Non-myeloablative conditioning with allogeneic hematopoietic cell transplantation for the treatment of high-risk acute lymphoblastic leukemia

Ron Ram,[1] Rainer Storb,[1,2] Brenda M. Sandmaier,[1,2] David G. Maloney,[1,2] Ann Woolfrey[1,2] Mary E. D. Flowers,[1,2] Michael B. Maris,[3] Ginna G. Laport,[4] Thomas R. Chauncey,[2,5] Thoralf Lange,[6] Amelia A. Langston,[7] Barry Storer,[1,2] and George E. Georges[1,2]

[1]Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [2]University of Washington School of Medicine, Seattle, WA, USA; [3]Rocky Mountain Cancer Center, Denver, CO, USA; [4]Stanford University, Stanford, CA, USA; [5]Veterans Affairs Puget Sound Health Care System, Seattle, WA, USA; [6]University of Leipzig, Leipzig, Germany, and [7]Emory University, Atlanta, GA, USA

Acknowledgments: we thank the research nurses Michelle Bouvier and Hsien-Tzu Chen and data manager Gresford Thomas for their invaluable help in this study; Helen Crawford, Bonnie Larson, and Sue Carbonneau for manuscript preparation; and especially the patients and their families, the transplantation teams, physicians, nurses, long-term follow-up team and support personnel for their dedicated care of patients in this study.

Funding: this work was supported by grants from the National Institutes of Health, Bethesda, MD (grants P01CA018029, P01CA078902, and P30CA015704). RR was a recipient of a fellowship award from the Davidoff Foundation.

Manuscript received on January 7, 2011. Revised version arrived on March 29, 2011. Manuscript accepted on April 11, 2011.

Correspondence: George E. Georges, M.D., Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, D1-100; Seattle, WA, 98109 USA.
Phone: international +1.206.6676886.
Fax: international +1.206.6676124.
E-mail: ggeorges@fhcrc.org

ABSTRACT

Background
Allogeneic hematopoietic cell transplantation is a potentially curative treatment for patients with acute lymphoblastic leukemia. However, the majority of older adults with acute lymphoblastic leukemia are not candidates for myeloablative conditioning regimens. A non-myeloablative preparative regimen is a reasonable treatment option for this group. We sought to determine the outcome of non-myeloablative conditioning and allogeneic transplantation in patients with high-risk acute lymphoblastic leukemia.

Design and Methods
Fifty-one patients (median age 56 years) underwent allogeneic hematopoietic cell transplantation from sibling or unrelated donors after fludarabine and 2 Gray total body irradiation. Twenty-five patients had Philadelphia chromosome-positive acute lymphoblastic leukemia. Eighteen of these patients received post-grafting imatinib.

Results
With a median follow-up of 43 months, the 3-year overall survival was 34%. The 3-year relapse/progression and non-relapse mortality rates were 40% and 28%, respectively. The cumulative incidences of grades II and III-IV acute graft-*versus*-host disease were 53% and 6%, respectively. The cumulative incidence of chronic graft-*versus*-host disease was 44%. Hematopoietic cell transplantation in first complete remission and post-grafting imatinib were associated with improved survival (P=0.005 and P=0.03, respectively). Three-year overall survival rates for patients with Philadelphia-negative acute lymphoblastic leukemia in first remission and beyond first remission were 52% and 8%, respectively. For patients with Philadelphia chromosome-positive acute lymphoblastic leukemia in first remission who received post-grafting imatinib, the 3-year overall survival rate was 62%; for the subgroup without evidence of minimal residual disease at transplantation, the overall survival was 73%.

Conclusions
For patients with high-risk acute lymphoblastic leukemia in first complete remission, non-myeloablative conditioning and allogeneic hematopoietic cell transplantation, with post-grafting imatinib for Philadelphia chromosome-positive disease, can result in favorable long-term survival. *(Clinicaltrials.gov identifier: NCT0036738)*

Key words: acute lymphoblastic leukemia, Philadelphia chromosome-positive, allogeneic hematopoietic cell transplantation, non-myeloablative conditioning, imatinib.

