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Day 15 bone marrow driven double induction in young adult patients with acute myeloid leukemia: feasibility, toxicity and therapeutic results.

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Day 15 bone marrow driven double induction in young adult patients with acute myeloid leukemia: feasibility, toxicity and therapeutic results.

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Table 2 – Therapeutic results and non hematologic toxicity

Double induction feasible	30/33 (91%)
CR	20 (67%)
Refractory	8 (27%%)
Toxic death	2 (6%)
Neut > 0.5x10E9/L, days* (range)	12 (9-20)
Plat > 20x10E9/L, days* (range)	17 (9-46)
FUO	22 (73%)
Documented infections	8 (27%%)
Bacterial	5
Fungal	3
Allo-SCT	11 (55%)
High dose Ara-C	9 (45%)
Alive/Death	11/9 (55%/45%)
Alive in CR	10 (33%)
Median OS	15 months
Median DFS from ASCT	12 months

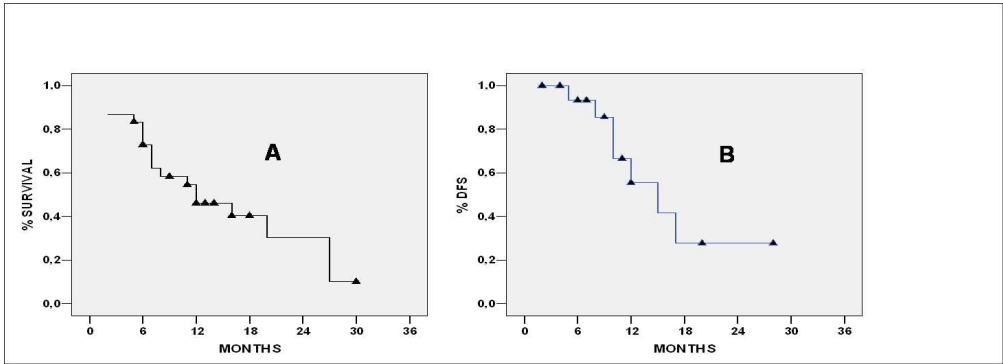
* From the start of double induction

Table 3 – Comparison of hematopoietic recovery and non hematologic toxicity between patients managed with double induction and a control groups of 30 patients, matched for age, achieving CR after one cycle of induction.

	Double induction	Controls	P value
Toxic death	2 (6%)	2 (6%)	1
Neut > 0.5x10E9/L, days* (range)	38(30-49)	21 (17-25)	0.001
Plat > 20x10E9/L, days* (range)	43 (33-48)	23 (16-29)	0.003
FUO	22 (73%)	20 (67%)	0.58
Documented infections	8 (27%)	6 (20%)	0.45
Bacterial	5	4	0.67
Fungal	3	2	
Grade 3 or 4 non hematological toxicity			
Liver	4 (13%)	2 (7%)	0.67
Stomatitis	4 (13%)	3 (10%)	0.99
Diarrhea	3 (10%)	3 (10%)	1
RBC units	15 (7-21)	9 (5-14)	0.32
Platelet Units	16 (7-26)	8 (5-13)	0.39
Days of hospitalization	44 (39-65)	30 (22-44)	0.41

* From the start of induction chemotherapy

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Peer Review

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3 **Day 15 bone marrow driven double induction in young adult patients with acute myeloid**
4 **leukemia: feasibility, toxicity and therapeutic results.**
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7 Felicetto Ferrara, Tiziana Izzo, Clelia Criscuolo, Cira Riccardi, Maria Celentano and Giuseppina Mele
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28 Short running title: Day 15 driven double induction in AML
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60**Abstract**

The strategy named double induction (DI) in acute myeloid leukemia (AML) consists of two courses of chemotherapy irrespective of the degree of cytoreduction in the bone marrow after the first course, unless severe complications prohibit its application. We describe treatment results from a series of 33 patients in whom DI was adopted only after demonstration of persistence of more than 10% blast cells at day 15 (D15) examination of bone marrow. All patients received as induction idarubicin, cytarabine and etoposide. As second induction, we administered the combination of fludarabine, intermediate dose cytarabine and G-CSF. The median blast count at D15 was 30 (15-90). Overall, 30 out of 33 patients were judged as eligible to receive DI, reasons for exclusion being in all cases active infection in the context of severe pancytopenia. Nineteen patients (63%) had unfavorable karyotype and 11 (37%) normal karyotype; 7 of these had FLT3/ITD mutation. Overall, CR was achieved in 20/30 patients (67%), while 8 patients (27%) were refractory and 2 died of infectious complications. All refractory patients had unfavorable cytogenetics. All patients achieving CR were programmed to receive allogeneic stem cell transplantation (allo-SCT), which was actually performed in 11 patients. Our study suggest that D15 driven DI represents a feasible and effective therapeutic strategy in young adult AML patients, improving therapeutic results and not compromising feasibility of allo-SCT. As compared to conventional DI, it offers the potential to avoid unnecessary toxicity in a consistent proportion of patients.

