Definitions

Patients were considered in morphological complete remission (CR) if they had normal neutrophil and platelet counts, less than 5% blast cells in a bone marrow (BM) smear and no extramedullary disease. All patients had a lumbar puncture before hematopoietic stem cell transplantation (HSCT) to document cerebro-spinal fluid CR.

Neutrophil and platelet engraftment were defined as the first of three consecutive days with a neutrophil count greater than 0.5 x 10^9/L and an unsupported platelet count greater than 50 x 10^9/L, respectively.

Acute and chronic GvHD (aGvHD and cGvHD) were diagnosed and graded according to established criteria (36, 37). Children with evidence of donor engraftment surviving more than 14 days and 90 days from transplantation were evaluated for the occurrence of aGvHD and cGvHD, respectively. Relapse was defined on the basis of morphological evidence of leukemia in BM, or at other extra-medullary sites. Transplantation-related mortality (TRM) was defined as all causes of non-leukemia death occurring after HSCT. Overall survival (OS) was defined as the interval between HSCT and either death or date of last follow-up, disease-free survival was defined as the interval between HSCT and either relapse, or death, or date of last follow-up, whichever occurred first.

Minimal Residual Disease analysis

DNA of BM mononuclear cells, obtained after Ficoll–Paque centrifugation, was extracted and purified using Gentra kit (Gentra System, Minneapolis, MN, USA). PCR analysis to detect specific TCRγ, TCRδ, and VDJH, DJH, VK and IRSS gene rearrangements was performed at diagnosis. Clonal gene rearrangements, identified by homo/heteroduplex analysis, were sequenced by dye-terminator cycle sequencing kit on ABI Prism 310 (Applied Biosystems, Foster City, CA, USA) (30). Minimal residual disease (MRD) levels in follow-up samples were analyzed by real-time quantitative PCR with hydrolysis (TaqMan) probes (31-33). Real-time PCR analysis of BM samples was performed in accordance with the guidelines published by the European Study Group on MRD detection in acute lymphoblastic leukemia (ALL) (34). Only markers with a cutoff level of at least 10^{-4} were considered.

Besides MRD-based stratification criteria, other high risk features used in previous protocols were also considered in the most recent ALL protocol, AIEOP-BFM 2000: patients with prednisone poor response [PPR; ≥1000 leukemic blasts per microliter in peripheral blood on day 8], or not achieving remission after induction treatment (i.e. ≥5%

leukemic blasts in BM on day 33), or with BCR/ABL or MLL/AF4 fusion gene transcripts. Such patients were treated in the HR arm, or with protocols specific for Ph-positive ALL, irrespective of MRD risk group assessment (27).

Statistical analysis

Patient-, disease-, and transplantation-related variables were expressed as median and ranges, or as percentages, as appropriate. For statistical analysis, all continuous variables, except for age, were categorized as follows: each variable was first divided into 4 categories at the 25^{th}, 50^{th} and 75^{th} percentiles. If the relative event rates (the ratio of the observed number of events to the expected number of events in the category) in two or more adjacent categories (and the median time to events) did not differ, those categories were grouped. If no clear pattern was observed for the primary outcomes, the median was taken as the cutoff point. Patients were censored at time of relapse, death or last follow-up. Death from any cause and graft rejection were competing risks to estimate the cumulative incidence of aGvHD and cGvHD. Death in remission was treated as a competing event to calculate the cumulative incidence of relapse. Relapse was considered to be the competing event for calculating TRM.

The following patient- or transplantation-related variables were analyzed for their potential impact on outcome: gender, age, white blood cells at diagnosis, immunophenotype, cytogenetics, interval between diagnosis and HSCT, first-line treatment, MRD, donor type, year of transplantation, stem cell source, use of total body irradiation, aGvHD and cGvHD occurrence.

Supplementary table 1.