Citation: Ram R, Storb R, Sandmaier BM, Maloney DG, Woolfrey A, Flowers MED, Maris MB, Laport GG, Chauncey TR, Lange T, Langston AA, Storer B, and Georges GE. Non-myeloablative conditioning with allogeneic hematopoietic cell transplantation for the treatment of high-risk acute lymphoblastic leukemia. Haematologica 2011;96(8):1113-1120. doi:10.3324/haematol.2011.040261

Introduction

Allogeneic hematopoietic cell transplantation (HCT) with a myeloablative conditioning regimen is an established potentially curative treatment for patients with high-risk acute lymphoblastic leukemia (ALL).[1,2] However, many older adults with ALL are not candidates for high-dose conditioning and HCT.[3,4] The use of non-myeloablative conditioning with fludarabine and low-dose total body irradiation can substantially decrease the toxicity of the preparative regimen and extends the possible use of allogeneic HCT to older or medically infirm patients.[5,6] This approach relies primarily on potent graft-*versus*-leukemia effects to prevent relapse of the disease. Other reduced intensity regimens have been reported by investigators for the treatment of patients with ALL.[3,7-9] However, relapse has remained a major problem following reduced intensity conditioning regimens.

In recent years, imatinib mesylate, and subsequently the newer tyrosine kinase inhibitors, dasatinib and nilotinib, combined with chemotherapy were found to be very effective for inducing disease remission in patients with Philadelphia chromosome-positive (Ph+) ALL.[10-13] Imatinib therapy after allogeneic HCT was well tolerated and improved relapse-free survival following myeloablative conditioning compared to that in historical controls not given imatinib therapy after HCT.[14] A recent study showed that patients with Ph+ ALL who received induction chemotherapy with imatinib followed by myeloablative conditioning and allogeneic HCT for Ph+ ALL in first complete remission had a better overall survival compared to patients who did not undergo HCT.[15]

Here we report on the multicenter experience with allogeneic HCT following non-myeloablative conditioning with fludarabine and 2 Gray (Gy) total body irradiation for patients with high-risk ALL. We identify risk factors for disease relapse and mortality. We also describe the causes of non-relapse mortality and the toxicity and efficacy of post-HCT imatinib for patients with Ph+ ALL.

Design and Methods

Eligibility

This analysis includes 51 consecutive patients with ALL who were prospectively enrolled and received non-myeloablative conditioning followed by allogeneic HCT on sequential multi-institutional protocols between February 1, 2000 and July 30, 2009. The protocols were registered as National Cancer Institute clinical trials. Patients treated with post-grafting imatinib were registered in NCT00036738. Other patients were enrolled in sequential protocols specific for donor type and with minor variations in planned duration of post-grafting immunosuppression. Patients were treated at six centers with the Fred Hutchinson Cancer Research Center (FHCRC) in Seattle (WA, USA) acting as the coordinating center. All patients signed informed consent forms approved by the local institutional review boards.

Patients with related or unrelated donors were eligible for non-myeloablative conditioning if they were older than 55 or 50 years, respectively. Younger patients were eligible if they had high-risk ALL and co-morbid conditions that excluded them from myeloablative conditioning or if they had disease relapse after a preceding myeloablative HCT. Adult high-risk ALL was defined as beyond first complete remission, or first complete remission and at least one of the following: (i) age greater than 35 years, (ii) white blood cell count greater than 30×10^9/L at diagnosis for B-cell ALL or greater than 100×10^9/L at diagnosis for T-cell ALL, or (iii) Ph+ ALL with t(9;22).[2] Pediatric high-risk ALL was defined as beyond first complete remission, or first complete remission and the addition of one of the following: (i) failure to achieve complete remission after the induction phase; (ii) t(9;22) or t(4;11) clonal abnormalities; and (iii) poor response to prednisone in T-cell ALL with a white blood cell count greater than 100×10^9/L.[16]

Pre-transplant characteristics

Patients referred for HCT in first complete remission had had a median of three cycles (range, 3-4) of various standard induction/intensification chemotherapy regimens.[17-19] Pre-transplant disease status was assessed within the 21 days prior to HCT. Complete remission was defined according to standard morphological criteria as outlined by the International Working Group.[20] For patients in complete remission, minimal residual disease was assessed by multiparametric flow cytometry (minimum four-color, cut-off level to establish minimal residual disease positivity ≥ 0.01%), karyotype analysis (G-banding) and fluorescence *in situ* hybridization (FISH). Quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) of p210 and p190 *BCR/ABL* mRNA was not part of the work-up for minimal residual disease in all of the patients with Ph+ ALL; thus, PCR results were not included in assessment of minimal residual disease. All patients and donors had high resolution HLA-allele level typing performed for ten HLA alleles (HLA-A, B, C, DRB1 and DQB1). Patients received grafts from the following donors: HLA identical siblings (n=9), 10/10 HLA-allele matched unrelated donors (n=31), single HLA allele mismatched unrelated donors (n=6) and single HLA-antigen mismatched unrelated donors (n=5). Pre-transplant comorbidities were assessed retrospectively, using the HCT comorbidity index (HCT-CI).[21]