Key words: Acute myeloid leukemia, early blast clearance, double induction

Introduction

For longer than 20 years, the combination of daunorubicin, given for 3 days, with continuous infusion of cytarabine for 7 days (3+7) has been the standard of induction therapy for acute myeloid leukemia (AML). Use of this regimen results in a complete remission (CR) rate of 60–70% in patients up to the age of 60 years (1-3). Because achievement of a complete response is an absolute prerequisite for long-term survival and cure, several attempts have been made to improve the effectiveness of this regimen in terms of both the rate and quality of remission. These investigations have included use of higher doses of daunorubicin or alternative anthracyclines, high-dose cytarabine, regimens that contain fludarabine, a third drug alongside daunorubicin and cytarabine (etoposide or tioguanine), haemopoietic growth factors, and, more recently, addition of monoclonal antibodies or other new agents (4-9). Following conventional induction therapy with 3 days of an anthracycline and 7 days of cytarabine (“3 + 7”) or therapies of comparable intensity, response assessment is commonly performed between day 21 and 28 after start of therapy (10). However, the exact timing may vary among protocols and should meet the specific objectives of the study. In a further attempt to improve the long-term prognosis of patients with AML, in the nineties the German AML Cooperative Group (AMLCG) introduced the concept of “double induction” (DI). This strategy is focused primarily on patients younger than 60 years and consists of 2 courses of chemotherapy irrespective of the degree of cytoreduction in the bone marrow after the first course, with the second course starting on day 21 unless severe complications prohibit its application (11). This approach resulted in a significantly longer remission duration and overall survival compared with standard induction in patients with unfavorable features at presentation (12).

An important factor of potential utility into clinical practice is the early assessment of marrow blasts after administration of induction chemotherapy (13-15). A threshold value of 10% at day 14-15 from the beginning of chemotherapy is highly prognostically relevant, in that the degree to which the blasts are cleared from the marrow in response to the first course of chemotherapy represents a surrogate for chemoresistance (16,17). In order to achieve better therapeutic results, avoiding unnecessary toxicity, from 2007 we decided to administer DI only to patients with persistent leukemia, defined as over 10 % of blasts in the bone marrow at day 15 morphological and/or immunophenotypic examination. Here we report therapeutic results from a series of 33

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3 consecutive patients found with more than 10 % blast cells in BM after conventional induction
4 chemotherapy and programmed to receive DI with alternative chemotherapy regimen.
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8 9 **Patients and Methods**