Univariate analyses of variables influencing the probability of DFS

Variables		No. of patients	Events	DFS probability	(95% CI)	Log rank test
Overall DFS probability		211	81	61	(54-68)	-
Gender						
	Female	73	23	68	(57-79)	
	Male	138	58	57	(49-66)	*0.13*
Age at diagnosis *(years)*						
	1-9	126	40	68	(60-76)	
	10-14	68	30	55	(43-67)	
	>15	17	11	34	(11-57)	***0.026***
Age at HSCT *(years)*						
	1-9	122	39	68	(59-76)	
	10-14	65	28	56	(44-68)	
	>15	24	14	41	(21-61)	*0.056*
WBC at diagnosis *($x10^9$/L)*						
	<50	86	30	64	(53-74)	
	50-100	20	6	70	(49-90)	
	>100	90	38	58	(48-68)	*0.36*
Immunophenotype						
	B	113	42	62	(53-71)	
	T	80	30	62.5	(52-73)	
	Unknown	18	9	50	(27-73)	*0.568*
t(9;22)						
	No	168	63	62	(55-70)	
	Yes	43	18	57	(42-72)	*0.76*
t(4;11)						
	No	197	76	61	(54-68)	
	Yes	14	5	64	(39-89)	*0.95*
Interval diagnosis-HSCT *(months)*						
	<6	62	28	55	(42-67)	
	6-12	134	51	62	(53-70)	
	>12	15	2	87	(69-100)	*0.11*
MRD						
	IR	27	7	74	(58-91)	
	HR	73	28	60	(48-72)	
	Unknown	111	46	58	(49-68)	*0.369*
Age at diagnosis by MRD *(years)*						
1-9						
	IR	10	1	90	(71-100)	
	HR	44	16	62	(47-77)	*0.12*
10-14						
	IR	15	5	67	(43-91)	
	HR	25	11	53	(32-74)	*0.59*
>15						
	IR	2	1	50	(0-100)	
	HR	4	1	75	(33-100)	*0.45*
Donor type						
	MFD	138	51	63	(55-71)	
	UD	73	30	59	(47-70)	*0.448*
Year of transplantation						
	1990-1999	69	27	61	(49-72)	
	2000-2004	58	23	60	(48-73)	
	2005-2008	84	31	63	(52-73)	*0.97*
Year of transpantation by donor type						
1990-1999						
	MFD	60	21	65	(53-77)	
	UD	9	6	33	(3-64)	*0.06*
2000-2004						
	MFD	37	13	65	(49-80)	
	UD	21	10	52	(31-74)	*0.3*
2005-2008						
	MFD	41	17	58	(43-73)	
	UD	43	14	67	(53-81)	*0.49*
Stem Cell source						
	BM	186	70	62	(55-69)	
	CB	14	5	64	(39-89)	
	PB	11	6	45	(16-75)	*0.469*
Use of TBI						
	yes	190	73	61	(54-68)	
	no	21	8	62	(41-83)	*0.94*

Acute GvHD						
	Absent	52	23	55	(41-69)	
	Grade I	67	21	68	(57-79)	
	Grade II	62	19	69	(58-81)	
	Grade III	19	9	53	(30-75)	
	Grade IV	11	9	18	(0-41)	***<0.00001***
Acute GvHD						
	Grade 0-II	181	63	65	(58-62)	
	Grade III-IV	30	18	40	(22-58)	***0.002***
Chronic GvHD						
	Absent	143	50	65	(57-73)	
	Limited	25	6	76	(59-93)	
	Extensive	27	9	66	(48-84)	*0.55*

Legend: DFS: disease-free survival; HSCT: hematopoietic stem cell transplantation; WBC: white blood cells; MRD: minimal residual disease; IR: Intermediate Risk; HR: High Risk; MFD: matched family donor; UD: unrelated donor; BM: bone marrow; PB: peripheral blood; CB: cord blood; TBI: total body irradiation; GvHD: graft-versus-host disease.

Supplementary table 2.

