Ann Hematol (2012) 91:73–82
DOI 10.1007/s00277-011-1252-x

ORIGINAL ARTICLE

Results of a lymphoblastic leukemia-like chemotherapy program with risk-adapted mediastinal irradiation and stem cell transplantation for adult patients with lymphoblastic lymphoma

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Received: 9 March 2011 / Accepted: 2 May 2011 / Published online: 11 May 2011

Abstract The therapeutic role of mediastinal radiotherapy and stem cell transplantation (SCT) in lymphoblastic lymphoma (LL) remains controversial. In a risk-oriented design, we adopted a flexible treatment program in which (1) patients with persistent mediastinal abnormality, evaluated by post-induction computed chest tomography, received mediastinal irradiation; and (2) those with persistence of minimal residual disease (MRD), evaluated by MRD analysis of the bone marrow, underwent SCT. Twenty-eight out of 30 patients (T-lineage, n=24; B-lineage, n=6) achieved a complete response. Of 21 patients with mediastinal mass, 13 (62%) achieved a complete response after chemotherapy alone, while 6 (28.5%) required additional irradiation. Eleven patients were evaluated for MRD: 6 were negative and 5 positive. On the basis of MRD findings and clinical risk characteristics, 14 patients underwent SCT, 13 received maintenance chemotherapy, and 1 had local radiotherapy. Five patients

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Electronic supplementary material The online version of this article (doi:10.1007/s00277-011-1252-x) contains supplementary material, which is available to authorized users.

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relapsed. Among the 14 non-irradiated patients with T-LL, the mediastinal recurrence rate was only 7%. After a median follow-up of 3.9 years, 21 patients who responded were alive without recurrence (75%). The projected 5-year survival, disease-free survival, and relapse rate were 72%, 77%, and 18%, respectively. This program induced high remission and survival rates, indicating the feasibility and the benefits potentially associated with a selective, response-oriented policy of mediastinal irradiation and a concurrent MRD-based strategy to assign adult LL patients to SCT.

Keywords Lymphoblastic lymphoma · Mediastinal irradiation · Minimal residual disease · Stem cell transplantation

Introduction

Lymphoblastic lymphoma (LL) is a rare tumor of B or, more often, T lymphoblasts that frequently involves the mediastinum (more than 70% of cases of T-LL). It is related closely to acute lymphoblastic leukemia (ALL), from which it can be differentiated by the predominant extramedullary involvement in LL and bone marrow infiltration by blast cells of less than 25% [1, 2]. In adults, LL is treated with regimens that are used to treat ALL (ALL-type regimens) [3–9], but these regimens give inferior results compared with those achieved normally in children [10–12]. One relevant issue is the role of mediastinal tumors in the failure of treatment, and debate persists as to whether all patients should undergo mediastinal irradiation or only those with residual disease post-induction. In a German study, the rate of mediastinal tumor progression was high, despite the use of intensive chemotherapy and early mediastinal irradiation [8]. With the Hyper-CVAD regimen from the M.D. Anderson Cancer Center (MDACC), the use of higher-dose irradiation (30–39 Gy) as an adjunct seems to reduce the incidence of relapse, although mediastinal progression still occurs [9]. A second issue related to treatment is whether the identification of prognostic factors, which are used to define patients with high-risk characteristics who are eligible for hematopoietic stem cell transplantation (SCT), can be used to predict outcome. All the risk factors studied so far have failed to predict outcome with respect to current ALL-type chemotherapy regimens [3]. In ALL, the analysis of minimal residual disease (MRD) through immunophenotypic or molecular techniques, which can identify as few as 1×10^{-5} leukemic cells in normal-looking bone marrow, provides a powerful means to predict relapse accurately in individual patients [13]. Currently, the intensification of treatment with SCT is considered to be the best treatment option for patients with ALL who are MRD-positive after induction plus early consolidation therapy. With respect to LL, the prognostic role of MRD has been studied in detail in childhood [14] but not in adult disease. In the present study, we adopted an ALL-type chemotherapy program together with the flexible application of early mediastinal irradiation. Only patients with residual tumors post-induction, as assessed by a computed tomography (CT) scan of the mediastinum, received mediastinal irradiation. MRD in the bone marrow was analyzed concurrently with the mediastinal evaluation to gather evidence of occult disease contamination of the marrow. We used the information obtained as an indicator of the risk of relapse and a tool to decide between post-consolidation maintenance therapy (MRD-negative patients) and SCT (MRD-positive patients).