10 From January 2007 to December 2009, 33 patients with AML were found with more than 10 %
11 blasts at morphological and immunophenotypic BM examination at day 15 after beginning
12 induction chemotherapy and were programmed to receive DI. The median age was 50 years
13 (range 16-60), 19 patients were males and 14 females. Cytogenetic RHG-banding analysis was
14 performed with a standard method and the definition of a cytogenetic clone and descriptions of
15 karyotypes followed the International System for Human Cytogenetic Nomenclature (18). A
16 minimum of 20 bone marrow (BM) metaphases/case were required to be examined and
17 definitively classified . Prognostic evaluation of cytogenetics was made according to UK Medical
18 Research Council criteria (19). Favorable karyotype included t(8;21) and inv(16) irrespective of other
19 abnormalities, while complex abnormalities, monosomies of chromosomes 5 and 7, deletions of
20 long arm of chromosome 5, or 3q aberrations were classified as unfavorable. The remaining
21 patterns including normal karyotype or structural or numerical changes not encompassed by
22 favorable or adverse risk group were considered as intermediate. Diagnosis was made according
23 to WHO classification, by using > 20 % bone marrow blasts as threshold for the diagnosis of AML
24 (20). In all cases, diagnosis was confirmed by immunophenotypic study, as previously described
25 (21). The analysis of FLT3 mutation was performed as previously described (22). Analysis of NPM1
26 mutations in AML cells was performed by fragment analysis according to molecular assay as
27 previously published (23). Patients aged up to 60 years with de novo AML were given induction
28 treatment consisting of ICE [idarubicin 10 mg/ sqm on days 1, 3 and 5; cytarabine (ARA-C) 100
29 mg/sqm as continuous infusion (c.i.) on days 1–7; etoposide 100 mg/sqm on days 1–4]. As second
30 induction, we administered the combination of fludarabine (F), intermediate dose cytarabine
31 (ARA-C) and G-CSF (FLAG). More in detail, F was given at 30mg/ m²/day as 30min intravenous
32 perfusion and ARA-C 2g/m²/day 3 hour and half after the end of F from day 1 to 5. G-CSF at 300
33 µg was concomitantly given from day 1 up to CR achievement. **Patients were judged as eligible to
34 receive DI, provided that neither active infections, nor non hematologic toxicities > 2 according to
35 WHO criteria were registered at day 15 from the start of induction.** Criteria for CR included
36 normal peripheral blood count in the absence of blast cells, bone marrow blast percentage less
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3 than 5 % and absence of extramedullary leukemia. Remission failures were classified as either due
4 to induction death (ID), that is death related to treatment and/or hypoplasia, or as resistant
5 disease (RD), i.e. related to the failure of therapy to eliminate the disease (including partial
6 remission with 5 to 15 % bone marrow blasts after FLAG) (24). A population of cells with
7 leukaemia-associated aberrant immunophenotype (LAIP) was identified in each patient from the
8 initial BM aspirate. At day 14, FC analysis was performed on BM in order to identify LAIP-positive
9 residual blasts. The degree of BM clearance was expressed as the ratio, converted to logarithmic
10 scale, between the percentage of LAIP positive blasts determined at diagnosis and day 15 (17).
11 Disease-free survival (DFS) was defined as the time from CR achievement until the month of
12 relapse, death from any cause or the date of last follow-up. Overall survival (OS) was defined as
13 the time from diagnosis to death or last follow-up. Either DFS or OS were calculated by Kaplan–
14 Meier method (25). All statistical comparisons used two-tailed p-value. The p-value used to define
15 statistical significance was 0.05.
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30 Results

