## Low-dose clofarabine in combination with a standard remission induction in patients aged 18-60 years with previously untreated intermediate and bad-risk acute myeloid leukemia or high-risk myelodysplastic syndrome: combined phase I/II results of the EORTC/GIMEMA AML-14A trial

The prognosis of younger patients with intermediate/high risk acute myelogenous leukemia (AML) or high-risk myelodysplastic syndromes (MDS) remains unsatisfactory.[1-4] Clofarabine is a purine nucleoside analog that is highly active as a single agent in AML.[5] Furthermore, synergy between clofarabine and Ara-C has been demonstrated *in vitro*[6] and in AML patients.[6-8] We have recently reported the results of the randomized phase I part of the EORTC/GIMEMA-AML-14A trial and identified clofarabine at 10 mg/m$^2$/day on days 2, 4, 6, 8 and 10 as the maximum tolerated dose (given either in a 1-hour infusion or as push injection) in combination with Ara-C and idarubicin.[9] We decided to administer clofarabine on these five days because we hypothesized that the synergy between clofarabine and Ara-C would be more effective when clofarabine was present in the leukemic cells during the entire period of Ara-C administration. Furthermore, we hypothesized that push injections of clofarabine might result in higher clofarabine peak levels than a 1-hour infusion schedule, leading to a better interaction with Ara-C and more pronounced anti-leukemic effects. We report the final results of the combined phase

**Table 1.** Baseline patients' characteristics.

| | Treatment arm | | |
|---|---|---|---|
| | 1-hour infusion (Arm A, n=31) | Push injection (Arm B, n=31) | Total (n=62) |
| Sex, n. of patients (%) | | | |
| Male | 16 (51.6) | 11 (35.5) | 27 (43.5) |
| Female | 15 (48.4) | 20 (64.5) | 35 (56.5) |
| Age, years | | | |
| Median | 46.0 | 50.0 | 49.5 |
| Range | 20.0 - 60.0 | 22.0 - 60.0 | 20.0 - 60.0 |
| Presence of poor prognosis features[a] at randomization, n. of patients (%) | 6 (19.4) | 5 (16.1) | 11 (17.7) |
| WHO performance status, n. of patients (%) | | | |
| 0 | 23 (74.2) | 23 (74.2) | 46 (74.2) |
| 1 | 7 (22.6) | 7 (22.6) | 14 (22.6) |
| 2 | 1 (3.2) | 1 (3.2) | 2 (3.2) |
| WHO classification, n. of patients (%) | | | |
| AML with multilineage dysplasia | 5 (16.1) | 6 (19.4) | 11 (17.7) |
| AML therapy related | 2 (6.5) | 3 (9.7) | 5 (8.1) |
| AML not otherwise specified | 23 (74.2) | 18 (58.1) | 41 (66.1) |
| MDS RAEB II | 1 (3.2) | 4 (12.9) | 5 (8.1) |
| Type of disease, n. of patients (%) | | | |
| *De novo* AML or MDS | 29 (93.5) | 28 (90.3) | 57 (91.9) |
| Secondary AML or MDS | 2 (6.5) | 3 (9.7) | 5 (8.1) |
| WBC at diagnosis, n. of patients (%) | | | |
| < 100 x10$^9$/L | 27 (87.1) | 30 (96.8) | 57 (91.9) |
| ≥ 100 x10$^9$/L | 4 (12.9) | 1 (3.2) | 5 (8.1) |
| Cytogenetics,[b] n. of patients (%) | | | |
| Normal | 19 (61.3) | 13 (41.5) | 32 (51.6) |
| With FLT3-ITD and unmutated/unk NPM1 | 3 (9.7) | 3 (9.7) | 6 (9.7) |
| Without FLT3-ITD and with mutated NPMI | 6 (19.4) | 5 (16.1) | 11 (17.7) |
| With FLT3-ITD and with mutated NPMI | 3 (9.7) | 1 (3.2) | 4 (6.5) |
| Other or unknown molecular markers | 7 (22.6) | 4 (12.9) | 11 (17.7) |
| Good risk | 0 | 0 | 0 |
| High risk | 3 (9.7) | 10 (32.3) | 13 (21) |
| Very high risk | 8 (25.8) | 5 (16.1) | 13 (21.0) |
| Unknown/ failure / missing | 1[c] (3.2) | 3 (9.7) | 4 (6.5) |
| Bone marrow blasts (%) | | | |
| Median | 70 | 57 | 60.5 |
| Range | 19-97 | 12-99 | 12-99 |

[a]Defined as white blood cell count (WBC) at diagnosis ≥ 100x10$^9$/L or very high-risk cytogenetics or FLT3-ITD positivity; [b]cytogenetics: good risk includes inv(16) or t(8;21); very bad risk includes complex abnormalitis (>3 abnormalities), monosomies 5, 7 and 5q-, 7q-, 3q, t(6;9), t(9;22), 11q23, t(9;11); bad risk includes all other chromosomal abnormalities; [c]47,XY,dup(1)(q1?1q4?2),+8[7] / 46,XY,-17,+mar2[4] / 46,XY,-17,+mar1[3].