Univariate analyses of variables influencing the cumulative incidence of RI

Variables		No. of patients	Events	RI	(95% CI)	Log-rank P
Overall CI of relapse		211	50	24	(19-30)	-
Gender						
	Female	73	14	19	(12-31)	
	Male	138	36	26	(20-35)	*0.25*
Age at diagnosis *(years)*						
	1 - 9	126	30	24	(17-33)	
	10 - 14	68	18	27	(18-39)	
	>15	17	2	12	(3-43)	*0.43*
Age at HSCT *(years)*						
	1-9	122	29	24	(17-33)	
	10-14	65	17	26	(17-39)	
	>15	24	4	17	(7-41)	*0.60*
WBC at diagnosis ($x10^9$/L)						
	<50	86	17	20	(13-31)	
	50-100	20	3	15	(5-44)	
	>100	90	26	29	(21-40)	*0.20*
Immunophenotype						
	B	113	23	21	(14-30)	
	T	80	22	27	(19-39)	
	Unknown	18	5	28	(13-59)	*0.21*
t(9;22)						
	No	168	38	23	(17-30)	
	Yes	43	12	28	(17-45)	*0.58*
t(4;11)						
	No	197	46	23	(18-30)	
	Yes	14	4	29	(12-65)	*0.51*
Interval diagnosis-HSCT *(months)*						
	<6	62	20	32	(23-46)	
	6-12	134	30	22	(16-31)	
	>12	15	0	0	-	***0.02***
MRD						
	IR	27	5	19	(9-42)	
	HR	73	17	23	(15-35)	
	Unknown	111	28	25	(18-35)	*0.77*
Age at diagnosis by MRD						
1-9						
	IR	10	1	10	(2-64)	
	HR	44	10	23	(13-39)	*0.66*
10-14						
	IR	15	3	20	(7-55)	
	HR	25	7	28	(15-53)	*0.61*
>15						
	IR	2	1	50	(12-100)	
	HR	4	0	-	-	*0.16*
Donor type						
	MFD	138	35	25	(19-34)	
	UD	73	15	21	(13-32)	*0.43*
Years of transplantation						
	1990-1999	69	18	26	(18-39)	
	2000-2004	58	12	21	(12-34)	
	2005-2008	84	20	24	(16-35)	*0.81*
Year of transpantation by donor type						
1990-1999						
	MFD	60	16	27	(18-41)	
	UD	9	2	22	(7-75)	*0.75*
2000-2004						
	MFD	37	6	16	(8-34)	
	UD	21	6	29	(15-56)	*0.31*
2005-2008						
	MFD	41	13	32	(20-50)	
	UD	43	7	16	(8-32)	*0.11*
Stem Cell source						
	BM	186	47	25	(20-32)	
	CB	14	2	14	(4-52)	
	PB	11	1	9	(1-59)	*0.30*
Use of TBI						
	Yes	190	45	24	(18-31)	
	No	21	5	24	(11-52)	*0.99*
Acute GvHD						

	Absent	52	17	33	(22-49)	
	Grade I	67	19	28	(19-42)	
	Grade II	62	11	18	(10-30)	
	Grade III	19	3	16	(6-45)	
	Grade IV	11	0	-	-	*0.13*
Acute GvHD						
	Grade 0-I	119	36	30	(23-40)	
	Grade II-IV	92	14	15	(9-25)	***0.013***
Chronic GvHD						
	Absent	143	42	29	(23-38)	
	Limited	25	4	16	(7-39)	
	Extensive	27	4	15	(6-37)	*0.12*

Legend: RI: relapse incidence; HSCT: hematopoietic stem cell transplantation; WBC: white blood cells; MRD: minimal residual disease; IR: Intermediate Risk; HR: High Risk; MFD: matched family donor; UD: unrelated donor; BM: bone marrow; PB: peripheral blood; CB: cord blood; TBI: total body irradiation; GvHD: graft-versus-host disease.

Supplementary table 3.

