[22] Various other small studies involving small numbers of patients explored azacitidine maintenance, but did not yield any conclusive data on its usefulness.[23,24]

Here we present the final analysis of the HOVON97 study. In this phase 3 study in older patients (≥60 years) with AML or MDS-refractory anemia with excess of blasts, subjects in CR/CR with incomplete hematologic recovery (CRi) after at least 2 cycles of intensive chemotherapy were randomly assigned to receive either azacitidine as postremission therapy or no further treatment (observation). The aim was to assess the value of maintenance treatment with respect to DFS (primary end point) and overall survival (OS; secondary end point).

Methods

Study design and treatment

In this study of the Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON97), patients who had entered CR or CRi after at least 2 cycles of remission-induction chemotherapy were randomly assigned to 12 cycles of azacitidine (50 mg/m^2 subcutaneously for 5 days every 4 weeks) or to observation (no further treatment). Randomizations were balanced by minimization with the factors hospital, platelet count ($<100 \times 10^9$/L vs $\geq 100 \times 10^9$/L) at randomization, and cytogenetic risk at diagnosis (favorable/intermediate vs unfavorable). Between 30 June 2009 and 1 December 2016 a total of 118 patients were registered in the study. Two patients were considered ineligible (1 was registered twice; the second patient had no CR/CRi), so that a total of 116 eligible patients were randomized and included in the analyses. Azacitidine was provided free of charge by Celgene. The study was approved by the ethics committees of the participating institutions, and was conducted in accordance with the Declaration of Helsinki. The HOVON97 study is registered with The Netherlands Trial Registry (NTR1810) and EudraCT (2008-001290-15). The database was locked on July 12, 2018.

Eligibility

Patients with an initial cytopathologically confirmed diagnosis of AML (M0-M2 and M4-M7) and a minimum of 20% blast infiltrate in the bone marrow, who were 60 years of age or older, were eligible, provided they had a World Health Organization (WHO) performance status of 2 or less and had given their written informed consent and had less than 5% bone marrow blasts after 2 cycles of induction chemotherapy. Eligibility also included an initial subtype of the MDS (ie, refractory anemia with excess of blasts) with an International Prognostic Scoring System score of 1.5 or higher and less than 5% bone marrow blasts after 2 cycles of induction chemotherapy. Patients with extramedullary disease, AML after previous polycythemia rubra vera or primary myelofibrosis, blast crisis of CML or AML-FAB-M3, or AML with cytogenetic abnormality t15,17, and patients with a concurrent severe and/or uncontrolled medical condition or cardiac dysfunction were considered not eligible. For randomization, postremission patients were required to be in CR/CRi after at least 2 cycles of intensive chemotherapy, and to have an absolute neutrophil count greater than 0.5×10^9/L and a platelet count greater than 50×10^9/L.

Patient characteristics and classification

On the basis of karyotype at diagnosis, patients were classified into distinct prognostic categories. Patients with core binding factor abnormalities [t8,21(q22;q22), inv16(p13.1q22), or t16,16(p13.1;q22)] were classified as favorable risk. Patients without cytogenetic abnormalities or with loss of X or Y as the only abnormality were classified as normal cytogenetics. Patients with complex karyotypes [≥3 abnormalities; −5(q), −7(q), abn (3q)] were classified as unfavorable risk. The remaining patients with AML were classified as intermediate risk.

Statistical analysis, criteria of response, and evaluation of outcome

The primary objective of this study in postremission patients was to compare the value, in relation to DFS, of azacitidine therapy (intervention group) and no further therapy (observation group). DFS was measured from the date of randomization to relapse or death from any cause, whichever came first. Cox regression analysis with adjustment for the stratification factors (except center) was the primary analysis for this comparison. According to the protocol, bone marrow aspirate had to be performed after 24 weeks (observation group) or after 6 cycles (azacitidine group), and in case of suspicion of relapse (in both groups). The compliance to these bone marrow evaluations was fairly reasonable, with 7 patients (observation) vs 8 patients (azacitidine) of whom no bone marrow aspirate was performed at approximately 6 months.

