Leukemia (2005) 19, 2043–2053

www.nature.com/leu

Treatment and long-term results in children with acute myeloid leukaemia treated according to the AIEOP AML protocols

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Since 1982, four consecutive studies on childhood acute myeloid leukaemia (AML) (namely LAM-82, -87, -87M and -92) have been conducted in Italy by the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP) group. The induction therapy of the first three studies consisted of daunorubicin and cytarabine structured in a 3+7 backbone. In the most recent protocol (LAM92), patients received two induction courses including idarubicin, cytarabine and etoposide. Patients with acute promyelocytic leukaemia (20% of diagnoses) were included in LAM-87 and 87M studies. Post-remissional therapy significantly changed over time, with an ever-increasing role given to stem cell transplantation (SCT). The long-term outcome of patients enrolled in the LAM-82, 87 and 87M studies was comparable, whereas that of children treated according to LAM-92 study was significantly better ($P<0.005$). Either allogeneic or autologous SCT was employed as consolidation therapy in more than 75% of cases enrolled in this latter study. Patients enrolled in the LAM-92 study were stratified in standard and high-risk groups with different outcome (67 *vs* 47%, respectively, $P=0.04$). Altogether, the results obtained in these four studies have permitted a progressive refinement of treatment, contributing to the structure of the ongoing LAM-2002 protocol that stratifies patients according to the presence of definite genetic anomalies and response to induction therapy.
Leukemia (2005) **19,** 2043–2053. doi:10.1038/sj.leu.2403869; published online 18 August 2005
Keywords: childhood acute myeloid leukaemia; stem cell transplantation; acute promyelocytic leukemia

Introduction

Both induction and consolidation treatment of children with acute myeloid leukaemia (AML) have significantly changed over time.[1,2] From 1982 to 2001, in Italy, four consecutive studies on childhood AML have been performed by the AIEOP (Associazione Italiana di Ematologia e Oncologia Pediatrica) cooperative group.

Table 1 presents some details of these four studies, including patient number per trial, number of centres involved, and their median patient accrual. These cases represent 55% of the expected number of patients with AML, aged 0–14 years, in Italy, and 72% of the observed cases in all AIEOP Centres, respectively.[3] In fact, some AIEOP Centres treated their patients according to different protocols.

Background and treatment strategy of the AIEOP AML studies

The treatment plan of the four consecutive studies is shown schematically in Figure 1, whereas the treatment details of each AIEOP AML protocol, including drug dosage, are summarised in Table 2.

The design of each study was partly inspired by the results of trials in childhood AML conducted by other centres (Dana-Farber Cancer Institute, DFCI and Saint Jude Children's Research Hospital, SJCRH) and cooperative groups (BFM, MRC) and adjusted according to the previous experience accumulated in a few selected AIEOP Centres since 1979. The intensity of treatment was gradually increased with each subsequent study and, starting with the LAM 87 protocol, both allogeneic and autologous stem cell transplant (SCT) were increasingly employed as consolidation treatment.

Before 1982

Until approximately 1974, children with AML in Italy had been treated either adapting protocols for acute lymphoblastic leukaemia (ALL) or employing, on a local basis, schemes that included drugs, such as anthracyclines and cytarabine, that were recognised to be more specifically active on myeloid blasts.

The experience subsequently accumulated in a number of centres during the late 1970s and early 1980s with an intensive induction chemotherapy, combining anthracyclines and cytarabine, and had resulted in complete remission (CR) rate of 60–80% in patients with AML. However, leukaemia relapse continued to be the major cause of treatment failure, which occurred, usually within 2 years after diagnosis, in more than three-quarters of children achieving CR. A variety of strategies was then proposed in an attempt to prevent the regrowth of residual leukaemia cells, thus delaying or possibly avoiding the occurrence of relapse. The VAPA protocol, developed at the DFCI, was the first to use the principle of intensive postremission chemotherapy, based on the use of sequential, noncrossresistant, combinations of active agents, administered in short monthly courses at myelosuppressive doses.[4] With this approach, approximately 40% of children achieving CR were predicted to survive, leukaemia-free, at 5 years.

Based on this background, the first cooperative AML AIEOP study was started on February 1982.

Study AIEOP LAM 82

In 1982, the AIEOP Cooperative Group activated a prospective multicentre study (named LAM 8204). This study aimed at increasing the proportion of long-term disease-free survivors with a chemotherapy programme designed to maximise leukaemia cell cytoreduction and to prevent emergence of drug-resistant clones. The treatment included two courses

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Received 20 December 2004; accepted 9 June 2005; published online 18 August 2005

Table 1 Patient accrual and follow-up in studies AIEOP LAM 82, 87, 87M and 92 (only patients <15 years at diagnosis)

Trial	*Accrual of patients: time period*	*Total number of patients (n)*	*Participating Centres (n)*	*Number of patients/ centre (median/ range)*	*Follow-up (median, range, years)*
AIEOP LAM-82	02/82–03/88	171	22	5 (1–17)	12 (5–14)
AIEOP LAM-87	01/87–02/93	151	29	3 (1–21)	13 (6–16)
AIEOP LAM-87M	02/89–05/93	77	23	3 (1–11)	10 (5–13)
AIEOP LAM-92	05/92–09/01	160	26	3 (1–31)	5 (1–10)