Conditioning regimen and treatment plan

The conditioning regimen consisted of fludarabine (30 mg/m^2/day on days -4 through -2 before HCT and 2 Gy total body irradiation given at a dose of 0.07 to 0.1 Gy/min from linear accelerator sources on day 0.[6] Patients received unmanipulated granulocyte colony-stimulating factor-mobilized peripheral blood stem cells shortly after total body irradiation. One patient received an unrelated bone marrow graft instead of peripheral blood stem cells. Post-grafting immunosuppression consisted of combined mycophenolate mofetil and a calcineurin inhibitor, cyclosporine or tacrolimus, as previously described.[5,22-25]

All patients received intrathecal methotrexate (12 mg/dose or 6 mg/dose if given via an Ommaya reservoir) for central nervous system prophylaxis, two doses pre-transplant (once per week) and six doses post-transplant (starting on day +30, once every 2 weeks). All men received 16 Gy of testicular irradiation in eight fractions during conditioning. All patients with a history of central nervous system disease, three with Ph– and four with Ph+ ALL, received cranio-spinal irradiation as part of the conditioning regimen.

The mean infused dose of peripheral blood stem cells was 8.5×10^6 CD34$^+$ cells/kg body weight (range, 0.9×10^6-24.4×10^6). Engraftment and donor chimerism were measured by variable number tandem repeat of microsatellite markers at days 28, 56 and 84 after HCT.

Acute and chronic graft-*versus*-host disease (GVHD) were assessed as described previously.[6,26] Toxicities occurring within the first 100 days were scored using the Common Terminology Criteria for Adverse Events v3.0. Disease response after HCT was monitored with standard marrow morphology, flow cytometry and conventional cytogenetics, FISH and, if indicated, PCR for

BCR-ABL transcripts.[27] Disease responses were assessed 1, 3, 6, 12 and 24 months after HCT and/or as clinically indicated.

Patients with Philadelphia chromosome-positive acute lymphoblastic leukemia

Twenty-five patients had the Ph+ cytogenetic abnormality detected at diagnosis. After the introduction of imatinib, 18 patients enrolled in a study evaluating the safety and efficacy of incorporating post-grafting imatinib into the treatment regimen. Patients were initiated on imatinib at a dose of 600 mg orally once daily either by their referring physician or at the transplant center before enrollment on the study. Imatinib was stopped on day –2 before HCT to avoid interaction with engraftment of donor hematopoietic cells. Imatinib was recommenced at a dose of 400-600 mg daily after HCT when the absolute neutrophil count was greater than 0.5×10^9/L or on day +15 if there was no neutropenia. Imatinib was continued for at least 1 year after HCT, unless there was toxicity or disease progression; all patients received imatibib for at least 1 month. Dose reduction of imatinib was allowed for mitigation of side effects/ toxicities.

Causes of death

In patients who relapsed or progressed with ALL, relapse/progression was listed as the primary cause of death regardless of other associated events. Relapse was defined as recurrence of malignancy based on one or more of the following parameters: marrow morphology, flow cytometry, cytogenetic studies, including FISH, or RT-PCR for *BCR/ABL* transcripts. All deaths occurring in the absence of relapse/progression were considered non-relapse mortality.

Statistical analysis

Data were analyzed as of October 1, 2010. Overall survival was estimated using the Kaplan-Meier method. Cumulative incidence estimates were calculated for acute and chronic GVHD, relapse and non-relapse mortality. Death was treated as a competing risk in the analyses of relapse/progression and acute and chronic GVHD. Relapse/progression was treated as a competing risk when analyzing non-relapse mortality. Cox regression was used for univariate analyses of risk factors for all time-to-event end points. For each analysis, hazard ratios (HR) and 95% confidence intervals (95% CI) are given together with *P* values for comparisons with the reference category. All *P* values are derived from likelihood ratio statistics and are two-sided.

Results

Fifty-one patients underwent allogeneic HCT. All had high-risk ALL including 19 who were beyond first complete remission. Six patients were under 18 years of age (1 in first complete remission and 5 beyond first complete remission). Table 1 summarizes the patients' characteristics. Twenty-five patients had Ph+ ALL. Of these, 18 received post-grafting imatinib (Table 2). The median follow-up for surviving patients was 43 (range, 14-98) months.