I and II parts of the trial.

EORTC/GIMEMA-AML-14A is an open label randomized 2-arm multicenter trial with a sequential phase I-II design (*clinicaltrials.gov identifier: 00838240*). The protocol was approved by the EORTC Protocol Review Committee and by the Ethical Committee of each participating center. The main objective of the phase II part of the trial was to explore the anti-leukemic activity of the aforementioned phase I selected dosage schedules of clofarabine given either as a 1-hour intravenous (iv) infusion (Arm A) or as a push injection (Arm B) over ten minutes. The primary end point was the complete remission (CR)/CR with incomplete blood count recovery (CRi) rate after 1 or 2 induction cycles. The aim was to determine whether, in each treatment group, the true CR/CRi rate was more than 65% or not. Thus, for each of the arms A and B, the regimen was considered as active and feasible if 23 or more of 30 (76.7%) patients achieved a CR/CRi (see *Online Supplementary Appendix* for detailed study design and methods of statistical analyses). Secondary end points included toxicity, overall survival (OS) from inclusion, OS from CR/CRi, relapse-free survival (RFS) from CR/CRi, and incidences of relapse and of death in CR/CRi.

Inclusion criteria included: age 18-60 years, primary or secondary intermediate or high-risk AML10 or MDS with 10%-19% blast cells in the bone marrow (BM), previously untreated disease, WHO Performance Status grade 0-2, and adequate organ functions. Main exclusion criteria included: good-risk AML (ie. AML-M3, or AML with t(8;21) or inv(16)) and a white blood cell count (WBC) at diagnosis of less than $100x10^9$/L), blast crisis chronic myeloid leukemia or AML supervening a myeloproliferative disorder, central nervous system leukemia, evidence of severe concurrent cardiac, pulmonary, and neurological disorder, and uncontrolled infection.

Clofarabine was administered at 10 mg/m$^2$ on days 2, 4, 6, 8 and 10 either as a 1-hour infusion (Arm A) or as a push injection (Arm B). Ara-C was administered at 100 mg/m$^2$/day on days 1-10 as a continuous infusion, while idarubicin was given at 10 mg/m$^2$/day on days 1, 3, and 5 as a 5-minute iv injection. A second identical course of induction chemotherapy was given in case of a partial response (PR). One cycle of consolidation chemotherapy consisting of Ara-C (500 mg/m$^2$ every 12 hours as a 2-hour iv infusion on days 1-6) and idarubicin (10 mg/m$^2$/day on days 4, 5 and 6) was administered in patients in both arms who achieved a CR/CRi. Post-consolidation treatment was left at the discretion of the local principal investigator, but it was recommended that the consolidation phase was followed by allogeneic hematopoietic cell transplantation (HCT) for patients