Patients and methods

Patients

Between the years 2000 and 2008, 30 consecutive adult patients with untreated LL were enrolled in the Northern Italy Leukemia Group (NILG) Protocol 09/00 (Table 1) [15]. The diagnosis was established by histological examination in accordance with the WHO criteria [1, 2]. The clinical evaluation included bone marrow biopsy, the examination of cerebrospinal fluid, a full blood count, and biochemistry, which included serum lactate dehydrogenase, plus CT scans of the chest and abdomen. Clinical staging was performed in accordance with the Ann Arbor System. Because of the difficulty in obtaining adequate diagnostic specimens for a detailed immunophenotypic and cytogenetic/genetic analysis for risk stratification in many LL patients, the high-risk (HR) subset included all the patients with T-LL, those with pro-B CD10-negative B-LL, and those achieving late complete remission (CR). Only the patients with pre-B CD10-positive B-LL were defined standard-risk (SR) in this study.

Analysis of MRD

Patient-specific molecular probes were identified using biopsy samples or bone marrow in patients with >5% infiltrating blast cells, and were tested for sensitivity and specificity as reported previously [15]. One or two probe(s) were used per patient, with the aim of achieving a sensitivity of at least 10^{-4} in the RQ-PCR (reverse quantitative polymerase chain reaction) assay. MRD in the bone marrow was examined at baseline and at predefined time points (TP) post-induction (before cycles 4, 6, and 8; i.e., weeks [w] 10, 16, and 22). The MRD findings were entered in an MRD risk model to distinguish between MRD-positive

Table 1 Diagnostic characteristics of the patients

Characteristics	Total (%) (n=30)	B-LL (n=6)	T-LL (n=24)	P value
Gender (male/female)	17/13	2/4	15/9	n.s.
Age (years), median (range)	27 (16–57)	26 (16–28)	32 (16–57)	0.04
Hemoglobin <12 (g/dL), no. (%)	5 (17)	1 (17)	4 (17)	n.s.
Platelets <120 ($\times 10^9$/L), no. (%)	0	0	0	–
Elevated LDH, no. (%)	18 (60)	1 (17)	17 (71)	0.01
Serum LDH (U/L), median (range)	530 (145–2,787)	298 (145–535)	620 (266–2,787)	0.006
Stage, no. (%)				
I	5 (17)	3 (50)	2 (8)	
II	8 (27)	0	8 (33)	
III	1 (3)	0	1 (4)	
IV	16 (53)	3 (50)	13 (54)	
B symptoms, no. (%)	8 (27)	0	8 (33)	n.s.
Mediastinal involvement, no. (%)	21 (70)	1 (17)	20 (83)	0.001
Bone marrow involvement, no. (%)	12 (40)	2 (33)	10 (42)	n.s.
Extranodal involvement,[a] no. (%)	14 (47)	4 (67)	10 (42)	n.s.
Pleural effusion	9	0	9 (37)	
Pericardial effusion	3	0	3 (12)	
Skin	2	2 (33)	0	
Ovary/testis	2[b]	2[b] (33)	0	
Thyroid	1	0	1 (4)	
High risk subset,* no. (%)	26 (87)	2 (33)	24 (100)	

n.s. not significant

[a] excluding mediastinum and bone marrow

[b] one each

*indicates eligibility to SCT by protocol design (modifiable by MRD study)

(MRD^{pos}) and MRD-negative (MRD^{neg}) patients (MRD^{neg}, MRD-negative at w22 and negative/low positive $<10^{-4}$ at w16; MRD^{pos}, any other MRD combination).

Early treatment (phase A) and MRD/risk-oriented therapy (phase B)

The NILG Protocol 09/00 (ClinicalTrials.gov identifier: NCT00358072) has been reported previously [15], and is detailed as Online Resource 1. Briefly, the induction plus early consolidation therapy (phase A) consisted of a prednisone–cyclophosphamide pre-phase (T-LL only) [16] followed by eight blocks of chemotherapy administered over 25 weeks, in association with central nervous system (CNS) chemo-radioprophylaxis after cycle 3. Cycles 4 and 7 included high-dose (HD) methotrexate and cytarabine. Autologous blood stem cells that had been primed with G-CSF were collected after cycle 4. Mediastinal irradiation (24 Gy planned) was delivered after cycle 2 only to patients with residual disease as confirmed by CT scan (>2 cm). Post-consolidation therapy (phase B) was MRD/risk-oriented. After completion of MRD analysis, and regardless of clinical risk class, MRD^{neg} cases were allocated to standard maintenance therapy, whereas MRD^{pos} patients were eligible to allogeneic SCT, or, when a compatible related or family unrelated donor was not found, to autologous HD therapy followed by maintenance. The HD sequence included up to four consecutive "hypercycles" with HD etoposide, 6-mercaptopurine, and melphalan (cycles 1 and 3), and HD methotrexate and cytarabine (cycles 2 and 4), each followed by the reinfusion of $1–2\times10^6$/kg CD34+ blood stem cells. The MRD^{neg} patients received a 2-year maintenance program with 6-mercaptopurine daily and methotrexate weekly, which was reinforced monthly during the first year by the addition of vincristine–prednisone (even cycles) and cyclophosphamide (odd cycles). Patients for whom the results of MRD analysis were not available were scheduled to receive phase B therapy on the basis of their clinical risk class (SCT if HR, maintenance if SR).

