Infants with acute myeloid leukemia treated according to the *Associazione Italiana di Ematologia e Oncologia Pediatrica* 2002/01 protocol have an outcome comparable to that of older children

Children under the age of one year (i.e. infants) with acute myeloid leukemia (AML) represent a distinct subgroup of patients with peculiar clinical and biological characteristics.[1] As compared to older children, a higher prevalence of unfavorable clinical and cytogenetic/molecular features has frequently been reported in this age group.[1,2] While the outcome of infants with acute lymphoblastic leukemia is still worse than that of older children,[3] in recent years, this may not be the case for infants with AML. In particular, consistent with the significant progress generally achieved in the outcome of childhood AML,[1] the event-free survival (EFS) of infants with AML has increased from less than 35% in the past[4] to more than 50% in recently published studies.[5] It is unclear whether and to what extent a wide use of allogeneic (ALLO) hematopoietic stem cell transplantation (HSCT) in first complete remission (CR), has contributed to the improved outcome of these particular patients. In the AML 2002/01 study of the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP)[6], infants achieving CR1 were most often offered either allo-HSCT or, less frequently, autologous (auto)-HSCT as post-remission therapy. Here we report clinical/biological characteristics, as well as outcome, of infants, comparing the results with those of older children treated in that protocol. We analyzed children aged one year or under year at diagnosis, with *de novo* non-promyelocytic AML, treated between December 2002 and June 2011 according to the AIEOP AML 2002/01 protocol; children with Down syndrome were not included in this study as they were treated with a specific protocol (i.e. AIEOP AML 2002/02). Details on the diagnostic workup, as well as on the cytogenetic/molecular characterization, have been reported elsewhere.[6]

Table 1. Clinical, cytogenetic and biological features of infants enrolled in the AIEOP AML 2002/01 protocol compared with those of older patients.

Age groups	Infants N (%)	1–<2 years N (%)	2–<10 years N (%)	>10 years N (%)	*P*
Number of patients	63	52	181	186	
Leukocytes (x10⁹/L), median (min–max)	71.2 (3.1-653)	46.9 (3.3-671)	45.9 (6.6-475)	39.7 (7-165)	
Sex: male	31/63 (49)	24/52 (46)	103/181 (57)	104/186 (56)	0.434
CNS leukemia	10/63 (16)	6/52 (12)	5/181 (3)	16/186 (9)	0.002
Extramedullary leukemia (Non-CNS)	9/63 (15)	6/52 (12)	30/181 (17)	32/186 (18)	0.809
Leukocytes >100x10⁹/L	13/63 (21)	9/52 (17)	25/181 (14)	30/186 (16)	0.598
FAB M0	8/63 (13)	4/52 (8)	9/181 (5)	13/186 (7)	0.231
FAB M1	8/63 (13)	5/52 (10)	31/181 (17)	44/186 (24)	0.042
FAB M2	3/63 (5)	1/52 (2)	45/181 (25)	42/186 (23)	<0.001
FAB M4	10/63 (16)	4/52 (8)	34/181 (19)	35/186 (19)	0.103
FAB M5	20/63 (32)	17/52 (33)	40/181 (22)	40/186 (22)	0.133
FAB M6	0/63 (0)	1/52 (2)	2/181 (1)	2/186 (1)	0.568
FAB M7	12/63 (19)	17/52 (33)	13/181 (7)	2/186 (1)	<0.001
Unclassifiable/not known	2/63 (3)	3/52 (6)	7/181 (4)	8/186 (4)	0.760
SR risk group	4/63 (6)	5/52 (10)	43/181 (24)	47/186 (25)	0.001
HR risk group	59/63 (94)	47/52 (90)	138/181 (76)	139/186 (75)	0.001
Patients with available cytogenetic data	58/63 (92)	46/52 (88)	156/181 (86)	158/186 (85)	0.553
Complex karyotype	6/58 (10)	3/46 (7).	9/156 (7)	11/158 (7)	0.685
Favorable cytogenetic t(8;21).t(16;16) or inv(16)	4/63 (6)	5/52 (10)	43/181 (24)	47/186 (25)	0.001
11q23/MLL-rearrangements	21/58 (36)	13/46 (30)	25/156 (13)	22/158 (14)	0.001
t(9;11)(p22;q23) and/or MLL-AF9	6/58 (10)	3/46 (7)	10/156 (6)	8/158 (5)	0.553
t(10;11)(p11.2;q23) and/or MLL-AF10	4/58 (7)	2/46 (4)	6/156 (4)	5/158 (3)	0.613
t(11;19) (q23;p13)	1/58 (1)	2/46 (4)	2/156 (1)	2/158 (1)	0.423
t(1;11) (variable;q23)	2/58 (4)	3/46 (7)	2/156 (1)	1/158 (1)	0.051
MLL rearrangement with other partners	8/58 (14)	3/46 (7)	5/156 (3)	6/158 (4)	0.023
t(1;22)(p13;q13)	3/58 (5)	1/46 (2)	0/156 (0)	0/158 (0)	0.020
Patients tested for FLT3 aberrations	50/63 (79)	40/52 (77)	140/181 (77)	154/186 (83)	0.694
FLT3-ITD mutations	1/50(2)	2/40 (5)	10/140 (7)	39/154 (25)	<0.001
Patients tested for NPM and CEBP aberrations	28/63 (45)	21/52 (40)	99/181 (55)	103/186 (55)	0.08
NPM mutations	1/28 (4)	0/21 (0)	7/99 (7)	7/103 (8)	0.035
CEBP mutations	1/28 (4)	0/21 (0)	5/99 (5)	12/134 (9)	0.022