Secondary objectives were to evaluate the effects of azacitidine after remission in relation to OS measured from the date of randomization, probability of relapse and death after inclusion from date of randomization (calculated as competing risks), and number and duration of hospitalizations, transfusion requirements (red cell and platelet transfusion), and adverse events. CR was defined as a cellular marrow with less than 5% blasts, no Auer rods, no evidence of extramedullary leukemia, and peripheral granulocyte and platelet counts of at least 1.0×10^9/L and 100×10^9/L, respectively. CRi was defined as CR except for residual neutropenia ($<1.0 \times 10^9$/L) or thrombocytopenia ($<100 \times 10^9$/L). Relapse was defined as recurrence of leukemia after CR or CRi. OS was measured from the date of registration until death from any cause. Patients known to be still alive at the date of last contact were then censored.

Based on our experience with the previous HOVON43 study, in the present study, we estimated that 40% of patients in the observation group would have a DFS of 12 months.[2] We hypothesized that 60% of patients in the azacitidine maintenance group would have a DFS of 12 months. A target number of 126 patients, with 97 events required, would give a power of 80% to detect this difference with a 2-sided test at 5% significance level, an accrual period of 3 years, and an additional follow-up of 1 year.

All analyses were performed according to intention to treat, irrespective of protocol compliance. The log-rank test and Cox regression analysis were used to analyze the differences between

Table 1. Patient characteristics

	Observation group (N = 60)	Azacitidine group (N = 56)
Sex, male/female	33/27 (55%/45%)	35/21 (63%/37%)
Age, median/range	69/60-79	69/64-81
WHO performance		
WHO 0	23 (38%)	29 (52%)
WHO 1	34 (57%)	17 (30%)
WHO 2	—	5 (9%)
Unknown	3 (5%)	5 (9%)
Unfavorable risk cytogenetic abnormalities at diagnosis*	14 (23%)	9 (16%)
CR(i) obtained after		
Induction cycle 1	45 (75%)	35 (63%)
Induction cycle 2	15 (25%)	21 (37%)
Platelet count ≥100 × 10^9/L	45 (75%)	38 (68%)
Neutrophils, ×10^9/L		
Median	4.1	3.3
Range	1.5-38	0.6-13.7
CR	45 (75%)	37 (66%)
MDS-refractory anemia with excess of blasts	6 (10%)	6 (11%)

All characteristics were obtained from randomization except unfavorable risk cytogenetic abnormalities, which were obtained at diagnosis.

*−7, −7q, −5, −5q, abn 3q, complex ≥3 abnormalities.

both groups with respect to OS and DFS. These analyses were performed without and with adjustment for platelet count (<100 vs ≥100) at randomization and cytogenetic risk classification at diagnosis. All *P* values reported are 2-sided.

Possible heterogeneity of the treatment effects between subgroups (poor-risk cytogenetic abnormalities at diagnosis [yes vs no], platelet count [≥100 vs <100 × 10^9/L] at randomization, age [younger vs older than median age 69 years], cycles to CR/CRi [1 vs 2], and performance status [0 vs ≥1]) were explored. For each of the variables, a multivariate Cox regression with treatment group, variable, and treatment group × variable interaction term was performed. Only if the hazard ratio (HR) for the interaction term was statistically significant different from 1 ($P < .05$) were subgroup analyses performed. Otherwise, subgroup analyses were not warranted, and the estimate of the overall treatment effect also was considered the best estimate for the treatment effect within a specific subgroup.