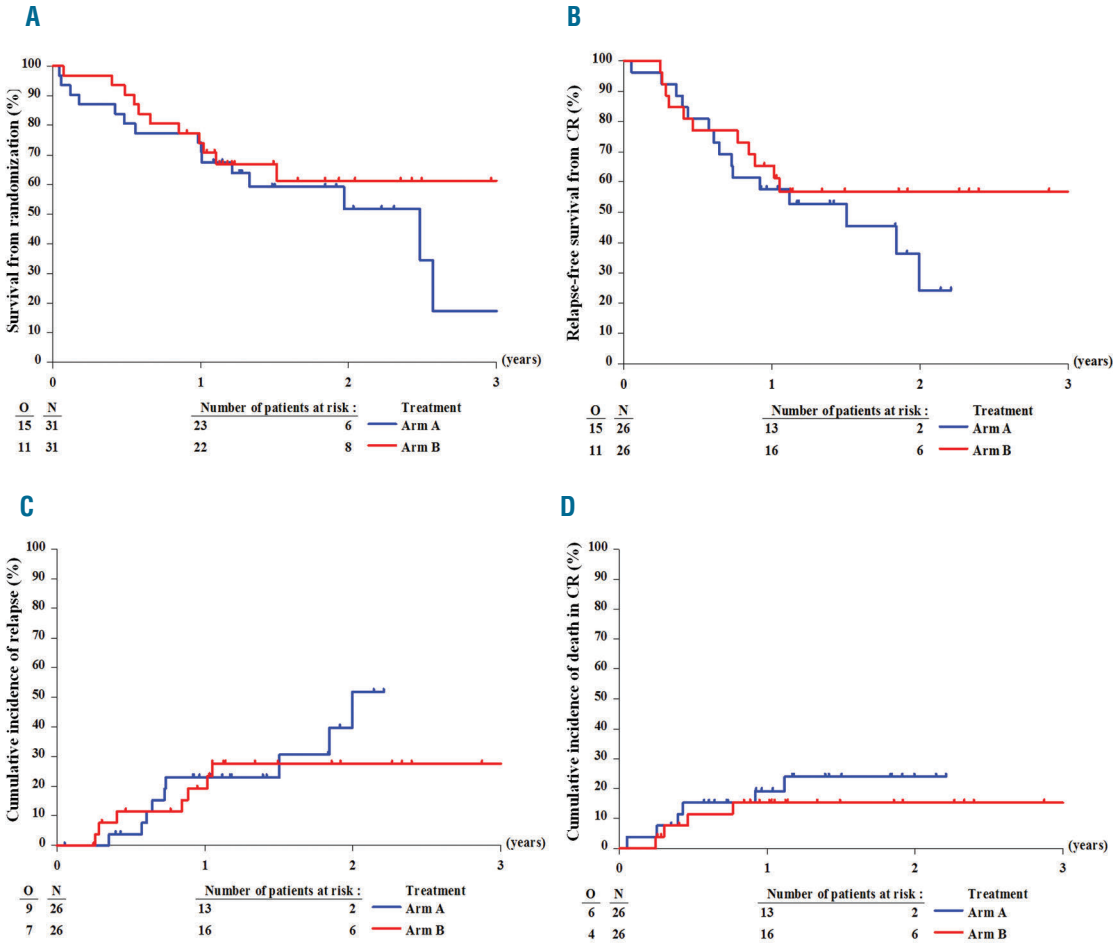


Figure 1. Overall survival (A), relapse-free survival from complete remission (CR) (B), cumulative incidence of relapse from CR (C) and cumulative incidence of death in CR (D) in the two arms.

with an HLA-identical related donor or for patients with very high-risk cytogenetics who had an HLA-compatible related or unrelated donor, or an autologous HCT in patients who were not candidates for allogeneic HCT.[1]

A total of 64 patients (12 in the phase I part and 52 in the phase II part of the study) were randomized at the dosage of clofarabine of 10 mg/m$^2$/day (*Online Supplementary Figure S1*). Two patients were excluded because they did not meet the inclusion criteria. Among the remaining 62 patients, 41 had AML not otherwise specified, 11 AML with multilineage dysplasia, 5 therapy-related AML while 5 patients were diagnosed with MDS-RAEB2. Median age was 49.5 (range 20-60) years. Baseline characteristics were generally well balanced between the two arms (Table 1).

After one induction course, the CR/CRi rates were 26 of 31 patients in arm A (84%) *versus* 25 of 31 patients in arm B (80%) (Table 2). The CR/CRi rate after 1 or 2 induction courses was 84% (95%CI: 66%-95%), in both arms, higher than the protocol-defined efficacy (>65%) (Table 2). Interestingly, combining the results from both arms, the CR/CRi rate after 1 or 2 courses of induction was similar in patients with very high-risk cytogenetics [11 of 13 patients (84.6%)], and in patients with normal or high-risk cytogenetics [38 of 46 patients (82.6%)]. These results are in the same range as those observed in

