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Flai (fludarabine, cytarabine, idarubicin) plus low-dose Gemtuzumab Ozogamicin as induction therapy in CD33-positive AML: Final results and long term outcome of a phase II multicenter clinical trial

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Abstract

The aim of this prospective clinical trial was to evaluate the efficacy and safety of a combination of Gemtuzumab-Ozogamicin (GO) and FLAI scheme (fludarabine, cytarabine, idarubicin) as a first-line therapy in CD33 positive AML. We treated 130 patients, aged <65, with a median age of 52 years. FLAI-GO induction regimen included fludarabine (30 mg/sqm) and cytarabine (2 g/sqm) on days 1–5; idarubicin (10 mg/sqm) on days 1, 3, and 5; and GO (3 mg/sqm) on day 6. SCT was planned for all high-risk AML patients, after consolidation with intermediate doses of cytarabine and idarubicin and a high dose of cytarabine. CD33 expression exceeded 20% in all cases. Primary endpoints of the study included feasibility, overall response rate (ORR) and toxicity. Secondary endpoints included the evaluation of MRD by WT1 expression, feasibility and outcome of consolidation with SCT, overall survival (OS) and disease-free survival (DFS).

After induction with FLAI-GO, complete remission (CR) rate was 82%. Four patients achieved partial remission (PR) and 12% were resistant (ORR 85%); death during induction (DDI) was 3%. The hematological and extra hematological toxicity of FLAI-GO was manageable; 45% of patients experienced transient and reversible GO infusion related adverse events. In the setting of patients who achieved a cytological CR after FLAI-GO, the mean of WT1 copies dropped from 8337±9936 copies/10^4ABL (diagnosis) to 182 ± 436 copies after induction therapy ($p = 0.0001$) showing a very good disease debulking. After a median follow-up of 54 months, 67/130 (52%) patients were alive. The probability of 1, 2, and 5-year OS was 80%, 63%, and 52%, respectively. The probability of 1, 2, and 5-year DFS was 77%, 58%, and 52%, respectively. Allogeneic and autologous SCT was performed in 60 (46%) and 23 (18%) patients, respectively.

In summary, the final results of this trial confirm that FLAI-GO is an active and safe treatment strategy for CD33-positive AML patients aged $\leq$ 65 years, allowing a high ORR, a good disease debulking, favorable safety profile, low DDI, and subsequent high SCT rate. The encouraging results of this trial, consolidated by a long follow-up, support the reintroduction of GO in clinical practice.

1 | INTRODUCTION

Despite considerable progress in the treatment of many hematological malignancies over the past three decades, in the field of acute myeloid leukemia (AML), the recommended standard approach for induction therapy is still based on the association of cytarabine and an anthracycline ("3 + 7").[1–3] In particular, the role of new emerging compounds specifically targeting defined molecular abnormalities, is still limited to

This study was registered at the Italian Trial Registry (number 07-005248-26) and at http://ClinicalTrials.com as NCT 0090916.

the setting of relapsed and refractory patients, or to elderly populations considered unfit for an aggressive and conventional approach.[1-3]

Nevertheless, over the last few years many attempts have been made to improve the efficacy, in terms of induction remission rates, of the conventional "3 + 7" schedule, which are usually not higher than 60%-70% in young patients.[2,3] These innovative approaches included the addition of multidrug resistance (MDR) modulators, the modification of anthracyclines and cytarabine doses, the addition of non-MDR related drugs such as fludarabine and the use, within clinical trials, of targeted agents, such as Gemtuzumab Ozogamicin (GO).[2,4-7] This drug, an anti-CD33 antibody conjugated with a cytotoxic antitumor antibiotic (calicheamicin), was first evaluated in the setting of relapsed elderly patients, showing an overall response rate (ORR) of 25%-35%.[5-10] Subsequently, this drug was tested in younger populations, in five randomized trials, in addition to induction schedules, showing positive results (in four of five trials) in terms of remission rates and safety profile.[11-15] Unfortunately, the regulatory history of GO has been complicated; in 2010, GO was prematurely withdrawn from the market, but, in 2017, it was resubmitted for review to the FDA and EMA, and, recently, it has been reapproved.[16-19]

We report the final results, with a long follow-up, of a multicenter, prospective, phase II, clinical trial based on the addition of a low-dose of GO (3 mg/sqm) to a FLAI schedule (fludarabine, cytarabine, idarubicin), as induction treatment for young, newly diagnosed and CD33-positive AML patients.