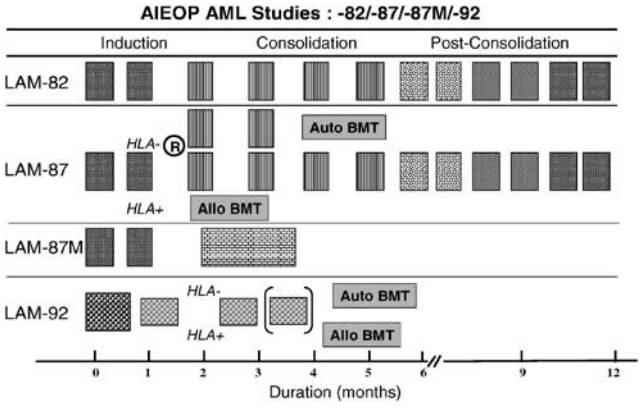

Figure 1 Flow diagram of AIEOP AML 82–92 studies. R indicates the time of randomisation.

of induction therapy with daunorubicin and cytarabine (DA), followed by four courses of consolidation therapy, which combined daunorubicin, thioguanine and escalating doses of cytarabine, and six courses of postconsolidation therapy with three paired drug combinations sequentially rotated (see Table 2 for further details). Prevention of CNS disease was performed through periodic administration of intrathecal cytarabine. The probability of event-free survival (EFS) at 5 years for children enrolled in this study was 31%.[5]

Study AIEOP LAM 87

According to the preliminary results of protocol AIEOP LAM 82, the LAM 87 trial was started and included two consecutive DA induction courses administered on a '3 + 7' and '2 + 5' days base. After induction therapy, patients, who achieved first CR and did not have an HLA-identical sibling received two courses of consolidation and were thereafter randomised to receive either autologous SCT (AUTO_SCT) or two more courses of consolidation chemotherapy followed by six courses of post-consolidation chemotherapy, similar to those employed in the previous study. The adopted conditioning regimen for children randomised to receive AUTO_SCT was based on the use of BCNU, M-AMSA, etoposide and cytarabine (BAVC regimen);[6] no indication was given about *in vitro* purging of harvested bone marrow cells. Allogeneic SCT (ALLO_SCT) in first CR after the second consolidation course was recommended for all children with an HLA-compatible sibling.

The rationale for using AUTO_SCT to consolidate remission in children and young adults with AML in first CR was based on the encouraging experience reported by the Baltimore group, using busulphan and cyclophosphamide as conditioning regimen.[7] The AIEOP LAM 87 represented the first randomised controlled trial comparing chemotherapy and AUTO_SCT.

The AIEOP LAM 87 trial did not significantly improve outcome with respect to the previous 82 study, as the overall 2-year EFS was 36%.[8] The AIEOP LAM 87 trial also documented that ALLO_SCT was the best strategy of consolidation therapy and that the outcome of children randomised either to AUTO_SCT or to chemotherapy was comparable. In view of these results, already in 1989, the AIEOP AML Committee decided to amend the protocol and to start the AIEOP LAM-87M protocol.

Study AIEOP LAM 87M

In the protocol AIEOP LAM 87M, DA induction therapy remained the same as in the previous study, while duration of consolidation was drastically reduced to a single course of chemotherapy employing high-dose cytarabine, combined with daunorubicin. In detail, an intensive postremission consolidation therapy was administered, which included high-dose cytarabine (36 g/m^2) and daunorubicin, as proposed by Wolff *et al*,[9,10] who had reported that, with this treatment, after a median follow-up of more than 3.5 years, 83% of patients younger than 25 years remained in continuous first CR. Owing to its remarkable antileukaemic activity, high-dose cytarabine was thought to provide effective consolidation of AML in first CR, with an expected outcome comparable to that of other approaches including SCT.

Unfortunately, application of the AIEOP LAM 87M protocol did not reproduce the results reported by Wolff *et al*, as the observed 3-year EFS probability was only to 23% (unpublished results). Therefore, in 1992, the AIEOP AML Committee decided to stop study AIEOP LAM 87M and proposed the AIEOP LAM-92 protocol.

Study AIEOP LAM 92

Patients enrolled in this study received one or two cycles of the 10-day induction, which included idarubicin, cytarabine and etoposide (ICE 3 + 5 + 10, see Table 2 for details), depending on haematopoietic reconstitution and bone marrow blast percentage at the time of recovery after first aplasia. In particular, patients who achieved CR after the first 10-day induction treatment were given only one course, while those who had a bone marrow blast percentage comprise between 5 and 25% were given two course of ICE 3 + 5 + 10. The rationale for using idarubicin was that *in vitro* and preclinical studies had suggested a possibly higher clinical benefit of using this drug, as compared to daunorubicin, and it is characterised by faster cellular uptake, increased retention and lower susceptibility to multidrug resistance glycoprotein.[11,12] Additional advantages seemed to