Results

Patient cohort

The study was terminated before the accrual of the planned 126 patients. Because of declining accrual of new patients, it was

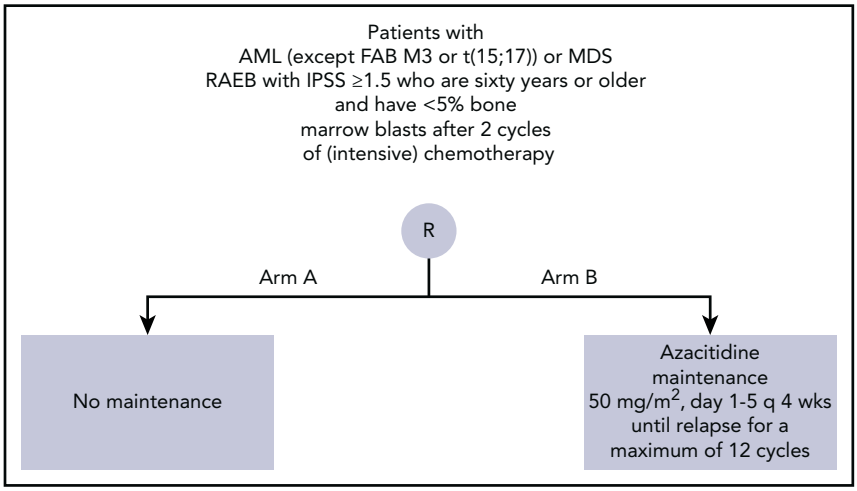

Figure 1. Trial design. IPSS, International Prognostic Scoring System.