2 | MATERIAL AND METHODS

2.1 | Patient population and study design

One hundred thirty consecutive patients from four institutions were enrolled over a 36-month period between 2007 and 2010. All patients received written information and provided written informed consent for the protocol approved by the Institutional Ethic Committee (Eudract number: 07-005248-26; ClinicalTrials.gov identifier: NCT00909168). All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

The main inclusion criteria were: age range between 18 and 65 years, and previously untreated primary or secondary AML (with bone marrow blasts $\geq$20%). All cases were required to express CD33 on blast cells, with a level of expression higher than 20% by flow cytometry. All patients had an ECOG performance status ≤ 2. Patients with documented central nervous system leukemia or known HIV-positive status were excluded from this study. Additional exclusion criteria included the presence of concurrent active malignancies, active uncontrolled infections, acute promyelocytic leukemia or blast crisis of chronic myeloid leukemia. In this protocol, AML was considered as high risk at diagnosis if there were one or more of the following features: therapy-related or secondary AML, unfavorable karyotype (complex cytogenetic abnormalities or any abnormality involving chromosomes 3, 5, 7, or 11), peripheral blast count $>30 \times 10^9$/L, over expression of P-glycoprotein (PGP; MFI/MRK16 > 6). For the conclusive data analysis, patients were also stratified according to cytogenetic molecular risk at diagnosis (2010 ELNet criteria).[20]

The induction regimen (FLAI-GO) included fludarabine (25 mg/sqm) and cytarabine (2 g/sqm) on days 1-5, idarubicin (10 mg/sqm) on days 1, 3, and 5; and single dose of GO (3 mg/sqm) on day 6. Hematopoietic stem cell transplantation (SCT) was planned for all high-risk AML patients in first complete remission (CR), after consolidation with intermediate doses of cytarabine and idarubicin (AC-IDA) and high doses of cytarabine (HDAC). Gemtuzumab Ozogamicin was administered over 2 hours through an infusion pump using all precautions suggested by the manufacturer. Premedication was administered with 40 mg methyl-prednisolone, paracetamol, and diphenhydramine to prevent or reduce infusion-related reactions. Patients with baseline blast cell counts of 30×10^9/L or more were allowed to initially receive hydroxyurea or a short course of low dose cytarabine to minimize the risk of tumor lysis syndrome. Supporting Figure S1 reports the flow chart of entire therapeutic program.

2.2 | Study end points and assessments

The primary endpoint of this prospective multicenter study was to evaluate feasibility, efficacy (ORR) and toxicity of an induction scheme including low dose of GO (3 mg/sqm) combined with a fludarabine based regimen (FLAI). The secondary endpoints included: (*a*) Evaluation of minimal residual disease (MRD) by WT1 expression after FLAI-GO; (*b*) Feasibility of consolidation with SCT; (*c*) Evaluation of overall survival (OS) and disease-free survival (DFS).

Cytogenetic, multidrug-resistance phenotype, FLT3 and NPM mutation status (only qualitative analysis) were assessed at diagnosis. WT1 quantitative expression analyses were performed in all patients at diagnosis and after induction (day +28-30) to detect MRD after FLAI-GO. The expression of WT1 was measured (in bone marrow samples) using Real Time quantitative PCR with the specific TaqMan probe; the WT1 expression was related to the ABL control gene and the "cut-off" for bone marrow samples was 70 copies WT1/10^4 copies ABL, as previously established.[21,22] The analysis of the FLT3 mutation was performed with PCR, as previously described.[23]

Multidrug resistance-related (MDR) proteins were assessed in all cases at onset, before induction therapy. After red cell lysing by FacsLysing solution, bone marrow or peripheral blood leukemic cells were washed in phosphate-buffered solution and evaluated for P-glycoprotein (PGP), lung related protein (LRP), and multidrug resistance protein 1 (MRP-1) expression by flow cytometry, using the anti-PGP MRK-16, anti-LRP LRP-56, and anti-MRP-1 MRP-m6 monoclonal antibodies (all from Kamiya), as previously described.[24,25] Staining reaction was expressed by the mean fluorescence index (MFI), obtained by the ratio of mean fluorescence intensity of the sample and of its negative control. Based on previous studies, cases over expressing PGP, LRP, and MRP were identified by an MFI higher than 6 for MRK-16, 5 for LRP-56, and 3 for MRP-m6, respectively.

Blood counts and biochemistry, including transaminases and bilirubin levels, were determined three times each week during the follow-

up period. A bone marrow aspiration was performed at days 7 and 15 to assess clearance of blasts. Final determination of remission status was assessed by blood and bone marrow examination as soon as normalization of blood counts was observed and/or at a maximum of 45 days after the induction treatment. Response criteria included those of the National Cancer Institute (NCI) revised by the International Working Group (IWG).[26] Complete Remission (CR) was defined by the absence of any tumor and <5% bone marrow blasts with polymorphonuclear cells (PMN) $>1 \times 10^9$/L, platelets $>100 \times 10^9$/L, and independence of transfusions. Partial remission (PR) was defined as 5%–15% blasts in bone marrow of adequate cellularity with evidence of trilineage regeneration. Patients that did not meet the criteria for CR or PR were categorized as resistant (RES) or non-responders (NR).

Therapy-related toxicity was evaluated according to the World Health Organization (WHO) guidelines. Early death was defined as death occurring during induction of therapy or before hematological recovery.