N: number of patients; CNS: central nervous system; SR: standard risk; HR: high risk.

Table 2. Outcome results of infants enrolled in the AIEOP AML 2002/01 protocol compared with older patients.

Age groups	Infants N (%)	1-<2 years N (%)	2-<10 years N (%)	>10 years N (%)	P
Number of patients	63	52	181	186	
CR achieved after induction	52/63 (82)	48/52 (92)	156/181 (86)	161/181 (88)	0.20
Non-responders	9/63 (14)	3/52 (6)	20/181 (11)	16/186 (9)	0.28
Early death	2/63 (3)	1/52 (2)	5/181 (3)	5/186 (3)	0.96
	% (SE)	% (SE)	% (SE)	% (SE)	P
OS at 8 years	73.7 (5.7)	66.4 (7.0)	62.8 (4.3)	65.4 (3.9)	0.81
EFS at 8 years	54.6 (6.1)	58.7 (7.2)	54.3 (4.6)	54.4 (4.0)	0.87
DFS at 8 years	64.9 (6.7)	62.1 (7.4)	62.1 (5.0)	62.4 (4.3)	0.87
CI of relapse at 8 years	31.3 (6.5)	25.7 (6.4)	30.5 (4.7)	22.9 (3.4)	0.61
CI of death in CR at 8 years	3.8 (1.0)	12.1 (5.2)	7.3 (2.5)	14.7 (3.3)	0.06

N: number of patients; SE: standard error; CR: complete remission; OS: overall survival; EFS: event-free survival; DFS: disease-free survival; CI: cumulative incidence.

Infants of the AIEOP AML 2002/01 study[6] received two courses of induction chemotherapy, including idarubicin, Ara-C and etoposide (see Pession *et al.*[6] for details). Children achieving CR received two consolidation courses, containing high-dose (HD) Ara-C, combined with either etoposide or mitoxantrone in the first and second course, respectively. The dosage of cytotoxic drugs was adapted for infants according to the following formula: weight (kg) x dose (x m^2)/30, the only exception being represented by the dose of HD-Ara-C, administered as 20%, 30%, 40%, 50% and 60% to infants aged under 3, 4-5, 6-7, 8-10 and 11-12 months, respectively, in view of the reduced Ara-C clearance of these patients.[7] At the end of consolidation, infants carrying t(8:21), inv(16) or t(16;16) (standard risk group) received a fifth course of HD Ara-C. The remaining high-risk (HR) infants were offered allo-HSCT from either matched family donors (MFD) or alternative donors. In 7 children lacking a matched related or matched unrelated donor, the treating physician decided not to perform an allograft, but opted for auto-HSCT.

Infants' clinical and biological features at presentation, compared with those of other age groups, are detailed in Table 1. Compared to older children, infants had significantly more central nervous system involvement at diagnosis (P=0.002) (Table 1). French-American-British (FAB) subtypes M1 (P=0.042) and M2 (P<0.001) were more common in older children, while infants presented more frequently with a FAB-M5 (P=0.008) or M7 subtype (P<0.001). In comparison to older children, infants were allocated more frequently to the HR group (P=0.001) (see Pession *et al.*[6] for details).