Table 2 Treatment elements in the four AIEOP AML Studies 1982–1992

Element	*Study AIEOP LAM-82*	*Study AIEOP LAM-87*	*Study AIEOP LAM-87M*	*Study AIEOP LAM-92*
Phase				
DA '3+7' Induction Introduced in Study-82	DA: *daunorubicin* 45 mg/m^2/day 30 min i.v. infusion on days 1, 2, 3; *cytarabine* 200 mg/m^2/day continuous i.v. infusion on days 1–7	DA: as in Study-82	DA: as in Study-82	Not given
DA '2+5' Induction Introduced in Study-82	DA: *daunorubicin* 45 mg/m^2/day 30 min i.v. infusion on days 1, 2; *cytarabine* 200 mg/m^2/day continuous i.v. infusion on days 1–5	DA: as in Study-82	DA: as in Study-82	Not given
ICE '3+5+10' Induction Introduced in Study-92	Not given	Not given	Not given	ICE (3+5+10)[a]: *idarubicin* 12 mg/m^2/day 1 h infusion on days 1, 3, 5; *etoposide* 100 mg/m^2/day 1 h i.v. infusion on days 1–5; *cytarabine* 25 mg/m^2/day i.v. push on day 1 followed by 100 mg/m^2/day continuous i.v. infusion on days 1–10
ICE '2+3+7' Induction Introduced in Study-92	Not given	Not given	Not given	ICE (2+3+7)[b]: *idarubicin* 12 mg/m^2/day 1 h infusion on days 1 and 3; *etoposide* 100 mg/m^2/day 1 h i.v. infusion on days 1–3; *cytarabine* 25 mg/m^2 i.v. push on day 1 followed by 100 mg/m^2/day continuous i.v. infusion on days 1–7
DAT Consolidation Introduced in Study-82	*Cycle 1*: *daunorubicin* 60 mg/m^2 i.v 1 h day 1, *cytarabine* 60 mg/m^2 s.c. every 8 h for 5 days; *thioguanine* 70 mg/m^2 os every 8 h for 5 days. *Cycle 2*: *daunorubicin* 60 mg/m^2 i.v. 1 h day 1, *cytarabine* 80 mg/m^2 s.c. every 8 h for 5 days; thioguanine 70 mg/m^2 os every 8 h for 5 days. *Cycle 3*: *daunorubicin* 60 mg/m^2 i.v. 1 h day 1, *cytarabine* 110 mg/m^2 s.c. every 8 h for 5 days; *thioguanine* 70 mg/m^2 os every 8 h for 5 days. *Cycle 4*: *daunorubicin* 60 mg/m^2 i.v. 1 h day 1, *cytarabine* 150 mg/m^2 s.c. every 8 h for 5 days; *thioguanine* 70 mg/m^2 os every 8 h for 5 days	DAT: as in Study-82	Not given	Not given
HDC-D 'Wolff' Consolidation Introduced in Study-87M	Not given	Not given	*High-dose cytarabine* 3 g/m^2 3 h infusion every 12 h for 6 consecutive days; *daunorubicin* 30 mg/m^2/day 30 min i.v. infusion on days 7–9	Not given

Table 2 *Continued*

Element	*Study AIEOP LAM-82*	*Study AIEOP LAM-87*	*Study AIEOP LAM-87M*	*Study AIEOP LAM-92*
Consolidation therapies in Study-92	Not given	Not given	Not given	Various schemas mostly including high-dose cytarabine
Postconsolidation Introduced in Study-82	*Cycles 1 and 2*: *etoposide* 100 mg/m^2 i.v. push days 1, 2, 3; *cytarabine* 150 mg/m^2 s.c. every 8 h for 3 days. *Cycles 3 and 4*: *thioguanine* 70 mg/m^2 os every 8 h for 5 days; *cytarabine* 150 mg/m^2 s.c. every 8 h for 5 days. *Cycles 5 and 6*: *daunorubicin* 40 mg/m^2 i.v. push day 1; *cytarabine* 300 mg/m^2 continuous i.v. infusion on days 1–3	As in Study-82	Not given	Not given
Cranial radiation	Not given	Not given	Not given	Not given
Intrathecal Chemotherapy	*Cytarabine (12 lumbar punctures with IT ARA-C)* 2 during induction on day 1 of each cycle; 4 during consolidation on day 1 of each cycle; 6 during postconsolidation on day 1 of each cycle. Dosage adjusted according to age, children <1 year $=20$ mg, 1–2 years $=30$ mg, 2–3 years 50 mg, $\geqslant 3$ years 70 mg	As in Study-82 for not transplanted patients and at the beginning of each cycle of chemotherapy for patients given bone marrow transplantation	*Cytarabine (2 lumbar punctures with IT ARA-C)* 2 during induction on day 1 of each cycle. Dosage adjusted according to age, as in Study-82	*Cytarabine (3–4 lumbar punctures with IT ARA-C)* 2 during induction on day 1 of each cycle; 1 during consolidation on day 1 of the cycle. Dosage adjusted according to age, as in Study-82
Haematopoietic stem cell transplant	Not scheduled	Allogeneic bone marrow transplantation if a sibling donor was available; autologous bone marrow transplantation on a randomised basis	Not scheduled	Allogeneic bone marrow transplantation if a sibling donor was available; autologous bone marrow transplant

[a]This cycle was repeated twice for children with partial remission.
[b]Only for patients achieving CR after first ICE.

arise from the long plasma half-life of 54 h of its main metabolic idarubicinol, which also showed antileukaemic activity in the cerebro-spinal fluid.[13] After achieving CR, patients received a 7-day ICE consolidation (2 + 3 + 7, see Table 2 for details), followed by one or two courses of consolidation therapy, depending on physician judgement. These courses mainly included high-dose cytarabine that was given at different dosages, together with other drugs (ie anthracyclines and epipodofillotoxins). ALLO_ or AUTO_SCT was then administered in more than three-quarters of patients, the choice depending on the availability of an HLA-matched family donor. Patients given an autograft were mainly prepared with a regimen including total body irradiation and received bone marrow cells that had been purged *in vitro* with mafosfamide. Thanks to this approach, a significant improvement of patient outcome was obtained, the overall Kaplan–Meier estimate of disease-free survival (DFS) reaching 60% at 5 years.