Response evaluation and statistics

The response to treatment was evaluated after cycle 1 in accordance with the method of Cheson et al. [17]. This included full clinical assessment, bone marrow biopsy in patients with prior involvement, and CT scans of the chest and abdomen as indicated in the protocol. CR was defined as the disappearance of any clinical manifestation of LL, as confirmed by imaging procedures, which included the recovery of normal marrow morphology (blasts <5%) in patients with infiltrating blast cells. A late CR was characterized by the achievement of a response only after cycle 2 and/or the need for mediastinal irradiation, whereas non-responsiveness indicated the persistence of LL. Overall survival (OS) was calculated from the date of diagnosis to death, and disease-free survival (DFS) from the date of CR

to relapse at any site or death in CR. The cumulative incidence of relapse (CIR) was calculated from the date of CR to recurrence. The DFS and survival curves were plotted by the Kaplan–Meier method and compared by the log-rank test.

Results

Patient characteristics

As shown in Table 1, the median age of the patients was 27 years, 17 patients were male, 80% had T-LL, and 70% presented with a mediastinal mass. Infiltration of the bone marrow by LL was documented in 40%, whereas no patient had CNS involvement. After the diagnostic work-up, 13 patients (43%) were classified as stage I/II (n=5/8) and 17 (57%) as stage III/IV (n=1/16).

Treatment response and mediastinal irradiation

Twenty-eight patients (93%) achieved CR and 2 had refractory T-LL (Table 2). Of the 21 patients with an enlarged mediastinum, 13 (62%) entered CR, and 8 (38%) had a residual tumor as defined by CT scan. Seven of the latter eight patients were irradiated and six achieved CR. All patients who achieved CR underwent successful collection of blood stem cells (median CD34+ cells 13.3×10^6/kg, range 2–55.3).

Analysis of MRD

Molecular probes for MRD-based risk definition were identified in 11 of 14 patients studied (Table 3). In 3 patients, no specific probe was detectable, and in 14 other CR patients, no diagnostic specimen was available for probe generation. A molecular probe was obtained successfully in one patient with normal marrow morphology (UPN 289). This finding was not inconsistent with the study methods, because rearrangements of the T-cell receptor gene can be detected with as few as 1% lymphoblasts [18]. The sensitivity of the probe was $\geq 10^{-4}$ in six patients (54.5%). However, given that low-sensitivity probes can be used to detect high levels of MRD, all patients were studied, principally to define those at greater risk of relapse. Interestingly, two of five patients with normal bone marrow on the basis of standard morphological criteria showed a positive PCR signal at baseline (TP0), which indicated subclinical dissemination of tumor cells into the marrow. Eventually, six patients were classified as MRD^{neg} (55%) and five as MRD^{pos} (45%). Four of the five MRD^{pos} patients were defined as such by the use of low-sensitivity probes, as opposed to only one of the six MRD^{neg} patients. Autologous blood stem cells collected after cycle 4 were available for MRD analysis in seven patients. Apheretic products were MRD^{neg} in all three MRD^{neg} patients, and MRD^{pos} in two of four MRD^{pos} patients (below quantitative range in one, Table 3).

MRD and risk-oriented therapy

All six MRD^{neg} patients received maintenance therapy. Four of the five MRD^{pos} patients received SCT, whereas one underwent maintenance therapy owing to the presence of a psychiatric disorder. Among the 17 patients with undefined MRD status, 15 received phase B therapy and 2 did not because of refusal and medical reasons, respectively. Phase B consisted of an allograft or the HD autologous therapy in 9 out of 12 patients classified HR (75%), and maintenance in 2 of 3 patients classified SR (67%). Maintenance and HD autologous therapy were instead prescribed to the remaining three HR and one SR patients, respectively, based on patient and/or physician's choice. Eventually, the protocol adherence rate for phase B was 75% (21 out of 28 patients), in relation to the MRD study results and the clinical risk classification in 10 and 11 patients, respectively.