Infants showed a significantly higher incidence (i.e. 36%) of 11q23/MLL rearrangements (P=0.001) than older children. All cases with t(1;22)(p13;q13) but one were infants belonging to the FAB-M7 subtype (Table 1). FLT-3 aberrations, namely internal tandem duplication and activating loop mutations, were present only in 2% and 5% of patients aged under one year and those aged one to under two years, respectively, while they were found in 7-25% of older children (P=0.001). Notably, 6 (9.5%) out of the 63 infants harbored inv(16)(p13.3q24.3) encoding for the cryptic fusion transcript *CBFA2T3-GLIS2* that we identified through whole-transcriptome sequencing and confirmed using Sanger techniques; 4 of them had a FAB-M7 blast morphology.[8] This cryptic translocation is much less common than the classical inversion of chromosome 16 and can be detected only by specific molecular/cytogenetic probes.[8,9]

Results of the outcome of infants compared to those of other age groups are reported in Table 2. CR, early death and induction failure rates were 82%, 3% and 14%, respectively, with no statistical difference to rates for older patients. Only 3 infants died in CR: 2 during consolidation due to Gram-negative sepsis and one after transplantation. Compared to older children, severe mucositis occurred less frequently in infants (25% *vs.* 35%, respectively) (P=0.04), while the incidence of the remaining chemotherapy-related toxicities was superimposable. The cumulative incidence of relapse was 31%.

Four patients were not offered HSCT as they carried a favorable cytogenetic profile. All these 4 children are alive and disease free.

Forty-six infants out of 63 received HSCT in CR1: 7 children were given auto- and 39 allo-HSCT. With a median follow up of 57 months (range 12-130 months), the 8-year overall survival (OS) and EFS probabilities of the whole population of infants were 74% (Standard Error (SE) 5.7) and 55% (SE 6.4), respectively, showing no difference to those of other age groups (Table 2). In multivariate analysis, only blast count over 5% at time of hematologic recovery after induction negatively affected prognosis (P=0.03).

Overall survival and EFS of infants undergoing HSCT in CR1 was 72% (SE 6.8) and 58% (SE 7.1), respectively. Neither type of donor nor stem cell source influenced the probabilities of OS and EFS in allo-HSCT recipients (*data not shown*). Notably, the small group of infants harboring the *CBFA2T3-GLIS2* fusion transcript had a significantly worse prognosis (EFS 32.3%, SE 19.2) compared with *CBFA2T3-GLIS2* negative infants (59.6%, SE 6.6) (P<0.05). Outcome of infants was not influenced be the presence of 11q23 MLL rearrangement (*data not shown*). Among transplanted infants, 14% experienced growth deficiency, 3% decreased cardiac function, 9% hypothyroidism and 6% have impaired cognitive function.

Besides confirming and extending previously reported observations, our data demonstrate for the first time that, in infants, *CBFA2T3-GLIS2* fusion transcript seems to be as frequent as *OTT-MAL* fusion in FAB-M7 AML, this

lesion predicting a grim prognosis. Moreover, our results suggest that a treatment strategy combining intensive chemotherapy and a widespread use of HSCT in CR1 is able to abolish the impact of unfavorable prognostic characteristics of infants. The outcome of our patients is similar to that reported by the I-BFM,[2] MRC[10] and Japanese groups,[5] in which, however, HSCT was not widely used. In the new AIEOP-AML study, post-remission treatment will be tailored to reduce the proportion of transplanted infants with predicted disease recurrence, in view of the more frequent and severe HCST-related long-term complications.[11]

Riccardo Masetti,[1] Roberto Rondelli,[1] Franca Fagioli,[2] Angela Mastronuzzi,[3] Paolo Pierani,[4] Marco Togni,[1] Giuseppe Menna,[5] Martina Pigazzi,[6] Maria Caterina Putti,[6] Giuseppe Basso,[6] Andrea Pession,[1] and Franco Locatelli[3,7]

[1]Department of Pediatrics, "Lalla Seràgnoli", Hematology-Oncology Unit, University of Bologna; [2]Oncoematologia Pediatrica, Ospedale Infantile "Regina Margherita", Torino; [3]Department of Pediatric Hematology-Oncology, IRCCS Ospedale Bambino Gesù, Rome; [4]Division of Pediatric Hematology and Oncology, Ospedale G. Salesi, Ancona; [5]Oncoematologia Pediatrica, Ospedale Pausillipon, Napoli; [6]Clinica Oncoematologia Pediatrica, Università di Padova; [7]University of Pavia, Italy

Correspondence: riccardo.masetti@gmail.com.
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