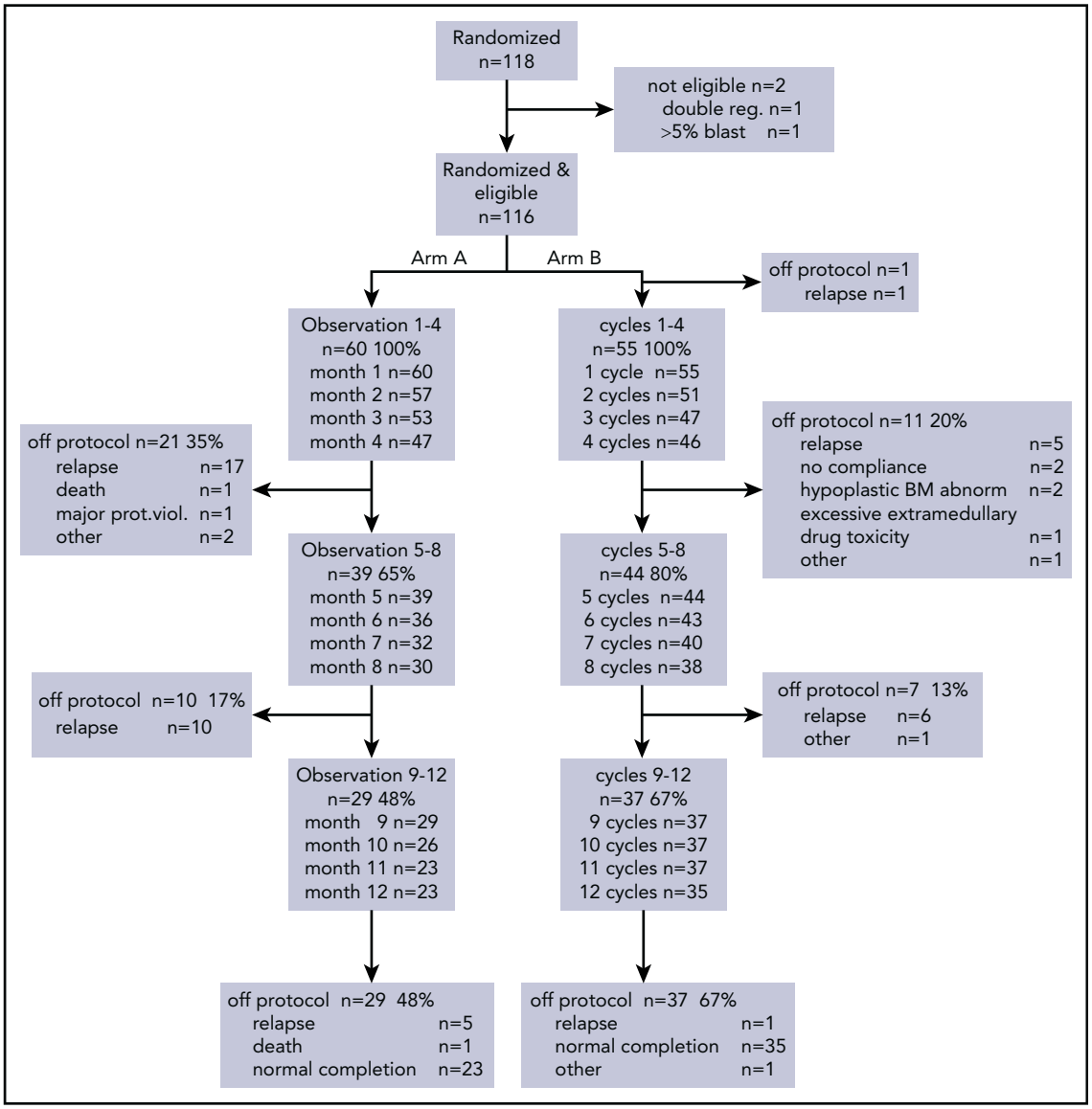

Figure 2. CONSORT study diagram. Arm A, observation; Arm B, azacitidine maintenance. The main reason for failure to complete protocol was intercurrent relapse.

estimated that the number of events, as defined in the original statistical plan, could not be reached within a reasonable time. The median follow-up time of the 116 evaluable and eligible patients still alive at the date of the last contact since the date of randomization was 41.4 months. Table 1 presents the characteristics of the patients enrolled in the observation and azacitidine maintenance groups.

Feasibility of azacitidine maintenance treatment

After randomization, 60 patients were assigned to the observation group and 56 patients to the azacitidine maintenance group (Figure 1). Because 1 patient had a relapse between randomization and start of azacitidine postremission treatment, 55 patients started azacitidine cycles 1 to 4. Subsequently, 44 patients started cycles 5 to 8 of azacitidine treatment, and 37 patients started cycles 9 to 12. After 4 cycles, 11 patients went off protocol (5 relapse, 2 no compliance, 2 hypoplastic bone marrow, 1 excessive extra-medullary toxicity, and 1 other reason); after 4 additional cycles, another 7 patients went off protocol (6 relapse, 1 other reason), and finally, after 12 cycles, 37 patients went off protocol (1 relapse, 1 other reason, 35 protocol completion). This is illustrated in the CONSORT flow diagram (Figure 2). Interestingly, in the azacitidine group, 35 (63%) of 56 patients completed protocol treatment, whereas in the observation group, this was feasible (ie, alive without relapse on protocol) in 23 (38%) of 60 patients.

The adherence to treatment according to the protocol was high. On average, 90% of the azacitidine cycles were given full dose according to schedule (mean, 90%; range, 81%-97%). The time intervals between 2 consecutive cycles was were 30 days for, on average, 86% of the cycles (mean, 86%; range, 76%-90%) and between 30 and 40 days for, on average, 10% of the cycles (median, 10%; range, 7%-14%).

Azacitidine maintenance was associated with a low transfusion dependence, a limited number of nights in the hospital, and a limited number of adverse events (AEs) and serious AEs (SAEs)

Table 2. Feasibility and safety

	Observation group (N = 60)	Azacitidine group (N = 56)
Transfusion requirements		
RBC (median/mean), units	0/1	0/1
No. of patients receiving no RBC	55 (92%)	48 (86%)
Platelets, median/mean	0/1	0/1
Patients receiving no platelets, n	56 (93%)	48 (86%)
Nights in hospital		
Median/mean	0/1	0/2
Patients without nights in hospital, n	55 (92%)	48 (86%)
AEs		
Median	1	2
AE ≥2 grade (total), n	449	510
Patients with SAEs		
0 SAE	56 (93%)	42 (75%)
1 SAE	4 (7%)	11 (20%)
2 SAE		2 (3%)
3 SAE		1 (2%)

RBC, red blood cells.