Table 2 Outcome to induction therapy according to LL subtype, presence of mediastinal mass, and mediastinal irradiation

Outcome	T-LL		B-LL		ALL		
	Med+(n=20)	Med−(n=4)	Med+(n=1)	Med−(n=5)	Med+(n=21)	Med−(n=9)	Total (n=30)
CR, no. (%)	18 (90)	4 (100)	1 (100)	5 (100)	19 (90.5)	9 (100)	28 (93)
After CHT	13 (65)	4 (100)	0	5 (100)	13 (62)	9 (100)	22 (73)
After CHT plus MRT	5 (25)	–	1 (100)	–	6 (28.5)	–	6 (20)
Refractory, no. (%)	2 (10)	0	0	0	2 (9.5)	0	2 (7)
After CHT	1 (5)	–	–	–	1 (4.75)	0	1 (3.5)
After CHT plus MRT	1 (5)	–	–	–	1 (4.75)	0	1 (3.5)

CR complete remission, *Med* mediastinal mass, *CHT* chemotherapy, *MRT* mediastinal radiation therapy (computed tomography-oriented)

Table 3 MRD study and clinical outcome

Patient UPN (age/sex)	Diagnosis[a] (stage)	Bone marrow (% blast cells)	MRD study			MRD study results					MRD risk class	Treatment	Outcome
			Sample	Probe(s)	Probe sensitivity	TP0	TP1	TP2	TP3	PB			
241 (36/M)	T-LL (IV)	Abn (10)	BM	TCRD	10^{-4}	pos	neg	neg	neg	NA	MRD^{neg}	CHT	A/W (61+)
				TCRD	10^{-3}	pos	neg	neg	neg				
909 (25/M)	T-LL (IV)	Abn (10)	BM	TCRD	10^{-5}	pos	neg	neg	neg	NA	MRD^{neg}	CHT	A/W (21+)
57 (43/F)	T-LL (I)	N	Med	TCRD	10^{-5}	NA	neg	neg	neg	neg	MRD^{neg}	CHT	A/W (89+)
74 (36/F)	T-LL (II)	N	PL	TCRG	10^{-3}	NA	neg	neg	neg	neg	MRD^{neg}	CHT	A/W (99+)
913 (23/M)	T-LL (II)	N	LN	TCRB	10^{-4}	pos	neg	neg	neg	neg	MRD^{neg}	CHT	A/W (42+)
				TCRB	10^{-3}	pos	neg	neg	neg	neg			
104 (46/M)	T-LL (IV)	Abn (16)	LN	TCRG	10^{-5}	NA	low–pos	NA	neg	NA	MRD^{neg}	CHT	D (35,4)[b]
				TCRD	10^{-5}	NA	low–pos	NA	neg				
12 (22/M)	T-LL (IV)	Abn (15)	BM	TCRG	10^{-3}	pos	NA	pos	pos	low–pos*	MRD^{pos}	CHT	A/W (112+)
331 (17/M)	T-LL (IV)	N	PL	TCRG	10^{-4}	NA	NA	pos	neg	pos	MRD^{pos}	Allo-SCT	A/W (50+)
				TCRG	10^{-3}	NA	NA	neg	neg	neg			
289 (30/M)	T-LL (I)	N	BM	TCRB	10^{-3}	pos	pos	pos	pos	neg	MRD^{pos}	Allo-SCT	A/W (56+)
375 (38/F)	T-LL (IV)	Abn (22)	BM	TCRG	10^{-3}	pos	pos	pos	pos	NA	MRD^{pos}	Allo-SCT	REL/D (19)
				TCRG	10^{-2}	pos	pos	pos	pos				
22 (16/F)	B-LL (IV)	Abn (10)	BM	TCRG	10^{-3}	pos	NA	pos	pos	neg	MRD^{pos}	HD-auto	REL/D (29)

N normal, *Abn* abnormal, *BM* bone marrow, *Med* mediastinum, *LN* lymphnode, *PL* pleural effusion, *TCR* T-cell receptor (*B* beta, *G* gamma, *D* delta) gene rearrangement, *pos* positive $\geq 10^{-4}$, *low–pos* positive $<10^{-4}$; *neg* negative, *NA* not available, *PB* peripheral blood stem cell harvest (*positivity below quantitative range) MRD^{neg}, MRD-negative; MRD^{pos}, MRD-positive, *CHT* chemotherapy, *allo-SCT* allogeneic stem cell transplantation, *HD-auto* high-dose therapy ("hypercycles") with autologous support, *A/W* alive and well in first complete remission, *REL* relapse, *D* death (survival in months)

[a] T-LL or B-LL

[b] Patient died of secondary AML

Duration of remission and survival

With a median follow-up period of 3.9 years (range, 0.8–9.2+ years), 22 patients (73%) who responded were alive at the time of writing: 21 in first CR and 1 in second CR. Post-remission failure was due to recurrence (n=5, 18%) in either the bone marrow (B-LL, n=1; T-LL, n=3; one irradiated and two non-irradiated patients) or mediastinum (n=1, non-irradiated T-LL patient), or to other malignancy (n=2, 7%; acute myeloid leukemia [AML] and large B-cell lymphoma, respectively). Of the five patients who relapsed, four died and one is alive in second CR after allogeneic SCT. The estimated OS rate at 5 years was 72%, 83% for B-LL, and 69% for T-LL. The 5-year DSF rate was 77%, 83% for B-LL, and 71% for T-LL; the CIR probability at 5 years was 18% (Fig. 1).