Patients and methods

Eligibility

The entry criteria for studies AIEOP LAM 82–92 included: newly diagnosed AML, age range from 0 to 15 years, and written informed consent of the parents. Patients with granulocytic sarcoma, secondary AML, myelodysplastic syndrome or Down syndrome, as well as patients with pretreatment duration longer than 14 days, were registered and treated with the standard study therapy – with reduced dosages whenever indicated – but have been excluded from the present analysis.

Patients with acute promyelocytic leukaemia (APL) were included in 87 and 87M studies, not recognised in the 82 study and excluded from the 92 study. In both LAM-87 and -87M studies, patients with APL did not receive all-*trans* retinoic acid (ATRA). Since the early 1990s, patients with APL defined as FAB M3 or M3v, with the demonstration of the PML/RARα fusion transcript alone or with t(15;17)(q22;q21) are enrolled in the AIDA 0493 protocol, which combines chemotherapy together with ATRA.[14]

Diagnosis

The initial diagnosis of AML with its subtype was established according to the FAB classification.[15] Since 1990, all smears obtained at the time of diagnosis were centrally reviewed at the Laboratory of Paediatric Haematology of the University Hospital in Padova and subjected to a specific panel review in controversial cases. Recently, the AIEOP-AML group performed a retrospective analysis on either the presence of t(8;21), inv(16), t(15;17) cytogenetic anomalies or the related molecular transcripts, which has been employed to obtain some of the results reported in this paper. Diagnosis of both M0 and M7 subtypes was always confirmed by immunophenotype.[16,17] Confirmation of the achievement of CR was also centrally reviewed.

Definitions and statistics

CR was defined as: $\leqslant 5\%$ leukaemic blasts in the bone marrow with evidence of normal haematopoiesis, no leukaemia cells in peripheral blood or anywhere else and clear signs of normal blood cell production (platelets $>50 \times 10^9$/l without support, neutrophil $>1.0 \times 10^9$/l) at the end of the induction phase.

Early death (ED) was defined as a fatal event occurring within the first 6 weeks of treatment. In particular, events were subdivided into (a) ED occurring before starting treatment, (b) ED registered within the first 14 days of treatment and (c) ED in aplasia between days 15 and 42. This subdivision reflects the ED rate due either to initial problems (hyperleukocytosis, leukostasis), treatment toxicity or aplasia secondary to induction therapy.

Patients who did not achieve CR and who survived after the first 6 weeks of treatment were defined as nonresponders (NR). Also, patients with CR lasting less than 4 weeks have been recorded as NR (type V failures according to CALGB criteria).

For the LAM 92 protocol, patients with FAB M1/M2 subtypes, with Auer rods, as well as patients with FAB M4eo were included in the standard-risk (SR) group, which comprised also patients who did not have the above-mentioned characteristics, but had t(8;21), inv(16) and t(15;17). All other children were assigned to the high-risk (HR) group.

EFS was calculated from date of diagnosis to last follow-up or first event (failure to achieve remission, demonstration of resistant leukaemia, relapse, second malignancy or death due to any cause, whichever occurred first). Patients who did not attain CR were considered failures at time zero.

Survival was calculated from date of diagnosis to time of death due to any cause or time of last contact. DFS for patients achieving remission was calculated from the date of remission to last follow-up or first event (relapse, second malignancy, death due to any cause, whichever occurred first). DFS of patients given SCT was calculated from the date of transplant to last contact or first event (relapse, second malignancy, death due to any cause, whichever occurred first).

Probabilities of survival were estimated using the Kaplan–Meier method and were compared using the log-rank test. Cumulative incidence of relapse and death in continuous CR (CCR) were constructed using the method of Kalbfleisch and Prentice. As we compared the outcome of patients given different type of consolidation therapy (namely chemotherapy, autologous or allogeneic transplantation), we used also the Landmark analysis, which takes into account the median time to transplantation to adjusted the different probabilities of EFS, in order to avoid bias related to the time censoring effect, which could favour the transplanted subgroups.

Analysis of efficacy data was performed according to the intent-to-treat principle. Computations were performed using SAS (Statistical Analysis System, Version 8.2, SAS Institute Inc, Cary, NC, USA).

Analysis used 15 June 2003 as the reference date, that is, the day at which all centres locked data on patient outcomes.

Results

Patient characteristics

From February 1982 to September 2001, 559 patients were enrolled in the four LAM-AIEOP studies. Comparison of data at diagnosis for LAM studies 82, 87, 87M and 92 did not reveal clinically important differences (see Table 3) except for patients with FAB type M3 or M3v who were included in the studies LAM–87 and 87M. Owing to the recent more precise definition of FAB subtypes with the introduction of M0 in 1991,[15] this subtype is present only for patients enrolled after 1989 in studies LAM-87M and -92.