2.3 | Statistical analysis

Continuous variables were analyzed using descriptive statistical methods (arithmetic mean, standard deviation, median, range, minimum, and maximum). Categorical variables were compared with chi-square test (2-tailed); a value of $P \leq 0.05$ was considered statistically significant. Fisher's exact test and *t*-test were used when appropriate. Survival curves were constructed using the Kaplan-Meier method. OS was measured from the time of diagnosis to all-cause death. For patients achieving CR after induction, DFS was calculated from the date of complete remission until disease relapse or death. Patients that did not relapse were censored at death date or last follow-up date, as appropriate. The log-rank test and Cox regression analysis were applied to analyze the differences between groups with respect to OS and DFS in univariate and in multivariate analysis. The following variables have been included into the model: age (< or > 55 years), PGP overexpression, cytogenetic-molecular risk at diagnosis, cytologic CR after FLAI-GO, molecular CR (WT1 < 70 copies) after FLAI-GO, allo SCT. The follow-up is updated to June 30, 2016. Data were analyzed by MedCalc software, version 12.5.0.0 (MedCalc Software bvba, Belgium).

3 | RESULTS

3.1 | Patient characteristics

One hundred thirty consecutive and untreated AML patients (66M/64F) were included, with a median age of 52 years (range 18–65). CD33 expression exceeded 20% in all cases, 24% of patients (29/123 evaluable cases) had an adverse karyotype, 26% (34/130) were secondary AML (secondary to Myelodysplastic Syndrome or Chronic Ph neg Myeloproliferative Disease), and 23% (27/118 evaluable cases) had an MDR phenotype with a PGP overexpression on blast cells. Thirty-three (28%) overexpressed MRP-1 and 61/118 (52%) LRP. A blast cell count $>30 \times 10^9$/L was documented in 32% of cases (41/130). Detailed demographics and baseline characteristics are shown in Table 1A.

3.2 | Treatment and response

After induction with FLAI-GO, CR rate was 82%; four patients (3%) achieved a PR and 16/130 patients (12%) were primary RES, with an ORR of 85%. There were only four cases (3%) of deaths during induction (DDI) (Table 1B). The achievement of CR after FLAI-GO was significantly influenced by cytogenetic molecular risk (Int2-high CR 73% vs low-Int1 CR 90%, $P = 0.02$, Fisher's exact test) and diagnosis of secondary AML (secondary leukemia-CR 67% vs de novo AML-CR 90%, $P = 0.003$). The response to induction was not affected by MDR-related protein (PGP, MRP-1, LRP) overexpression at diagnosis. Similarly, cell blast count $>30 \times 10^9$/L at onset and age >55 years did not have a significant impact on CR.

After induction, 120 patients received consolidation therapy with cytarabine (2 g/sqm, days 1–5) and idarubicin (10 mg/sqm, days 1–3) followed, after hematological recovery, by a high dose of cytarabine (6 g/sqm, days 1–4) and allogeneic SCT when indicated (high risk AML according to protocol criteria and donor availability). Four patients died, while in CR, after the second and third course of chemotherapy (2 septic shock, 1 H1N1 virus pneumonia, and 1 cerebral hemorrhage) with an overall chemotherapy related mortality (induction plus consolidation) of 6% (8/130).

In this study, 83 patients (64%) underwent SCT. Median time between FLAI-GO and SCT was 6.2 months (range, 3–18). Twenty-three patients (18%) received autologous SCT (14/23 while in first CR), whereas 60/130 (46%) patients received allogeneic SCT (46/60 in first CR), 31 from sibling donors and 29 from unrelated donors. None of these patients developed veno occlusive disease (VOD) before or after SCT. Relapse Rate after SCT was 31% (26/83). Supporting Figure S1 reports a flow diagram summarizing the entire therapeutic program and post SCT outcome.

3.3 | WT1-minimal residual disease after FLAI-GO

Overexpression of WT1 at diagnosis was observed in 94% evaluable cases (115/122), with a median value of 5848 copies WT1/10^4 copies ABL (range 235-81111). Bone marrow samples from patients in CR after induction showed significantly lower WT1 expression levels (mean 182 ± 436) compared to WT1 expression levels at diagnosis (mean 8337 ± 9936) ($P = 0.0001$, *t*-test) (Supporting Figure S2). Of note, 51% (54/106) of patients who obtained a cytological CR after FLAI-GO, reached complete molecular remission with a number of WT1 copies in bone marrow samples lower than 70/10^4 ABL. Moreover, there was a complete concordance between normalization of karyotype and clearance of WT1, confirming both the value of WT1 as an MRD marker and the optimal tumor debulking induced by the FLAI-GO regimen.

3.4 | Hematological and extra-hematological toxicity

Hematological and extra-hematological toxicity after FLAI-GO are listed in Table 2.