Prognostic analysis

The probability of OS and DFS was not affected significantly by any of the factors examined (Table 4). The achievement of a molecular remission was associated with a better outcome; DFS at 5 years increased from 60% in the MRD^{pos} group to 80% in the MRD^{neg} group, although the difference was not statistically significant owing to the small number of patients (Fig. 2). The DFS was 83% and 81% in patients who received allogeneic SCT and maintenance therapy, respectively, and 50% in those who underwent the HD autologous phase. The CIR was 8% and 17% in the groups who received maintenance therapy and allogeneic SCT, respectively, compared with 37% in the group given HD autologous therapy. The outcome of the 11 MRD-studied patients is detailed in Table 3. The outcome of the 12 HR patients treated outside the MRD study varied according to cell subset and treatment. Of the ten T-LL patients, three fared well following allogeneic SCT, six received HD autologous therapy (with one CR death, two relapses, and three patients alive and well), and the last one relapsed after maintenance. Both HR B-LL patients did well on maintenance, and all three SR B-LL patients experienced a prolonged DFS (two maintenance, one HD autologous therapy).

Discussion

We analyzed the long-term outcome of 30 adult patients with LL who were enrolled prospectively in the NILG study 09/00. LL is rare in adult patients, the most recent prospective series dealing with 27–34 total patients (a

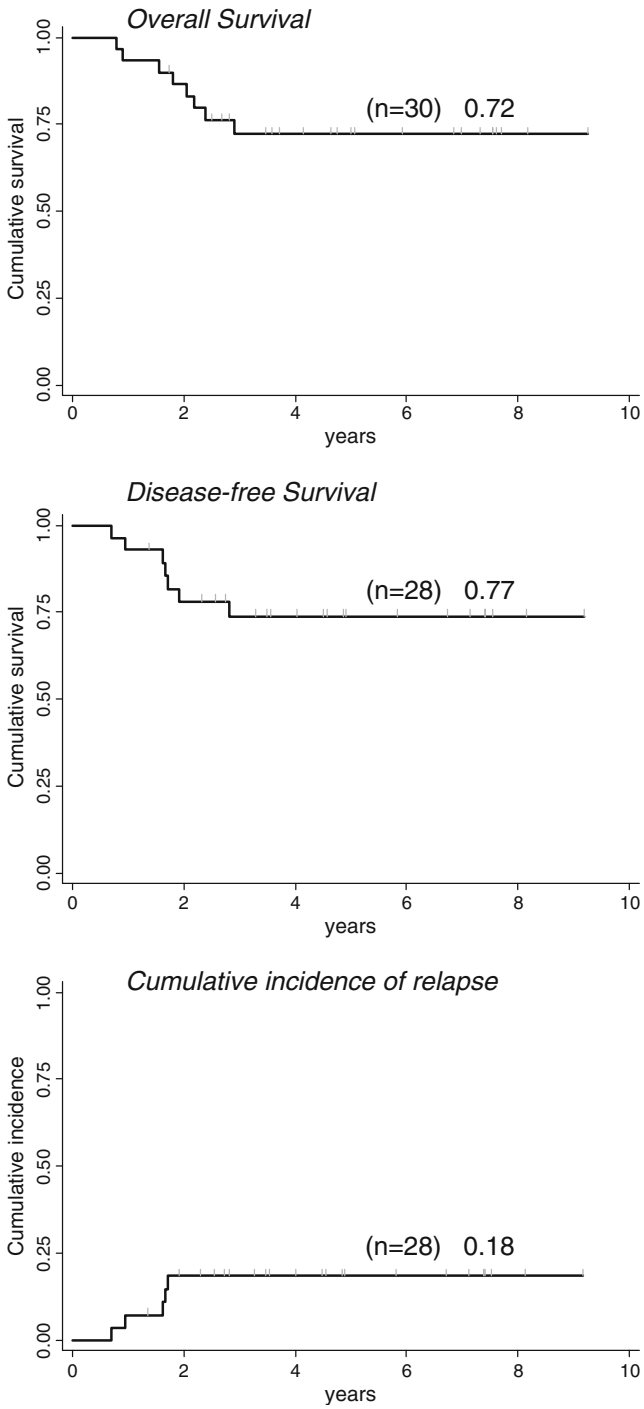

Fig. 1 Kaplan–Meier estimates of overall survival, disease-free survival, and cumulative incidence of relapse (probability at 5 years is indicated)