**Table 2.** Disease response (primary end point) and adverse events.

| | Treatment arm | | |
|---|---|---|---|
| | 1-hour infusion (Arm A, n=31) | Push injection (Arm B, n=31) | Total (n=62) |
| Response[a] to induction n.1, n. of patients (%) | | | |
| CR | 23 (74.2) | 24 (77.4) | 47 (75.8) |
| CRi | 3 (9.7) | 1 (3.2) | 4 (6.5) |
| PR | 1 (3.2) | 1 (3.2) | 2 (3.2) |
| Failure due to resistant disease | 0 (0.0) | 4 (12.9) | 4 (6.5) |
| Failure due to complication from aplasia | 2 (6.5) | 1 (3.2) | 3 (4.8) |
| Hypoplasia | 1 (3.2) | 0 (0.0) | 1 (1.6) |
| Not assessable | 1 (3.2) | 0 (0.0) | 1 (1.6) |
| Response to inductions n.1 / 2, n. of patients (%) | | | |
| CR | 23 (74.2) | 25 (80.6) | 48 (77.4) |
| CRi | 3 (9.7) | 1 (3.2) | 4 (6.5) |
| PR | 1 (3.2) | 0 (0.0) | 1 (1.6) |
| Failure due to resistant disease | 0 (0.0) | 4 (12.9) | 4 (6.5) |
| Failure due to complication from aplasia | 2 (6.5) | 1 (3.2) | 3 (4.8) |
| Hypoplasia | 1 (3.2) | 0 (0.0) | 1 (1.6) |
| Not assessable | 1 (3.2) | 0 (0.0) | 1 (1.6) |
| Most frequent (>5% in at least one arm) grade III-IV[b] biochemical abnormalities, n. of patients (%) | | | |
| Bilirubin | 4 (12.9) | 6 (19.4) | 10 (16.1) |
| ALT | 4 (12.9) | 4 (12.9) | 8 (12.9) |
| Alkaline phosphatase | 0 | 2 (6.5) | 2 (3.2) |
| Most frequent (>5% in at least one arm) grade III-IV[b] adverse events, n. of patients (%) | | | |
| Febrile neutropenia | 23 (74.2) | 13 (41.9) | 36 (58.1) |
| Documented infection | 13 (42.0) | 22 (70.9) | 35 (56.5) |
| Anorexia | 6 (19.4) | 10 (32.3) | 16 (25.8) |
| Diarrhea | 7 (22.6) | 6 (19.4) | 13 (21.0) |
| Dyspnea | 3 (9.7) | 1 (3.2) | 4 (6.5) |
| Fatigue | 1 (3.2) | 2 (6.5) | 3 (4.8) |
| Rash | 3 (9.7) | 1 (3.2) | 4 (6.5) |
| Nausea | 2 (6.5) | 1 (3.2) | 3 (4.8) |
| Hemorrhage | 3 (9.7) | 0 | 3 (4.8) |
| Deshydratation | 0 | 2 (6.5) | 2 (3.2) |
| Documented grade II-IV fungal infection, n. of patients (%) | 4 (12.9) | 9 (29.0) | 13 (21.0) |
| Causes of death | | | |
| Acute myeloid leukemia | 4 (12.9) | 4 (12.9) | 8 (12.9) |
| Toxicity | 4 (12.9) | 1 (3.2) | 5 (8.1) |
| Transplant-related mortality | 7 (22.6) | 5 (16.1) | 12 (19.4) |
| Other | 0 | 1 (3.2) | 1 (1.6) |

[a]Evaluation of response was scheduled around day 31 after the start of the induction course. CR was defined as less than 5% marrow blasts and recovery of normal hematopoiesis with a neutrophil count ≥ 1x10$^9$/L and a platelet count ≥ 100x10$^9$/L in addition to disappearance of all clinical, laboratory, or radiological evidence of disease. The term incomplete CR (CRi) was used to define patients who met all CR criteria, but had neutrophil counts between 0.5 and 1.0x10$^9$/L and/or platelet counts between 50 and 100x10$^9$/L. Finally, partial remission (PR) was defined as 5%-25% blast cells in the bone marrow and a reduction of at least 50% of blasts in the bone marrow, irrespective of count recovery. [b]Adverse events were graded with Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 scoring system (*http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf*).

two recent phase II studies investigating the efficacy of higher dosages of clofarabine combined with AraC (and idarubicin) as remission induction regimen for younger AML patients.[11,12]

With a median follow up of 1.8 (range 1-5.3) years, 15 of 31 (48%) patients in arm A and 11 of 31 patients (36%) in arm B died. One-year OS from inclusion was 74% (95%CI: 55%-86%) in each arm, while median survival was 2.5 (1 not reached) years in arm A, *versus* not yet reached in arm B (Figure 1A).

Consolidation chemotherapy was given in all of 26 patients who achieved a CR/CRi in arm A, and in 23 of 26 patients (88.5%) in arm B. Following consolidation chemotherapy, 12 of 26 patients in arm A received an allogeneic HCT, while, in arm B, 14 patients received an allogeneic HCT and 2 an autologous HCT. Among a total of 52 patients in CR/CRi, 1-year OS and RFS from CR/CRi were 77% (95%CI: 56%-89%) and 58% (95%CI: 37%-74%), respectively, in arm A *versus* 73% (95%CI: 51%-86%) and 65% (95%CI: 44%-80%), respectively, in arm B (Figure 1B). One-year incidences of relapse and of death in CR were 23% (95%CI: 7%-39%) and 19% (95%CI: 4-34%), respectively, in arm A, *versus* 19% (95%CI: 4%-34%) and 15% (95%CI: 2-29%), respectively, in arm B (Figure 1C and D). As expected, the incidence of relapse was higher in CR/CRi patients with very high risk cytogenetics (5 of 11: 45%) than in those with intermediate-/high-risk cytogenetics (10 of 38: 26%).