TABLE 1 (A) Patient's characteristics. (B) Response to FLAI-GO and outcome

[A]	
Total number (sex)	130 (66M/64F)
Median age (range), years	52 (18–65)
Mean age (DS), years	49 ± 14.4
FAB subtype	
• M0–M1	36/130 (28%)
• M2	21/130 (16%)
• M4–M5	55/130 (42%)
• Sec	18/130 (14%)
Cytogenetic-Molecular Risk at diagnosis (evaluable 118/130)	
• Favorable	23/118 (19%)
• Intermediate 1	51/118 (43%)
• Intermediate 2	15/118 (13%)
• Adverse	29/118 (25%)
Hyperleukocytosis[b] (evaluable 130/130)	41/130 (32%)
Secondary AML[a] (evaluable 130/130)	34/130 (26%)
Unfavorable karyotype (evaluable 123/130)	29/123 (24%)
FLT3 (evaluable 121/130)	
• ITD +	24/121 (20%)
• D835 +	14/121 (12%)
MDR-overexpression (evaluable 118/130)	
• PGP	27/118 (23%)
• MRP-1	33/118 (28%)
• LRP	61/118 (52%)
WT1 positivity at onset (evaluable 122/130)	115/122 (94%)
[B]	
RESPONSE to FLAI-GO	
• COMPLETE REMISSION	106/130 (82%)
• PARTIAL RESPONSE	4/130 (3%)
• REFRACTORY	16/130 (12%)
• Death During Induction	4/130 (3%)
• OS 2 and 5 years	63%, 52%
• DFS 2 and 5 years	58%, 52%
STEM CELL TRANSPLANT (SCT)	83/130 (64%)
• Allo-SCT[c]	60/130 (46%)
• Auto-SCT	23/130 (18%)
• TIME Diagnosis-BMT Median-months (range)	6.2 (3–18)
• 1° CR at SCT	60/83 (72%)
Outcome after SCT	
• Alive	55/83 (66%)
• Death	28/83 (34%)

[a]Secondary to Myelodysplastic Syndrome or Chronic Myeloproliferative Disease.
[b]Blast Cells more than 30×10^9/L. MDR = Multidrug Resistance.
[c]HLA Identical Family Donor 31; Matched Unrelated Donor 29.

As expected, all patients experienced grade IV hematological toxicity: median time to neutrophil ($>1 \times 10^9$/L) and platelet ($>50 \times 10^9$/L) recovery was 24 (range 19–40) and 25 days (range 18–44), respectively. Supportive treatment consisted of a median of 11 packed red cell units (range 5–27) and 7 platelet units (range 3–16). G-CSF was used in 63/130 (48%) patients because of prolonged myelosuppression and/or infection for a median of 9 days (range 1–18).

Documented infections occurred in 56/130 (43%) patients, including 34 episodes of bacteremia (15, gram positive bacteria; 14, gram negative bacteria; and 5, polymicrobial) and 22 cases of pneumonia (5, mycotic pneumonia). Infectious death after FLAI-GO occurred in two patients who developed septic shock and multiorgan failure due to bacteremia (1, *Enterococcus* and *Staphylococcus* sp.; 1, *Pseudomonas aeruginosa*). Fever of unknown origin (FUO) was reported in 68/130 (52%) cases. Oral mucositis grade II–III (WHO) was reported in 22/130 (17%) patients, and oral or labial herpes simplex virus reactivation was documented in 24/130 (18%) cases. Gastrointestinal toxicity, as reported in Table 2, was not relevant. Common non-hematological adverse events included GO infusion-related reactions (58/130, 45%), mainly transient fever and chills. No cases of VOD occurred during chemotherapy or after allogeneic SCT. No patient experienced grade IV hepatic toxicity, but 10 (8%) had a transient elevation in liver function tests, specifically bilirubin and/or transaminases. No treatment-related cardio toxicity was observed. After FLAI-GO treatment, median time to hospital discharge was 30 days (range 22–59).

3.5 | Outcome

After a median follow-up of 54 months (range 1–120 months), 67/130 (52%) patients are alive. OS and DFS Kaplan-Meier curves for all patients are shown in Figure 1. The median OS and DFS were 63 and 61 months, respectively (Figure 1A,B). The probability of 1-, 2-, and 5-year OS was 80%, 63%, and 52%, respectively. The probability of 1, 2, and 5-year DFS was 77%, 58%, and 52%, respectively. As reported in Figure 1C,D, after a long-term follow-up, we did not find significant differences in OS and DFS between patients with favorable cytogenetic molecular risk at diagnosis and other cytogenetic molecular risk groups (OS, Log-rank 0.22; DFS, Log-rank 0.16).

In a Cox univariate analysis, there are the following favorable prognostic factors for OS: achievement of cytological CR after FLAI-GO, age <55 years, molecular remission (MRD-WT1 <70 copies) after FLAI-GO and consolidation with an allogeneic SCT. In a Cox multivariate analysis as reported in Table 3A, age, molecular remission after FLAI-GO, and allogeneic SCT retained statistical significance. The same variables were significantly favorable prognostic factors for DFS as reported in Table 3B. It should be highlighted that PGP overexpression of blast cells and cytogenetic molecular risk at diagnosis did not influence the OS and DFS in this clinical trial.