31 Among 33 consecutive patients found with more than 10 % blast cells at morphological and or
32 immunophenotypic evaluation, 30 (91%) were judged as clinically eligible to receive further
33 chemotherapy with FLAG. Reasons for exclusion in three patients included in all cases active
34 pulmonary infection (bacterial in two and fungal in one patient) in the context of severe
35 pancytopenia. Notwithstanding, as summarized in table 1, all patients were severely neutropenic
36 at day 15 (median WBC count: $0.4 \times 10^9/L$, range 0.1-1.0; median neutrophil count $0.3 \times 10^9/L$,
37 range 0-0.5). The median percentage of day 15 bone marrow blasts, as evaluated at morphological
38 examination, was 35 % (15-90). Of note, there was no statistically significant difference between
39 morphological and immunophenotypic evaluation of day 15 blast cells (30 % by morphology, 28 %
40 by immunophenotype, $p:0.86$). According to cytogenetic examination, 11 patients had normal
41 karyotype (median blast count: 18%), while 19 patients were found with unfavorable patterns,
42 including complex karyotype in 14 patients and chromosome 5 or 7 abnormalities in 5 patients). In
43 this subgroup, the median day 15 blast count was 58 % and was significantly higher than the group
44 with normal karyotype ($p:0.004$). Among patients with diploid karyotype, 6 patients had FLT3/ITD
45 mutation, which was found as concomitant with NPM1 mutation only in 1 patient; 4 patients had
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3 neither FLT3 nor NPM1 mutations. Of note, in this series no patient had favorable cytogenetics,
4 i.e. either t(8;21) or inv(16) or NPM1 isolated mutation.
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7 Therapeutic results and non hematologic toxicity are summarized in table 2. Overall, CR was
8 achieved in 20 patients (67%), while 8 patients (27%) were classified as refractory. Finally, two
9 patients (6 %) died from severe infectious complications while severely cytopenic. Of interest, all
10 refractory patients had unfavorable cytogenetics. All patients experience fever; more in detail,
11 there were 22 episodes of FUO (fever of unknown origin) and 8 documented infections (5 bacterial
12 and 2 fungal, both lung aspergillosis). The median number of days for recovery to neutrophils > 0.5
13 $\times 10^9/l$ and platelets $> 20 \times 10^9/l$ from the start of DI was 20 and 22, respectively, while from the
14 start of the first induction 38 (30-49) and 43 days (33-48) were needed, respectively. Patients
15 received a median of 15 packed red cells (range: 7-21) and 16 platelet units (range: 7-26). Data
16 concerning hematopoietic recovery and supportive therapy are summarized in table 3, in which a
17 comparison between DI patients with a series of 30 patients matched for age, in whom CR was
18 achieved after one course of chemotherapy, is also showed. **The control group, in which CR was**
19 **achieved in 25 out of 30 patients (83%), was selected within the same sequential cohort as the**
20 **patients with persistent blasts. Of note, in this group more patients presented with favorable and**
21 **intermediate cytogenetics as well as with favorable molecular findings (p:0.001 and 0.02,**
22 **respectively) as indicated in table 1.**
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37 Either WBC or platelet recovery from the start of induction chemotherapy was significantly longer
38 for the group managed with double induction than controls (38 days vs. 21 days for neutrophils
39 and 43 days vs. 23 for platelets, p=.001 and 0.003, respectively). In addition, the need for RBC and
40 platelet support was significantly higher for DI patients as opposed to controls (median RBC Units:
41 15 vs 9, p 0.01; median platelet units: 16 vs. 8, p:0.006, respectively); finally, the duration of
42 hospitalization was significantly longer for DI patients as opposed to those in the control group 44
43 days (39-65) vs. 30 days (22-44), p: 0.005. Notwithstanding, there was neither difference in
44 induction death rate, nor in the overall incidence of FUO and documented infections (see table 3).
45 Finally, the incidence of grade 3 or 4 non hematologic toxicity, consisting of diarrhea, liver enzyme
46 elevation and stomatitis was comparable. All remitters were consolidated with an additional
47 course of FLAG and were programmed to receive allogeneic stem cell transplantation, given that
48 all of them were considered at high risk because of persistent leukemia at day 15 BM examination
49 (26). Overall, 11 out of 20 patients (55%) received allogeneic BMT; of these, 7 are alive in
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3 continuous CR, 3 relapsed and died; finally, 1 patient died for severe graft versus host disease after
4 allo-SCT. Nine patients received consolidation with FLAG, followed by two courses of high dose
5 ARA-C. Among these, three are in continuous CR, 6 relapsed while waiting for allo-SCT from
6 unrelated donor. One patient achieved second CR and is in a waiting list for allo-SCT. Eight
7 relapsed patients died in the setting of refractory disease. OS and DFS for the whole patient
8 population are 12 and 15 months, respectively, as shown in fig 1.
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16 Discussion