The toxicity profile of the two tested remission-induction chemotherapy regimens was acceptable and comparable in the two arms (Table 2), confirming data observed in the phase I of our study.[9] Median time to neutrophils of $0.5x10^9$/L or more and of $1.0 x10^9$/L or more were 28 (range 22-96) and 31 (range 22-99+) days, respectively, in arm A, *versus* 27 (range 20-50) and 29 (range 21-50) days, respectively, in arm B (Table 2). Further, median time to platelet levels of $20x10^9$/L or more or of $100x10^9$/L or more were 28 (range 24-83) and 31.5 (range 24-99+) days, respectively, in arm A, *versus* 27 (range 23-44) and 31 (range 24-51) days, respectively, in arm B. Besides hematologic recovery, the toxicities over grade 2 observed in the AML-14A patients were in the same range as currently observed after standard remission-induction chemotherapy, with the possible exception of a higher incidence of over grade 2 hyperbilirubinemia that was observed in 18% of the AML-14A patients.

We finally compared the outcomes of the AML14A patients to those of a subgroup of 201 patients from the standard arm of the previous EORTC/GIMEMA-AML-12 study (combining standard dose Ara-C, daunorubicin and etoposide; see *Online Supplementary Appendix*)[1] who met the same inclusion criteria as current patients, and were treated in the centers that contributed patients to the current AML14A study. As shown in the *Online Supplementary Table S1*, patients' characteristics were comparable in the 2 groups. However, since these analyses were not planned beforehand in the protocol, they should be seen as indicative. The rate of CR/CRi after 1 or 1-2 cycles of induction chemotherapy were 82.3% and 83.9%, respectively, in current AML14A patients *versus* 66.7% and 72.6%, respectively in the cohort of AML12 patients (*Online Supplementary Table S2*). A higher proportion of patients included in the AML-14A (50%) than in AML-12 (30%) were offered an allogeneic HCT, probably reflecting, at least in part, the higher CR/CRi rate achieved in AML-14A patients. One-year OS and RFS rates were 74.1% (95%CI: 61.3%-83.3%) and 61.5% (95%CI: 47.0%-73.2%), respectively, in current AML14 patients, and 58.0% (95%CI: 50.9%-64.5%) and 54.1% (95%CI: 45.7%-61.8%), respectively, in AML12 patients. Finally, among patients who reached a CR, the 1-year cumulative incidences of relapse and of death in CR were 23.3% and 17.3%, respectively, in AML-14A, as compared with 37.7% and 9.6%, respectively, in AML-12.

In conclusion, the two tested clofarabine containing regimens yielded an impressive CR/CRi rate and encouraging 1-year OS/RFS rates among patients with intermediate/high-risk AML or high-risk MDS. These results are worth confirming in a large phase III study.

*Dominik Selleslag,[1] Stefan Suciu,[2] Giovanna Meloni,[3] Petra Muus,[4] Constantijn J.M. Halkes,[5] Adriano Venditti,[6] Safaa M. Ramadan,[2,7] Hans Pruijt,[8] Liv Meert,[2] Marco Vignetti,[9] Jean-Pierre Marie,[10] Sébastian Wittnebel,[11] Theo de Witte,[12] Sergio Amadori,[6] Roelof Willemze[5*] and Frédéric Baron[13*]*

*[1]Department of Hematology AZ St-Jan Brugge-Oostende, Belgium; [2]EORTC Headquarters, Brussels, Belgium; [3]Hematology, Sapienza University, Rome, Italy; [4]Department of Hematology, Radboud University, Medical Center, Nijmegen, the Netherlands; [5]Department of Hematology, Leiden University Medical Center, the Netherlands; [6]Department of Hematology and of Biomedicine and Prevention, Tor Vergata University, Rome, Italy; [7]Department of Hematology, Istituto Europeo di Oncologia, Milan, Italy; [8]Jeroen Bosch Medical Center, Den Bosch, the Netherlands; [9]Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA), Rome, Italy; [10]Department of Hematology, Hôpital Saint-Antoine, APHP, Paris, France; [11]Department of Hematology, Institut Jules Bordet, ULB, Brussels, Belgium; [12]Department of Tumorimmunology, Radboud University, Medical Center, Nijmegen, the Netherlands and [13]GIGA-I3-Hematology, University of Liège, Belgium*

**RW and FB contributed equally to this work.*

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*Correspondence: f.baron@ulg.ac.be*

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