Table 3 Initial patient data of studies AIEOP LAM 82, 87, 87M and 92 (only patients <15 years at diagnosis)

	AIEOP LAM 82		*AIEOP LAM 87*		*AIEOP LAM 87M*		*AIEOP LAM 92*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Number eligible patients	171	100	151	100	77	100	160	100
Gender (male)	93	54.4	90	59.6	38	49.3	81	50.6
Age								
<2 years	33	19.3	27	17.9	17	22.1	39	24.4
2–9 years	92	53.8	75	49.7	38	49.3	73	45.6
⩾10 years	46	26.9	49	32.4	22	28.6	48	30.0
Leukocytes ($\times 10^3/mm^3$)								
<20	74	43.3	79	52.3	45	58.4	75	46.9
20–<100	68	39.8	47	31.1	20	26.0	60	37.5
⩾100	29	17.0	25	16.6	12	15.6	25	15.6
CNS leukemia (yes)	6	3.5	13	9.8	11	14.9	6	3.8
*(*ND *or questionable)*	(7)		(8)		(3)		(1)	
FAB types								
M0	0	0	0	0	2	2.6	7	4.4
M1	44	25.7	21	13.9	8	10.4	16	10.0
M1 (with Auer)							7	
M2	49	28.7	36	23.8	10	13.0	41	25.6
M2 (with Auer)							23	
M3	2[a]	1.2[a]	27	17.9	20	26.0	2	1.2
M4	27	15.8	24	15.9	10	13.0	36	22.5
(M4eo)							12	
M5	44	25.7	28	18.5	21	27.2	44	27.5
M6	2	1.2	4	2.7	2	2.6	6	3.8
M7	3	1.7	2	1.3	2	2.6	4	2.5
Other (–)	0	0	9	6.0	2	2.6	4	2.5
Karyotypes ND	171		45		23		40	
Cytogenetic favorable[b]			23	21.7	20	37.0	31	25.8
t(8;21)			6	5.7	4	7.4	22	18.3
inv(16)			3	2.8	4	7.4	8	6.7
t(15;17)			14	13.2	12	22.2	1	0.8
Normal			68	64.1	18	33.3	59	49.2
Other			15	14.1	16	29.7	30	25.0
t(9;11)			0	0	0	0	2	1.6

ND = no data; NA = not applicable.
[a]M3v identified after central morphological review.
[b]Definition of favorable cytogenetics: t(8;21), inv16, t(15;17).

Overall outcome in studies AML-AIEOP 82, 87, 87M and 92

The main outcomes of the four studies are detailed in Table 4. Overall, the probabilities of survival and EFS have improved over time, with the remarkable exception of study 87M (see Figures 2 and 3).

The overall percentage of NR is 10.4% and changes significantly among the different studies: it was 8.8% in the study LAM-82, 12.6% in the study -87, 20.8% in the study -87M and 5.0% in the study -92 (see also Table 4).

The overall ED rate is 7.9%; considering the LAM-87 and -87M studies together, a decrement from 8.8% in the study LAM-82 to 6.2% in the study LAM-92 is observed. The greatest decrement was seen with the LAM-92 protocol for ED occurring before day 15, only two out of 160 (1.25%) children dying before day 15 in this protocol. This was possibly mainly due to exclusion of APL cases. However, as we do not have detailed information on cause of death, we cannot further speculate on variations of ED over time.

Table 5 shows the outcomes detailed separately for different risk parameters. Patients with $<100\,000/\mu l$ leukocytes at diagnosis, t(8;21), FAB M2 and M4, as well as those assigned to the SR group, had better outcome than others when treated according to the LAM-92 protocol.

Results of study LAM 82

The results of this first Italian paediatric prospective multicentre study, starting in 1982, demonstrated the possibility of achieving CR in more than 80% of patients with two DA induction courses. The observed ED rate of only 8.8% was remarkably low for that time period, also in view of the multicentre nature of the study. Repeated courses of consolidation and postconsolidation therapy led to a DFS at 5 years of children achieving CR of 38%. It is noteworthy that less than 10% of patients were given an allograft from an HLA-identical sibling, while none received an AUTO_SCT.

Table 4 Results in studies AIEOP LAM 82, 87, 87M and 92 (only patients <15 years at diagnosis)

	AIEOP LAM 82		*AIEOP LAM 87*		*AIEOP LAM 87M*		*AIEOP LAM 92*	
	n	*% (s.e.)*	*n*	*% (s.e.)*	*n*	*% (s.e.)*	*n*	*% (s.e.)*
Number of patients	171	100	151	100	77	100	160	100
Median follow-up of patients in CCR (years, range)	12.0	(6.7–14.3)	13.0	(1.5–16.1)	10.1	(5.0–13.2)	5.5	(0.8–10.8)
Early deaths (total)[a]	15	8.8	8	5.3	11	14.3	10	6.2
Nonresponse	15	8.8	19	12.6	16	20.8	8	5.0
CR achieved	141	82.4	124	82.1	50	64.9	142	88.8
Death in CCR (cumulative incidence)	11	6.4 (1.8)	11	9.6 (4.4)	4	5.3 (6.5)	11	7.1 (2.5)
Relapse (cumulative incidence)	78	45.6 (4.2)	73	57.6 (4.7)	34	68.3 (6.6)	45	32.4 (4.7)
(with CNS involvement)	4	2.3 (1.0)	5	3.6 (2.2)	3	5.3 (3.7)	3	1.5 (1.3)
Lfu in CCR	1	0.6	5	3.3	5	6.5	3	1.9
Probability of survival								
At 5 years	69	41.5 (3.8)	60	41.8 (4.1)	25	35.1 (5.4)	56	59.5 (4.0)
At 10 years	56	36.6 (3.7)	51	41.1 (4.1)	16	32.2 (5.4)	2	54.8 (0.6)
Probability of EFS								
At 5 years	53	31.1 (3.5)	39	27.4 (3.7)	10	15.6 (4.1)	49	53.5 (4.0)
At 10 years	46	29.0 (3.5)	31	26.0 (3.6)	7	15.6 (4.1)	2	51.6 (4.3)
Probability of DFS								
At 5 years	53	38.4 (4.1)	38	33.4 (4.3)	10	24.0 (6.0)	47	60.4 (4.2)
At 10 years	46	35.0 (4.0)	31	31.6 (4.2)	6	24.0 (6.0)	2	58.1 (4.6)
Allogeneic BM in first CR	14	8.2	25	16.6	7	9.1	47	29.4
Autologous BMT in first CR	0	0	34	22.5	3	3.9	76	47.5

[a]Early deaths are defined as death until day 42.
CR, complete remission; CCR, continuous complete remission; lfu, lost to follow-up.