4 | DISCUSSION

In the last two decades, few new compounds have been approved for the treatment of AML (Gemtuzumab-Ozogamicin, decitabine, azacitidine, midostaurin), whose therapeutic approach still relies on the administration of conventional chemotherapy ("3 + 7" regimens as induction, followed by cytarabine-based consolidation courses)

TABLE 2 Hematologic and extra-hematologic toxicity

PMN > 0.5 × 10^9/L Mean ± SD, days Median (range), days	23 ± 3.3 23 (19–36)	PRC, No. Mean ± SD, Median (range)	11 ± 4.5 11 (5–27)	FUO	68/130 (52%)	HSV infectious	24/130 (18%)
PMN > 1 × 10^9/L *Mean ± SD, days* *Median (range), days*	26 ± 5 24 (19–40)	*PU, No.* *Mean ± SD* *Median (range)*	7 ± 4,2 7 (3–16)	*BACTEREMIA*	34/130 (26%)	*PNEUMONIA*	22/130 (17%) (5 mycotic pneumonia)
PLT > 20 × 10^9/L *Mean ± SD, days* *Median (range), days*	24 ± 4 24 (18–38)	*G-CSF vials* *Mean ± SD* *Median (range)*	8 ± 6 9 (1–18)	*MUCOSITIS* *Grade II WHO* *Grade III WHO* *Grade IV WHO*	22/130 (17%) 20/130 (15,5%) 2/130 (2,5%) 0/30	*ENTERITIS* *Grade II WHO* *Grade III WHO* *Grade IV WHO*	16/130 (12%) 13/130 (10%) 3/130 (2%) 0/130
PLT > 50 × 10^9/L *Mean ± SD, days* *Median (range), days*	26 ± 5 25 (18–44)	*Hospitalization* *Mean ± SD, days* *Median (range), days*	30 ± 8 30 (22–59)	*LIVER toxicity* *Grade II WHO* *Grade III WHO* *Grade IV WHO*	10/130 (8%) 9/130 (7%) 1/130 (1%) 0/130	*Fever during GO infusion*	58/130 (45%)
				VOD	0/130	*Other (Encephalitis)*	1/130

PRC= Packed red cells; PU= Platelets units.

followed or not by allogeneic SCT based on the cytogenetic and molecular risk stratification.[1–3] In the scenario of innovative compounds, GO was the first antibody targeted therapy to be developed and approved, showing promising results both in the setting of elderly relapsed or refractory AML patients, and in the context of induction schedule for the treatment of the young AML population, in association with standard chemotherapy regimens.[4–7,10,13–15,27–36]

Specifically, four European prospective studies, in which 2744 patients were randomized (MRC/NCRI AML15 and AML16 trials, GOELAMS AML2006 IR trial, ALFA-0701 trial), showed that the addition of GO to induction chemotherapy improved DFS and OS particularly in a subset of young patients with newly diagnosed AML.[10,12–15] Overall, in most studies that addressed the combination of a low dose of GO (3–6 mg/sqm) with intensive induction chemotherapy, both in pediatric and young AML patients, a survival benefit was observed mainly in the subgroups of patients with favorable and intermediate cytogenetic-molecular risk.[4,5,10,12–15,34,37] Nevertheless, GO was withdrawn from the U.S. and European markets due to