figure comparable to our study), with only a single study from Germany including exceptionally 45 patients [8, 9, 19, 20]. In the NILG program, induction chemotherapy was integrated with early mediastinal irradiation in patients with suboptimal tumor regression, whereas the molecular analysis of MRD was used for the first time in adult LL to decide between SCT and standard maintenance for post-induction therapy. The general results reflected the high rate of CR that can be attained in LL with ALL-like regimens [4–6, 8–12] in contrast to regimens for non-Hodgkin lymphoma [7, 21].

The rate of 93% for CR and the projected 5-year OS and DFS rates of 72% and 77%, respectively, compare favorably with those obtained in recent German and MDACC studies, which reported rates of 91–93% for CR and 5-year DFS rates between 62% and 66% [8, 9]. Although ALL-like induction regimens can be associated with pronounced morbidity, none of our patients died early, during induction chemotherapy, as compared with 8% of patients with ALL who were treated with the same schedule [15]. Two patients (7%) did die in CR from other neoplasms. Remission mortality in LL has been reported previously to range from 5% to 9%, which included secondary AML [8, 9]. The median ages for the patients in the German and MDACC series were 25 and 28 years, respectively, which were similar to the median age in our study (27 years). The distribution of other clinical characteristics was also similar, and no patient in our series had CNS disease, as in the German study but in contrast to the MDACC study (9%). In the present study, all patients received prophylactic cranial irradiation (18 Gy) plus 12 triple intrathecal injections, and none suffered from CNS relapse. In studies that employed five to eight intrathecal injections with or without cranial irradiation, the rate of CNS relapse was <5% [8, 9]. The lowest rate of CNS relapse (1.8%) was observed in one study that employed higher-dose methotrexate (5 g/m^2 instead of 1–1.5 g/m^2) plus intrathecal prophylaxis [22].

In LL, a key therapeutic issue is how mediastinal tumors, which are both the cause and the site of early recurrence, can be managed best [3]. In the current series, 20 out of 24 patients with T-LL (83%) had mediastinal involvement, but only a proportion of them (n=8, 35%) had residual disease post-induction, as confirmed by a positive CT scan, that required additional radiation therapy to increase the overall response rate in this patient cohort from 62% to 82%. Given that mediastinal recurrence occurred in only 1 out of 14 non-irradiated patients in CR (7%) to confirm the adequacy of the present risk-adapted consolidation regimen (with or without SCT) in patients with chemosensitive mediastinal disease, we recommend the use of selective irradiation on the basis of the early CT result. The omission of mediastinal irradiation in patients who have been identified as responsive to chemotherapy through the CT scan would reduce the delay in the application of chemotherapy, which is theoretically of benefit in patients with extensive extranodal and marrow involvement. Moreover, long-term cardio-pulmonary complications of mediastinal irradiation could be avoided in a significant

Table 4 Univariate prognostic analysis

	Overall survival			Disease-free survival		
Prognostic factors	No.	Probability at 5 years	*P* value	No.	Probability at 5 years	*P* value
Age, years						
≥30	13	0.67	0.69	12	0.73	0.88
<30	17	0.75		16	0.74	
Gender						
Male	17	0.62	0.19	15	0.63	0.26
Female	13	0.85		13	0.85	
Phenotype						
B	6	0.83	0.49	6	0.83	0.57
T	24	0.69		22	0.71	
Mediastinum						
Not involved	9	0.60	0.65	9	0.60	0.54
Involved	21	0.76		19	0.79	
Mediastinum involved						
With effusion[a]	9	0.89	0.29	8	0.87	0.50
Without effusion	12	0.67		11	0.73	
LDH						
Normal	11	0.61	0.43	10	0.67	0.74
Abnormal	18	0.78		17	0.76	
Clinical stage						
I/II	13	0.85	0.20	13	0.77	0.81
III/IV	17	0.62		15	0.70	
Bone marrow						
Not involved	18	0.83	0.13	17	0.82	0.25
Involved	12	0.54		11	0.59	

[a] pleural and/or pericardic

proportion of younger patients, more than 50% according to our results [23]. To improve this strategy, positron emission tomography (PET) could be used to optimize the evaluation of residual mediastinal disease in T-LL [24] and refine the decision criteria for irradiation. In the German study, 41 out of 45 patients with T-LL (91%) had mediastinal involvement, and all received radiation therapy at a dose of 24 Gy [8]. Seven out of 42 patients who achieved CR (17%) suffered a relapse in the mediastinum. As a result, the radiotherapy dose was increased to 36 Gy in the subsequent trial; the results of which are awaited. Notably, a regimen for childhood LL that avoided irradiation and used a higher dose of methotrexate (5 g/m^2) resulted in a low rate of mediastinal relapse (7%) [11]. Leukemic T-lymphoblasts are exquisitely sensitive to methotrexate concentrations of approximately 65 μmol/l, which are achievable in vivo with 5 g/m^2 methotrexate infusions [25, 26]. As a consequence, this type of systemic intensification therapy should be evaluated further for the optimal prevention of mediastinal recurrence in T-LL.