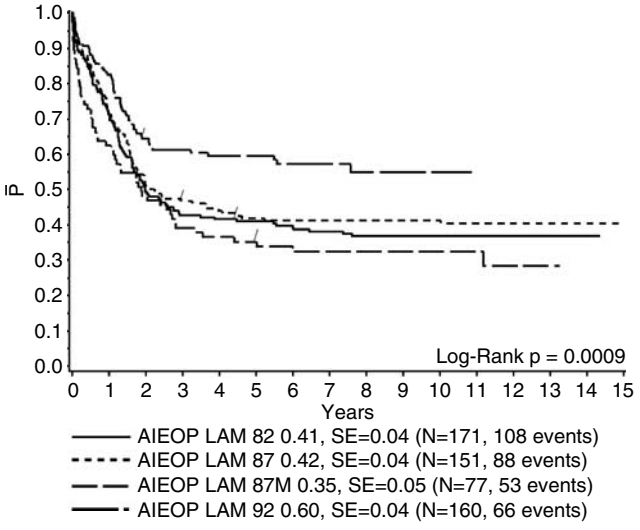

Figure 2 Estimated probability of 5-year survival in patients of AIEOP AML 82–92 studies. Slash indicates the patient alive with the shortest follow-up.

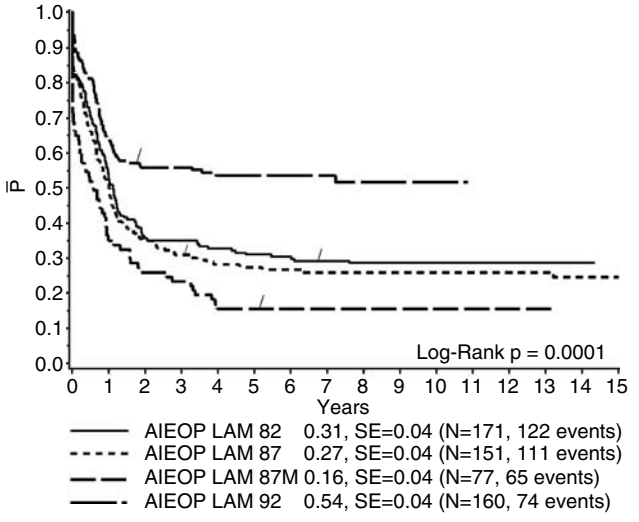

Figure 3 Estimated probability of 5-year EFS in patients of AIEOP AML 82–92 studies. Slash indicates the patient without event with the shortest follow-up.

Results of study LAM 87

As mentioned above, this study compared the use of ALLO_SCT, AUTO_SCT and sequential postremission chemotherapy as consolidation in children with *de novo* AML achieving first CR. From January 1987 to February 1993, 151 patients were enrolled in this study. Overall, 124 of 151 patients attained CR (82%). The estimated probabilities of survival and EFS at 5 years for all patients were 42 and 27%, respectively (median follow-up, 13 years). For the 124 children obtaining first CR, the 5-year probability of DFS was 33%, while the cumulative risk of relapse was 58%. The 5-year DFS was 52% for the ALLO_SCT group ($n=25$), significantly higher ($P=0.03$) than that observed for the other cohorts: 21% for AUTO_SCT ($n=34$), 26% for CT ($n=37$) and 34% for a group of 28 patients who were not randomised due to either parental or medical decision. Bone marrow relapse was the most frequent cause of failure in the three subgroups, including the ALLO_SCT cohort, in which no death attributable to toxicity of the procedure was recorded.

Table 5 Results according to different risk parameters in studies AIEOP LAM 82, 87, 87M and 92 (only patients <15 years at diagnosis): 5-year probability of EFS (%), only for subgroups $n \geqslant 10$

Presenting feature	*AIEOP LAM 82*		*AIEOP LAM 87*		*AIEOP LAM 87M*		*AIEOP LAM 92*	
	Total number of patients	*pEFS (s.e.)*	*Total number of patients*	*pEFS (s.e.)*	*Total number of patients*	*EFS (s.e.)*	*Total number of patients*	*EFS (s.e.)*
SR	NA		NA		NA		52	67.1 (6.6)
HR	NA		NA		NA		108	47.0 (4.9)
Cytogenetics favourable[a]	ND		23	26.1 (9.2)	20	10.0 (6.7)	31	67.7 (8.4)
t(8;21)			6		4		22	68.2 (9.9)
inv(16)			3		4		8	
t(15;17)			14	21.4 (11.0)	12	8.3 (8.0)	1	
Normal karyotype	ND		68	28.7 (5.6)	18	11.1 (7.4)	59	49.0 (6.8)
Other cytogenetic abnormalities	ND		15	40.0 (12.6)	16	18.7 (9.8)	30	55.3 (9.2)
t(9;11)			0		0		2	
FAB M0	0		0		2		7	
FAB M1	44	25.1 (6.5)	21	38.1 (10.6)	8		16	37.5 (12.1)
FAB M2	49	39.5 (7.0)	36	25.0 (7.2)	10		41	72.9 (7.0)
FAB M3	2		27	27.4 (8.8)	20	15.0 (8.0)	2	
FAB M4	27	33.6 (4.0)	24	39.4 (10.2)	10		36	52.1 (8.5)
FAB M4eo	ND		ND		ND		ND	
FAB M5	55	25.0 (6.5)	28	17.9 (7.2)	21	23.8 (9.3)	44	43.5 (7.7)
FAB M6	2		4		2		6	
FAB M7	3		2		2		4	
Leukocytes $<100\,000/\mu l$	142	33.0 (8.0)	126	30.6 (4.2)	65	15.4 (4.5)	135	58.1 (4.3)
Leukocytes $\geqslant 100\,000/\mu l$	29	21.5 (7.5)	25	12.0 (6.5)	12	16.7 (10.8)	25	28.5 (9.7)
Age <2 years	33	30.0 (8.0)	27	23.7 (8.4)	17	23.5 (10.3)	39	50.8 (8.1)
Age $\geqslant$2 years	138	31.5 (3.9)	124	28.3 (4.1)	60	13.3 (4.4)	121	54.3 (4.6)
CNS positive	6		13	15.4 (10.0)	11	0	6	
CNS negative	165	30.3 (3.6)	130	29.0 (4.0)	63	15.9 (4.6)	153	56.1 (4.1)