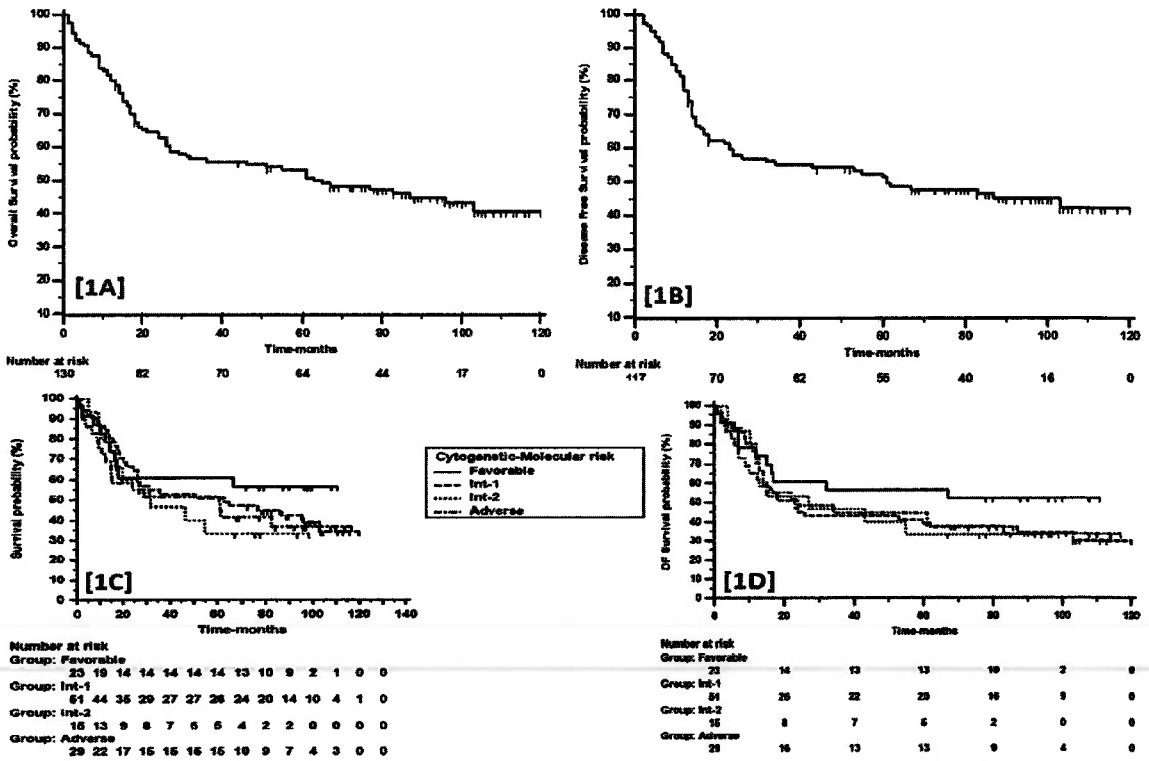

FIGURE 1 (A) Overal survival (OS). Median OS = 63 months. Probability of OS at 12, 24, and 60 months was 80%, 63%, and 52%, respectively. (B) disease free survival (DFS). Median DFS = 61 months. Probability of DFS at 12, 24, and 60 months was 77%, 58%, and 52%, respectively. (C) OS and (D) DFS according to cytogenetic molecular risk at diagnosis

TABLE 3 Cox regression analysis of variables affecting OS [3A] and DFS [3B]

[3A] VARIABLES	OS-UNIVARIATE ANALYSIS			OS-MULTIVARIATE ANALYSIS		
	HR	95% CI	P	HR	95% CI	P
Cytologic CR	2,23	1,29-3,84	0,007	0,96	0,41-2,23	0,94
Age (≤ 55 vs > 55)	2,45	1,54-3,93	0,0002	2,11	1,22-3,64	0,007
Cytogenetic-Molecular Risk (low/Int-1 vs Int-2/high)	1,14	0,89-1,45	0,27	1,02	0,76-1,39	0,85
Molecular Remission after FAI-GO (WT1 ≤ 70)	2,73	1,60-4,64	0,0005	2,39	1,10-5,10	0,027
P-Glycoprotein (PGP) overexpression	1	0,57-1,79	0,97	0,87	0,46-1,64	0,68
Allo-SCT	0,5	0,32-0,83	0,007	0,58	0,32-1,03	0,05
[3B] VARIABLES	DFS-UNIVARIATE ANALYSIS			DFS-MULTIVARIATE ANALYSIS		
	HR	95% CI	P	HR	95% CI	P
Cytologic CR	2,3	1,34-3,98	0,002	1,43	0,62-3,31	0,39
Age (≤ 55 vs > 55)	1,98	1,26-3,1	0,003	1,72	0,99-2,99	0,05
Cytogenetic-Molecular Risk (low/Int-1 vs Int-2/high)	1,07	0,85-1,35	0,53	0,97	0,71-1,33	0,88
Molecular Remission after FAI-GO (WT1 ≤ 70)	2,68	1,58-4,54	0,0003	2,21	1,05-4,64	0,03
P-Glycoprotein (PGP) overexpression	1,03	0,58-1,84	0,89	1,05	0,56-1,96	0,86
Allo-SCT	0,49	0,31-0,78	0,003	0,53	0,30-0,95	0,03

PRC= Packed red cells; PU= Platelets unit.

the negative results, in terms of toxicity and safety profile, coming from the Southwest Oncology Group (SWOG) study-S0106. This study was designated and performed to expand the GO indications after the first FDA approval.[11,38] However, several reasons may justify the unfavorable outcome of this trial. Firstly, the dose of daunorubicin in the GO arm was lower compared to the control arm (45 mg/sqm vs 60 mg/sqm), and this may negatively impact the response rate. Secondly, in the control arm of this trial, an unusually low mortality rate was observed (1%) compared with a mortality rate of 6% in the GO arm, which corresponded with other standard induction regimens. Thirdly, in the four favorable studies mentioned above, GO was administered at fractionated and/or lower doses, resulting in a better toxicity profile.[11]

The aims of our phase II clinical trial that began in 2007, before the availability of the previously discussed results, were to explore the feasibility, response rate (CR and ORR) and toxicity of a FLAI plus GO schedule as induction therapy in young and CD33 positive AML patients. DFS and OS were also assessed as secondary endpoints.