Prognostic factors for adult LL are poorly understood. In our series, immunophenotype and clinical stage did not have a significant effect, although survival was slightly better in patients with B- rather than T-LL, as reported previously [27, 28], and in patients with stage I/II (85%) rather than stage III/IV (62%) disease. Given that, in patients with ALL, MRD is the best available means to predict relapse and to select high-risk patients for allogeneic SCT [15], we elected to investigate the use of this parameter in adult LL. Up to 30% of patients with LL are at risk of failing to respond to the chemotherapy regimen used [3]; hence, we looked for persistent signs of MRD in the bone marrow, which denote the chemo-resistant dissemination of LL and an increased risk of relapse. The protocol was designed such that patients who were found to be positive for MRD were switched from standard chemotherapy to allogeneic SCT (or alternatively to HD therapy with autologous stem cell support). Past studies have confirmed the therapeutic efficacy of SCT in adults with LL [19, 29, 30], with survival rates of 55–72%, which almost overlap with the best results obtained with chemotherapy. Although allogeneic SCT might no longer be a routine choice in the current era of improved chemotherapy results [3, 8, 9], it remains the most effective treatment modality for acute lymphoid malignancies beyond CR1 [31]. Hence, it is appropriate to

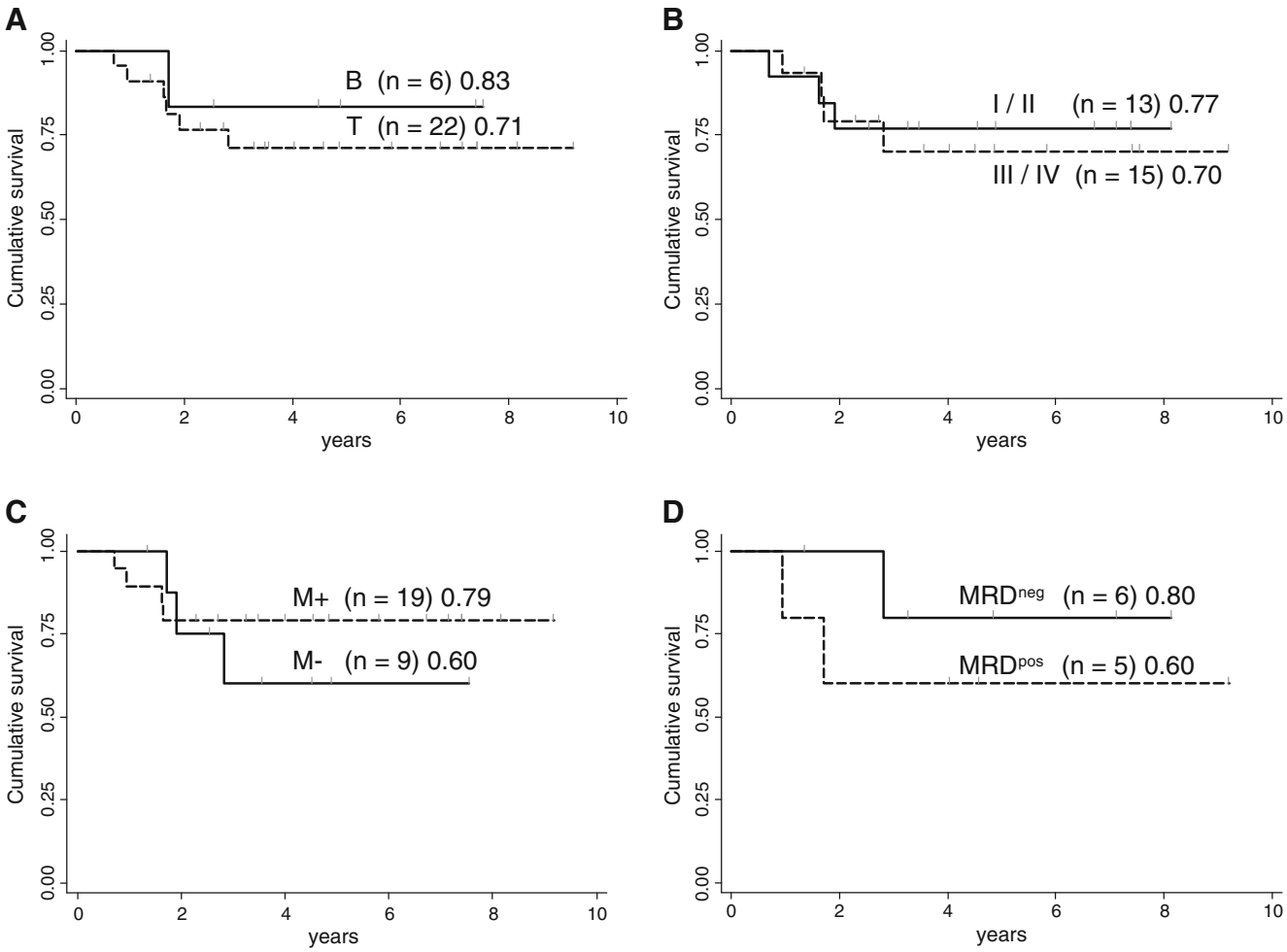

Fig. 2 Kaplan–Meier estimates of disease-free survival according to (**a**) immunophenotype, (**b**) clinical stage, (**c**) mediastinal involvement (M+/−, present/absent), and (**d**) MRD risk classification (probability at 5 years is indicated)

consider allogeneic SCT in MRD-positive patients, who are at the highest risk of relapse.

Only a small proportion (39%) of the patients with LL underwent MRD analysis, in contrast to 79% of the adult patients with ALL who were treated concurrently with the same protocol [15]. In many cases, this can be explained by the lack of an adequate biopsy specimen, together with the low incidence of bone marrow involvement (40% of the patients, all with blast cells <25%), which, by itself, precluded the identification and testing of probes. Aside from this, the survival of the MRD^{neg} patients, who were treated with chemotherapy, was better than that of the MRD^{pos} patients, whereas MRD^{pos} patients who received allogeneic SCT fared better than those who were unable to have an allograft. Thus, it appears, in keeping with the rationale of the study, that the analysis of MRD allows the early identification of the relatively few patients with LL who are highly likely to fail chemotherapy, and in whom SCT can have a greater chance of success. In this regard, patients with