[a]Definition of favorable cytogenetics: t(8;21), inv16, t(15;17).

Results of study LAM 87M

Only 10 out of the 77 children included in this modified (M) version of protocol LAM-87 were alive and disease-free 5 years after diagnosis. CR rate was only 65%: both ED and nonresponse contributed to this poor outcome (see also Table 4 for details). It is noteworthy that the percentage of patients with CNS involvement at diagnosis was the highest of the all AIEOP studies.

The Kaplan–Meier estimate of DFS at 5 years after achievement of CR was 24%, documenting that leukaemia recurrence after induction also adversely affected patient outcome.

Results of study LAM 92

Despite the fact that only the first courses of induction therapy were strictly defined, while freedom of choice was left to the Centres participating in this study regarding subsequent therapy, this trial has been the most successful one. In fact, the CR rate was almost 90%, with a 5-year EFS for the overall cohort of 53%. ED occurred in only 6% of patients. The Kaplan–Meier estimate of DFS at 5 years for patients achieving CR was 60%, autologous and allogeneic transplantation having been employed as consolidation therapy in more than 75% of cases. Also, in children enrolled in the LAM-92 study, we stratified patients in SR and HR groups, which show different outcome (67 *vs* 47%, respectively, $P=0.04$); leukocyte count at diagnosis $>100\,000/\mu l$ was a factor predicting low probability of EFS (see Table 5 for details).

Discussion

Since 1982, four consecutive multicentre studies for treatment of children with newly diagnosed AML have been performed in Italy. A majority of Italian paediatric patients with this disease, diagnosed in AIEOP centres, were included in these studies. As shown in Table 1, the median number of patients enrolled per center was low in all studies and this fact can explain the minimal decrement of ED rate observed over time.

A centrally reviewed diagnosis has become available only since 1990 and this fact, at least in part, explains some of the differences in the observed distribution of subtypes. The exclusion of APL cases since the early 1990s (ie in the ATRA era) also contributed to this different subdivision. The percentage of APL cases confirms previously published studies, which reported a greater incidence of this AML variant in children of Latin origin.[18] Environmental and/or genetic factors could explain such differences.

The CR rate after induction therapy was substantially comparable in the four consecutive studies, with the only exception of the LAM-87M protocol, where a high, unexplained, incidence of both ED and NR was observed and a single course of high-dose cytarabine, combined with three days of daunorubicin, did not prove to be sufficient for preventing leukaemia recurrence in patients achieving first CR. The high percentage of patients with APL before the introduction of ATRA could have partially contributed to the high incidence of ED. The comparable CR rate of our studies is in agreement with previously published reports documenting that substitution of daunorubicin with idarubicin, despite favouring blast clearance on day 15, does not substantially improve the CR rate.[19] Also,

neither addition of a third drug to the classical combination of cytarabine and an anthracyclin, nor use of etoposide rather than an antimetabolite drug have been reported to offer an advantage in terms of CR and OS for patients with AML.[20,21]

The main change in the overall treatment strategy of the four consecutive AIEOP studies regarded postremissional treatment. In fact, in the first study, patients were given repeated courses of mild-intensity chemotherapy, only a minority of children receiving ALLO_SCT in first CR. The LAM-87 trial had the great merit of being the first paediatric study that tried to comparatively assess, in a randomised way, the safety and efficacy of autologous transplantation with respect to repeated courses of chemotherapy. The results of this trial did not demonstrate any advantage for the transplant arm, as also found in other randomised studies.[22,23] However, the recommended preparative therapy for patients enrolled in the LAM-87 study and given AUTO_SCT was the BAVC regimen, which, in a recently published retrospective analysis on the role of autologous transplantation in childhood AML, was found to be associated with an increased risk of relapse and a low probability of DFS.[24] Moreover, in the LAM-87 trial, *in vitro* purging of haematopoietic progenitors was not performed in the majority of patients (data not shown) and several studies have documented that a lower risk of leukaemia recurrence is observed when patients are given a purged graft.[24–26]

The best probability of sustained remission for patients enrolled in the LAM-87 trial was observed when an allograft from an HLA-identical sibling was employed as part of postremissional therapy. The advantage of ALLO_SCT over the other two forms of consolidation therapy, namely chemotherapy and AUTO_SCT, has been consistently found also in other randomised studies on childhood AML.[22,23,27] In view of the results obtained in the LAM-87 trial and in other controlled studies,[22,23,27] this type of consolidation therapy has been increasingly employed when an HLA-identical sibling was available, and it is now considered the most powerful form of antileukaemia treatment for children with AML.