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18 Double induction treatment is based on early administration of a second course of induction,
19 which is usually given independently of bone marrow status (10). In a randomized study, Buchner
20 and coworkers administered either conventional or more aggressive therapy based on high-dose
21 cytarabine as second induction on day 21 after therapy was started (11). Toxic effects were
22 substantial, and a survival advantage from receiving the more aggressive treatment was limited to
23 poor-risk patients. More recently, the AMLCG has piloted a dose-dense induction regimen
24 sequential high-dose AraC and mitoxantrone followed by pegfilgrastim (S-HAM) in which two
25 induction cycles are applied over 11 to 12 days instead of 25 to 29 days as used in conventional
26 double induction, thereby increasing dose density two-fold (27). Importantly, the compression of
27 the 2 induction cycles into the first 11 to 12 days of treatment was beneficial for normal
28 hematopoiesis as demonstrated by a significantly shortened duration of critical neutropenia of 31
29 days compared with 46 days after conventionally timed double induction (27).
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41 The main difficulty of double induction is that it relies on the judgment of the attending physician
42 as to the eligibility of patients for the second induction. Although eligibility by itself can be an
43 important outcome to determine, it is conceivable that the physician's personal attitude will
44 significantly affect the results—especially given the variety of the clinical status of patients, mainly
45 in terms of suspected or documented infections (1). Because the data from the German trials
46 suggested that the survival advantage of double induction was limited to the poor-risk category,
47 use of double induction to treat all patients with AML seems inappropriate: a substantial
48 proportion might have unnecessary life-threatening toxic effects, not justified by stratification for
49 risk factors at diagnosis. The potential utility of DI was also investigated in a randomized study
50 aimed at comparison of double induction and timed-sequential induction to a “3+7” induction in
51 adults with AML. Treatment related mortality, either during the induction or the postremission
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3 phase, was not significantly higher for the more intensive arms; notwithstanding, event-free
4 survival and overall survival were similar in the 3 randomization arms (28).
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7 Our approach was unique in that administration of DI was based on bone marrow status at day 15.
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9 The timing was established on the basis of previous observations, which did clearly demonstrate
10 that patients with absence or low blast count day 15 BM, which reflects major sensitivity to
11 induction therapy, had a significantly better clinical outcome as compared to those with persistent
12 leukemia (13-16). As DI, we decided to employ the FLAG regimen, which has been proven as
13 useful and safe for poor prognosis AML as well as for high risk MDS. In addition, FLAG regimen
14 delivers high-dose treatment without increasing overall toxicity, an approach which is of particular
15 interest in patients potentially candidate to receive further chemotherapy, while being severely
16 pancytopenic from previous conventional induction (29-33). Of note, 30 out of 33 patients (91 %)
17 of patients were considered as clinical eligible to receive a second induction therapy, causes of
18 non eligibility being in all cases severe active infections. In spite of severe pancytopenia at the
19 start of DI, toxic death rate was low and comparable to that we observed after conventional
20 induction, confirming the tolerability of the FLAG regimen. The absence of anthracyclines could
21 account for the favorable results we observed in terms of treatment related mortality (TRM) and
22 eligibility to the programmed allo-SCT. In this regard, it is noteworthy that the only reason for
23 exclusion from allo-SCT was early relapse while patients were in the waiting list.
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27 Even though we cannot exclude that some patients would achieve CR without receiving DI, the
28 67% CR rate we observed is remarkable, if we consider that 65 % of patients had unfavorable
29 cytogenetics and no one of them were found positive for NPM1 mutation in absence of FLT3/ITD
30 mutations. Indirectly, our findings seem to confirm previous data suggesting that NPM1+/FLT3-
31 AML is associated with peculiar sensitivity to conventional chemotherapy, mainly to anthracyclines
32 (34,35). Such an assumption was recently suggested by data in young adults with AML, in whom
33 intensifying induction therapy with a higher daily dose of daunorubicin improved the rate of
34 complete remission and the duration of overall survival, as compared with the standard dose, only
35 in patients with intermediate cytogenetics, in whom most NPM1+ AML cases are found (36,37).
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37 As expected, hematologic toxicity was substantially higher in patients receiving DI as compared to
38 historical controls, who achieved CR after one course of chemotherapy. This did also result in
39 need for more transfusion support and prolonged hospitalization. On the contrary, there was no
40 difference in the overall non hematological toxicity and all remitters were able to receive further
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3 intensification followed by stem cell transplantation. Better therapeutic results were achieved in
4 patients who did actually receive allo-SCT, confirming that persistent leukemia at day 15
5 represents a main adverse prognostic factor in AML, needing appropriate management based on
6 more aggressive induction followed by allo-SCT. **In this study, donor research was started after CR
7 achievement. However, it seems more reasonable to start research as soon as persistent leukemia
8 at day 15 is documented; in addition, any potential alternative stem cell source, including
9 haploidentical donors or cord blood, would be considered.**

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16 In conclusion, day 15 bone marrow driven DI represents a safe and effective therapeutic strategy
17 in AML. As compared to conventional DI, which is usually administered irrespectively of bone
18 marrow status, it offers the potential to avoid unnecessary toxicity in a consistent proportion of
19 patients. Finally, any effort should be made to offer allogeneic stem cell transplantation to
20 patients with persistent leukemia at day 15 examination.
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Legend to figure 1: Overall survival (A) and disease free survival of 30 AML patients receiving day 15 driven double induction

For Peer Review

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Table 1 – Main characteristics of 30 patients with persistent leukemia at day 15 receiving double induction

	DI	Controls	P value
Patient number	30	30	n.a.*
Sex (M/F)	18/12 (57%/43%)	20/10 (67%/33%)	0.79
Median age, range	50 (16-60)	48 (16-60)	0.45
Cytogenetic findings			
Favorable	0	3	0.001
Intermediate (all normal)	11 (37%)	22	
Unfavorable	19 (63%)	5	
Molecular data			
NPM1+/FLT3-	0	8	0.02
NPM1+/FLT3+	4 (13%)	4	
NPM1-/FLT3+	12 (40%)	7	
NPM1-/FLT3-	14 (47%)	11	
Median blast % at day 15	35 (15-90)	3 (0-8)	< 0.001
Median neutrophil count** (x 10E9/l)	0-4 (0.1-1.0)	0.4(0.1-0.8)	0.68
Median platelet count** (x 10E9/l)	14 (3-60)	11 (2-53)	0.21

* Not applicable

** At the start of double induction