Univariate analyses of variables influencing the cumulative incidence of TRM

Variables		No. of patients	Events	TRM probability	(95% CI)	Log-rank P
CI of TRM		211	31	15	(11-21)	-
Gender						
	Female	73	9	13	(7-23)	
	Male	138	22	16	(11-24)	0.467
Age at diagnosis *(years)*						
	1- 9	126	10	8	(4-15)	
	10-14	68	12	18	(11-31)	
	>15	17	9	54	(34-84)	***<0.00001***
Age at HSCT *(years)*						
	1-9	122	10	8	(5-15)	
	10-14	65	11	18	(10-30)	
	>15	24	10	42	(26-68)	***0.0002***
WBC at diagnosis *($x10^9$/L)*						
	<50	86	13	16	(10-27)	
	50-100	20	3	15	(5-43)	
	>100	90	12	13	(8-23)	*0.95*
Immunophenotype						
	B	113	19	18	(12-27)	
	T	80	8	10	(5-19)	
	Unknown	18	4	22	(9-53)	*0.165*
t(9;22)						
	No	168	25	15	(11-22)	
	Yes	43	6	15	(7-32)	0.87
t(4;11)						
	No	197	30	16	(11-22)	
	Yes	14	1	7	(1-47)	0.44
Interval diagnosis-HSCT *(months)*						
	<6	62	8	13	(7-25)	
	6-12	134	21	16	(11-24)	
	>12	15	2	13	(4-48)	*0.84*
MRD						
	IR	27	2	8	(2-29)	
	HR	73	11	17	(10-30)	
	Unknown	111	18	16	(11-25)	*0.53*
Age at diagnosis by MRD *(years)*						
1-9						
	IR	10	0	0	-	
	HR	44	6	15	(7-32)	*0.46*
10-14						
	IR	15	2	13	(4-48)	
	HR	25	4	19	(7-47)	*0.82*
>15						
	IR	2	0	0	-	
	HR	4	1	25	(5-100)	*0.52*
Donor type						
	MFD	138	16	12	(7-19)	
	UD	73	15	21	(13-32)	*0.067*
Years of transplantation						
	1990-1999	69	9	13	(7-24)	
	2000-2004	58	11	19	(11-32)	
	2005-2008	84	11	13	(8-23)	*0.7*
Years of transplantation by donor						
1990-1999						
	MFD	60	5	8	(4-19)	
	UD	9	4	44	(21-92)	***0.0043***
2000-2004						
	MFD	37	7	19	(10-37)	
	UD	21	4	19	(8-46)	*0.90*
2005-2008						
	MFD	41	4	10	(4-25)	
	UD	43	7	16	(8-32)	*0.40*
Age at diagnosis by years of transplantation						
1-9 years						
	1990-1999	52	3	6	(2-17)	
	2000-2004	26	2	8	(2-29)	
	2005-2008	48	5	10	(4.5-24)	*0.61*
10-14 years						
	1990-1999	15	4	26	(11-61)	

	2000-2004	23	3	13	(4.5-37)	
	2005-2008	30	5	16	(7.5-37)	*0.52*
>15 years						
	1990-1999	2	2	100	-	
	2000-2004	9	6	66	(42-100)	
	2005-2008	6	1	16	(3-99)	*0.03*
Stem Cell source						
	BM	186	23	13	(9-19)	
	CB	14	3	21	(8-58)	
	PB	11	5	45	(24-87)	***0.0062***
Use of TBI						
	Yes	190	28	15	(11-21)	
	No	21	3	14	(5-41)	*0.99*
Acute GvHD						
	Absent	52	6	12	(6-26)	
	Grade I	67	2	3	(1-14)	
	Grade II	62	8	13	(7-25)	
	Grade III	19	6	32	(16-61)	
	Grade IV	11	9	82	(62-100)	***<0.00001***
Acute GvHD						
	Grade 0-I	119	8	7	(4-14)	
	Grade II-IV	92	23	25	(18-36)	***0.0002***
Chronic GvHD						
	Absent	143	8	6	(3-12)	
	Limited	25	2	8	(2-30)	
	Extensive	27	5	19	(9-42)	*0.079*

Legend: TRM: transplantation-related mortality; HSCT: hematopoietic stem cell transplantation; WBC: white blood cells; MRD: minimal residual disease; IR: Intermediate Risk; HR: High Risk; MFD: matched family donor; UD: unrelated donor; BM: bone marrow; PB: peripheral blood; CB: cord blood; TBI: total body irradiation; GvHD: graft-versus-host disease.

Supplementary Table 4.

Causes of transplantation-related mortality according to the period of transplantation and the donor employed

	UD	MFD	Combined
Transplant between 1990 and 1999			
No. of deaths	**4**	**5**	**9 (100%)**
Infections	2	1	3 (35%)
GvHD	0	3	3 (35%)
Bleeding	1	1	2 (20%)
Others	1	0	1 (10%)
Transplant between 2000 and 2004			
No. of deaths	**4**	**7**	**11 (100%)**
Infections	2	2	4 (35%)
GvHD	2	3	5 (45%)
Bleeding	0	0	0 (0%)
Others	0	2	2 (20%)
Transplant between 2005 and 2008			
No. of deaths	**7**	**4**	**11(100%)**
Infections	3	2	5 (45%)
GvHD	3	2	5 (45%)
Bleeding	0	0	0 (%)
Others	1	0	1 (10%)

Legend: UD: unrelated donor; MFD: matched family donor; GvHD: graft-versus-host disease.