In the LAM-92 study, either autologous or allogeneic transplantation was employed to consolidate the state of remission in 54 and 33% of patients, respectively. The wide use of SCT resulted in a significant improvement in the outcome of patients enrolled in the LAM-92 study over that of patients treated with the previous protocols (see also Figures 2 and 3). We also calculated the Kaplan–Meier estimates of DFS, performed taking into account the median time to transplant, of patients enrolled in the studies LAM 87, 87M and 92 and we found that patients given either allogeneic or autologous transplantation have a better probability of long-term outcome in comparison to those who were given chemotherapy alone for consolidation (see Figure 4 for details). Possibly, also the introduction of repeated courses of high-dose cytarabine during consolidation before SCT had a favourable impact on these results. However, all these data should be interpreted with caution due to the retrospective and uncontrolled nature of our analysis.

Most patients of the LAM-92 study who were given AUTO_SCT received melphalan, either alone[28] or more frequently associated with total body irradiation,[29] as part of the preparative regimen, whereas those receiving ALLO_SCT were given a busulphan-based regimen.[30] It must be noted also that children given an AUTO_SCT received *in vitro* purged haematopoietic progenitor cells.[24,29] In the LAM-92 study, we have been able to subdivide patients in SR and HR groups, according to the criteria mentioned above. SR and HR groups included one-third and two-third of patients (see Table 5),

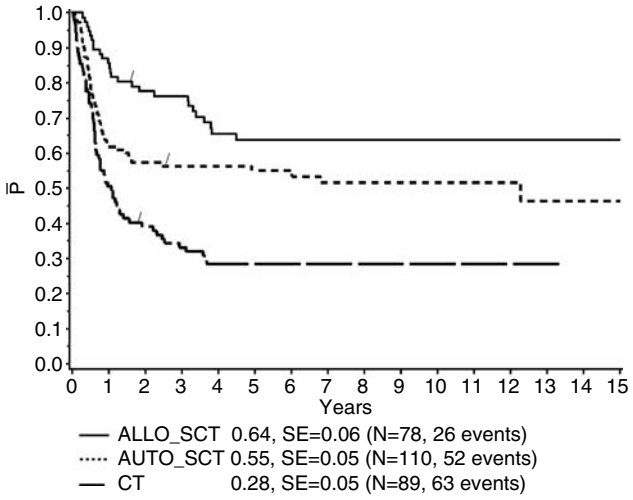

Figure 4 Estimated probability of 5-year disease-free survival in patients of AIEOP AML 87–92 studies by postremissional treatment: allogeneic stem cell transplant (ALLO_SCT) *vs* autologous stem cell transplant (AUTO_SCT) *vs* chemotherapy (CT). Slash indicates the patient without event with the shortest follow-up.

respectively, and the outcome of these two cohorts showed an advantage for patients belonging to the SR group. These results allow to consider tailoring of treatment according to definite morphological and cytogenetic/molecular criteria. In fact, several studies have demonstrated that patients with favourable genetic anomalies have a better probability of survival and may benefit from less-intensive postremissional therapies, such as those not including SCT, especially if high-dose cytarabine is employed in repeated courses.[31–34] Thus, in the AIEOP 2002 study, recently started children with AML1-ETO fusion transcript or anomalies of CBF-β are considered to be at lower risk of treatment failure and assigned to receive two courses of induction chemotherapy and three courses of consolidation which include high-dose cytarabine, SCT being reserved for relapsed patients only. This choice is also supported by the consideration that both ALLO_ and AUTO_SCT may be associated with the occurrence of long-term sequels (namely growth impairment, endocrine complications and, for children given an allograft, chronic graft-versus-host disease).

In the last decade, children with APL have been treated with a special protocol employing ATRA. Excellent results on these patients have been reported using a combination of chemotherapy and ATRA,[14,35,36] which is able to induce, despite a remarkable but manageable ATRA-related toxicity observed in some cases, a stable CR, with low ED rate. In view of these results, SCT is no longer considered to be electively indicated in patients with this type of leukaemia in molecular remission during treatment or at the time of treatment discontinuation.

Overall, the experience gained in the four AIEOP studies has inspired the recent AIEOP 2002 study, which, as mentioned above, stratify patients with *de novo* and non-APL AML in SR and HR groups. Patients belonging to the former are those with AML1-ETO fusion transcript or anomalies of CBF-β achieving CR after the first induction course, whereas all the others are assigned to the HR group. Patients of this latter cohort are given two courses of induction chemotherapy and two courses of consolidation which include high-dose cytarabine, followed by either ALLO_ or AUTO_SCT, depending on the availability of the family donor. The use of unrelated donors for ALLO_SCT in Italy is presently reserved to patients in second CR or to the few

patients with M7 AML, as a recent study has documented a particularly dismal outcome for this latter subtype.[37]

Acknowledgements

Until 2000, the coordinator of the AIEOP studies was professor Franco Mandelli, whom we thank for his valuable contribution to the improvement of results in childhood AML in Italy. We are indebted also with Dr Laura Sainati and Dr Emanuela Frascella who performed most of the cytogenetic and molecular biology evaluation on centralised marrow/blood samples.

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