This clinical trial, even if not randomized, has specific features compared to other published studies; it combines, as a first-line AML therapy, a low dose of GO with a fludarabine based regimen (fludarabine, cytarabine, idarubicin) placing GO after chemotherapy (day 6). Furthermore, it includes only CD33-positive AML cases (CD33 >20% classified as positive) and evaluates the expression of Multidrug resistance (MDR) phenotype and response to FLAI-GO according to MDR (P-glycoprotein-PGP) status (negative versus positive cases). In addition, this study also aimed at evaluating, as secondary endpoints, the depth of response to FLAI-GO induction regimen, in terms of MRD, by WT1 panleukemic marker expression.[1,21,22]

The rationale for fludarabine and cytarabine combinations is that fludarabine, a fluorinated purine analogue, enhances cytarabine cytotoxicity by increasing cellular concentration of Ara-C 5-triphosphate, thus inhibiting DNA repair. Moreover, fludarabine is toxic against MDR overexpressing blast cells, particularly against PGP-positive leukemic cells both in cell lines and in leukemic blasts.[24,25,39] Unfortunately, the MDR overexpression also affects GO activity[40–43] Recently, Walter et al. documented that the blast cells of patients responding to GO had significantly lower PGP activity and higher CD33 expression than non-responsive cases.[42,44] However, in the majority of published trials, patients were not selected according to CD33 expression status and CD33 expression did not appear to have a predictive value for survival.[5,45] Despite this, van der Velden et al. found that high CD33-antigen loads in peripheral blood limit the efficacy of GO in bone marrow blasts.[46,47] In clinical daily practice, this suggests that GO might have a higher efficacy in bone marrow blasts if administered after the reduction of CD33 positive blast cells in peripheral blood by standard chemotherapy.[5] For all these reasons, in order to increase the efficacy of a first-line therapy, our FLAI-GO scheme combined both MDR related (anthracyclines) and MDR unrelated drugs (fludarabine), and we adminstered GO on day 6 after the AML debulking with standard chemotherapy.

Interestingly, in our study, 22% of patients had an MDR phenotype with a PGP overexpression on blast cells. However, we found that the MDR PGP overexpression at diagnosis did not have a significant impact

on the response to induction therapy, thus supporting the role of FLAI-GO in overcoming this mechanism historically related to chemoresistance.

In terms of efficacy, our data confirmed, in line with MRC AML15 trial, a high ORR rate after induction with FLAI-GO (85%), with a CR of 82%. Notably, 51% (54/106) of patients that obtained a cytological CR after induction, reached complete molecular remission, as assessed by WT1 expression. These findings highlight the ability of the FLAI-GO regimen to induce a good debulking effect and a deep response, supporting the value of WT1 as a marker of MRD, although WT1 is not still worldwide considered a standard tool for MRD assessment.[48,49] Furthermore, in the present study both univariate and multivariate analysis showed that the achievement of a molecular response after FLAI-GO (WT1 less than 70 copies) significantly improved OS and DFS (Table 3).

Additionally, in our experience, the cytogenetic molecular risk affects the achievement of CR after FLAI-GO. In particular, Int2-high patients obtained a CR rate of 73% vs low-Int1 patients, in which a CR was reached in 90% of the cases ($P = 0.02$, Fisher's exact test). Moreover, as expected, a diagnosis of secondary AML was related to a lower CR rate, when compared to de novo AML (67% vs 90%, $P = 0.003$).

In terms of toxicity, after FLAI-GO all patients experienced a grade IV hematological toxicity that required supportive treatment with a median of 11 packed red cell units (range 5–27) and 7 platelet units (range 3–16). Furthermore, documented infections occurred in 38% of the patients, leading to death in only two cases. Hepatic toxicity was extremely low and manageable as reported in Table 2, confirming a good toxicity profile of GO when it is given at lower doses (3 mg/sqm) as reported in a recent meta-analysis.[10] To confirm the safety profile of FLAI-GO, the DDI rate was very low, accounting for only 3%.

The tolerability coupled with efficacy of FLAI-GO induction approach allowed a high proportion of patients (64%) to proceed to a consolidation and, according to risk stratification, to a SCT procedure (60 patients received an allogeneic SCT, and 23 patients an autologous SCT), with an OS and DFS at 2 and 5 years of 63% and 52%, and of 58% and 52%, respectively. Of note, none of the transplanted patients developed VOD before or after SCT.[50] The high transplantation rate of high and intermediate risk patients of this trial could explain why in this experience the cytogenetic and molecular risk at diagnosis did not significantly impact DFS and OS (Figure 1C,D, Table 3).

In summary, GO was withdrawn from the U.S. and European markets in 2010 due to post marketing concerns about drug safety and lack of efficacy (according to the SWOG-S0106 trial). However, after withdrawal, several large and randomized clinical trials, combining lower and fractionated doses of GO to standard first-line chemotherapy, have been completed and showed better tolerability and clear efficacy with significant improvement of DFS and OS, particularly in AML with favorable and intermediate-risk cytogenetics, leading to reintroduction of this drug into clinical practice.

Our study confirms these positive results and provides some additional information to better select AML patients, who would most likely benefit from the combination of GO and induction chemotherapy. In our opinion, taking into account all the available data, the best AML candidate to receive GO plus induction chemotherapy should have the following characteristics: age less than 60 years, no hepatic diseases, de novo AML, first induction phase, favorable or intermediate cytogenetic risk, no PGP overexpression (no MDR phenotype), and expression of CD33 on blast cells over 20%. The preferred schedule of GO, in addition to induction chemotherapy, should include lower doses (eg, 3 mg/sqm) and repeated administrations (2–3 doses) avoiding toxicity without affecting efficacy as proposed by the ALFA group.[12,18,32,51,52] Chemotherapy in association with GO should include cytarabine plus daunorubicin based regimens (eg, DA, DAE) or FLAI scheme (our preference) that is well tolerated and includes MDR reversing drugs, such as fludarabine and a more potent and less PGP-sensitive anthracycline (idarubicin).