MRD levels $\geq 10^{-4}$ after early consolidation have the highest risk of recurrence [13, 15]. Low-sensitivity probes ($\geq 10^{-3}$), which are unsuitable for the confirmation of a negative status for MRD, are nonetheless useful to detect high levels of residual disease, and should be employed to identify candidates suitable for salvage therapy by SCT. Given that multiparametric flow cytometry allows the degree of marrow contamination by LL cells to be evaluated in almost all patients with a sensitivity of at least 10^{-4} [14], this technique should be integrated with, if not substituted for, the molecular analysis of MRD in future studies. The analysis of MRD also confirmed that bone marrow of normal morphology can harbor LL cells at diagnosis. Although this concept is not new [32], and has been emphasized recently in childhood T-LL [14], these findings underline the importance of the analysis of MRD during the diagnostic process. This analysis should increase the accuracy of clinical staging even in patients with early-stage disease and without apparent bone marrow involvement. While SCT was a successful choice in MRD^{pos} patients, standard consolidation therapy plus maintenance

resulted in a high chance of cure (DFS 80% at 5 years) in the MRD^{neg} subset. Extending this observation in a large patient number could also allow to assess the need for prolonged maintenance therapy, that could not be even necessary in T-LL patients who achieve an early mediastinal response and maintain an MRD^{neg} status. This perspective should be best evaluated with the support of CT/PET analysis and an optimized MRD monitoring.

The prospective NILG study confirmed the favorable therapeutic outcome of an ALL-type regimen in adults with LL. This treatment, in combination with response-oriented mediastinal irradiation, reduced the incidence of mediastinal recurrence to only 4.5% (1/22 patients with T-LL in CR). Concurrently, the molecular analysis of MRD allowed patients at higher risk of progression to be identified, which both improved the classification of clinical risk and offered the best rationale for the selective application of SCT. The combination of CT/PET scans with flow cytometry analysis of MRD to assess response in the two most critical sites for LL, the mediastinum and bone marrow, could provide another advance in the management of adult LL.

Acknowledgments We are grateful to the following colleagues who contributed to this study: A. Gallamini (Cuneo), E. Angelucci (Cagliari), and K. Aprile (Milan), Italy. The work was supported by grants from AIRC (Associazione Italiana per la Ricerca sul Cancro), Milan, and AIL (Associazione Italiana Leucemia contro le Leucemie) sezione "Paolo Belli", Bergamo, Italy.

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