(Table 2; supplemental Table 1, available on the *Blood* Web site). Red blood cell transfusions were not given to 92% of control patients and 86% of patients in the azacitidine group, whereas 93% and 86%, respectively, did not require any platelet transfusions. Furthermore, 92% of patients in the observation group and 86% in the azacitidine treatment group did not require clinical hospital admission. The number of AEs and SAEs were also comparable between both groups: 93% of patients in the observation group and 75% of those in the azacitidine treatment group did not experience any SAEs (ie, 4 patients in observation group and 14 patients in the azacitidine group experienced SAEs).

Treatment outcome according to postremission randomization

DFS was significantly improved after azacitidine maintenance treatment (64% vs 42% at 12 months; logrank; $P = .04$; Figure 3). DFS at 24 and 36 months was estimated at 44% and 32% for the azacitidine group and 20% and 16% for the control group, respectively. Cox regression analysis, with adjustment for poor-risk cytogenetic abnormalities at diagnosis and platelet count of at least 100×10^9/L (according to protocol), confirmed the significant improvement in DFS after azacitidine maintenance (Cox regression; HR, 0.62; 95% confidence interval, 0.41-0.95; $P = .026$).

Multivariate Cox regression analysis was performed to investigate possible heterogeneity of the treatment effects (DFS) between subgroups (poor-risk cytogenetic abnormalities at diagnosis [yes vs no], platelet count [≥100 vs <100 × 10^9/L], age [younger vs older than median age 69 years], response CR[i] reached after induction cycle 1 vs induction cycle 2, cycles to CR/CRi [1 vs 2], performance status [0 vs ≥ 1]). Only a statistically significant interaction between treatment group and platelet count was found, which revealed that patients with a platelet count of at least 100×10^9/L at inclusion (and not those with a platelet count <100 × 10^9/L) had a significant better DFS after azacitidine maintenance (supplemental Figure 2). In line with this, a significant interaction between treatment group and CR (and not CRi) was observed (supplemental Figure 3).

This significant improvement in DFS did not translate to a significant improvement in OS (84% vs 70% at 12 months; logrank; $P = .69$) (Figure 4). Cox regression analysis confirmed the lack of improvement in OS after azacitidine maintenance (Cox regression; HR, 0.91; 95% confidence interval, 0.58-1.44; $P = .69$). At the same time, we noted an imbalance in the use of salvage therapy after relapse between the study groups. Thirty-two

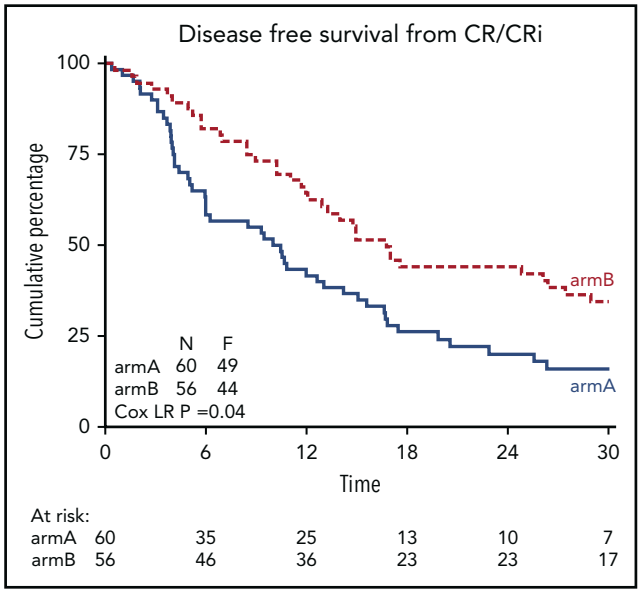

Figure 3. Kaplan-Meier estimates for DFS. armA, observation group; armB, azacitidine maintenance group. Azacitidine maintenance treatment increased the median DFS by 5.6 months (armA, 10.3 months; armB, 15.9 months).

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