The data of this study were presented in part at the EHA 2014 (oral presentation) and published in abstract form (Haematologica 2014).

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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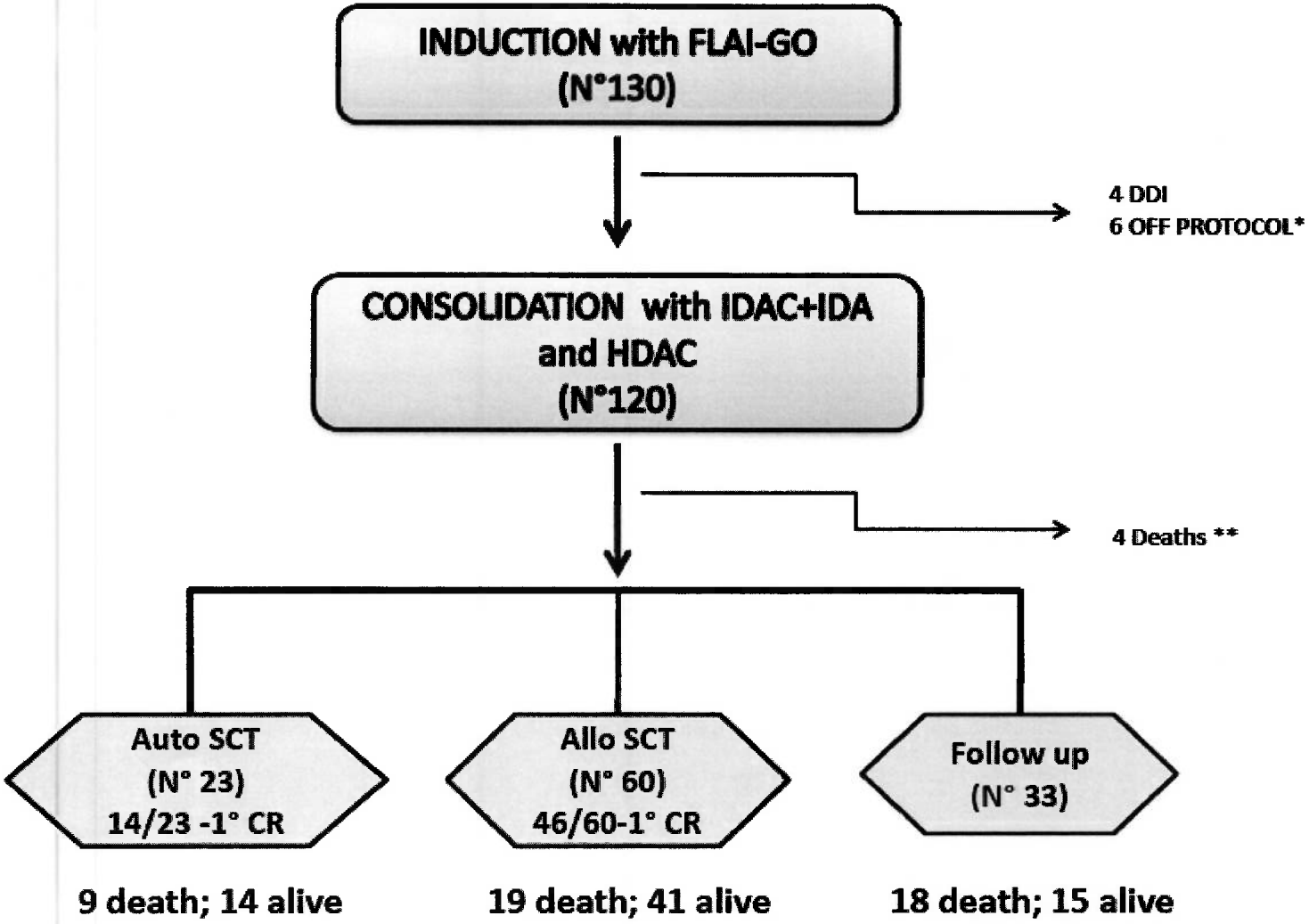
Supplementary FIGURE S1. Flow diagram showing therapeutic program and patients allocation.



Supplementary FIGURE S2. Amount of Leukemia debulking according to decrease of MRD panleukemic marker WT1. Comparison between mean values (±SD) of WT1 before and after induction (FLAI-GO) of patients who were WT1-positive at diagnosis (WT1 >70 copies/10^4ABL) and who achieved cytologic complete remission (cCR). WT1 at diagnosis: mean 8337±9936, median 5876 (81111-255); WT1-post FLAI-GO: mean 182 ±436, median 57(3.5-3318). T-Test: P< 0.0001.