Engraftment

Fifty patients achieved sustained donor engraftment. One patient with Ph+ ALL had non-fatal primary graft rejection. This patient received an HLA matched unrelated marrow graft with a dose of 0.9×10^6/kg CD34+ cells. After graft rejection, this patient was treated with imatinib, chose not to undergo a second HCT, relapsed and died 20 months after HCT. The median donor CD3+ T-cell chimerism levels for the 50 peripheral blood stem cells recipients at day 28 and day 84 were 79 (range, 15-100)% and 88 (range, 48-100)%, respectively. The chimerism and engraftment patterns were not different in patients treated with imatinib.

Graft-versus-host disease

By day 120 after HCT, 53% of patients had developed grade II acute GVHD and 6% had developed grades III-IV acute GVHD (Figure 1A). Among the patients who received HLA-identical sibling, HLA-allele-matched unrelated donor, and HLA-allele/antigen mismatched unrelated grafts, the overall incidences of grade II-IV acute GVHD were 33%, 50% and 91%, respectively. The cumulative incidence of chronic extensive GVHD at 3 years was 42% (Figure 1B).

Among the patients with Ph+ ALL treated with post-grafting imatinib (n=18), ten (56%) developed grade II-IV acute GVHD. There was no significant difference between the incidences of acute GVHD among patients who received or did not receive imatinib (HR=0.65, 95% CI 0.3-1.4; *P*=0.25). Ten of the 18 patients (56%) developed chronic extensive GVHD and five (28%) developed limited chronic GVHD. There was no significant difference in the incidence of chronic GVHD among patients who received or did not receive imatinib (HR=1.4, 95% CI 0.6-3.3; *P*=0.45). Five patients (36%) developed chronic skin GVHD while receiving imatinib treatment. Discontinuation of imatinib after HCT was not associated with new onset or exacerbation of chronic GVHD.

Table 1. Characteristics of ALL patients, disease and transplantation.

Characteristics	Ph- ALL (*n*=26)	Ph+ ALL (*n*=25)
Median age: years (range)	56 (8-65)	57 (38-69)
Disease status at time of HCT: *n*, (%)		
CR1 without MRD	12 (46%)	13 (52%)
CR1 with MRD	1 (4%)	6 (24%)
>CR1 (CR2/CR3)	13 (50%)	5 (20%)
Persistent disease	0	1 (4%)
Months from diagnosis to HCT: median, (range)		
CR1	7.7 (4-10.7)	7.6 (4.4-10.9)
Beyond CR1	30.6 (10.7-90.7)	38.7 (8.9-126.1)
History of myeloablative HCT (%)	4 (15%)	2 (8%)
HCT-CI[1] (%)		
0-1	9/17 (53%)	14/18 (78%)
≥2	8/17 (47%)	4/18 (22%)
Recipient gender (male/female)	11/15	16/9
Female donor to male recipient: (%)	5 (19%)	6 (24%)
Donor type: (%)		
HLA-identical sibling	4 (15%)	5 (20%)
Unrelated HLA matched	14 (54%)	17 (68%)
1 HLA allele mismatched	3 (12%)	3 (12%)
1 HLA antigen mismatched	5 (19%)	0
Cell dose × 10^6 CD34+ cells/kg: median, (range)	8.8 (2-20.2)	8.2 (0.9-24.4)
Cell source (marrow/PBSC)	0/26	1/25

ALL: acute lymphoblastic leukemia, CR1: first complete remission, HCTCI: hematopoietic cell transplantation comorbidity index, MRD: minimal residual disease, PBSC: peripheral blood stem cells, Ph: Philadelphia chromosome. [1]Data were available for 17 Ph– ALL patients and for 18 Ph+ ALL patients.

Non-relapse mortality

The rate of non-relapse mortality at 3 years after HCT among all patients was 28% (Figure 1C). The following causes of death were included under non-relapse mortality: GVHD (n=4), GVHD-associated infections (n=8), sepsis (n=1), congestive heart failure (n=1) and suicide (n=1). There was no significant difference in non-relapse mortality between patients who were or were not treated with post-HCT imatinib (HR=0.5, 95% CI 0.2-1.5; *P*=0.20). The four deaths in the imatinib group were due to GVHD-associated infections: bacterial pneumonia, sepsis with pancreatitis, respiratory syncytial virus pneumonia and community-acquired H1N1 viral pneumonia.

Disease relapse/progression

Among the 51 patients, 22 (43%) had relapse/progression at a median of 5 (range, 0.3 to 58) months after HCT. None of the patients developed isolated central nervous system relapse. None was treated with donor lymphocyte infusion for disease relapse. The median time from the diagnosis of relapse to death was 4 (range, 0.3-15) months in the 20 patients who had died by the time of analysis.

Overall, the 3-year estimated probability of relapse/progression was 40%. Univariate analysis identified risk factors for relapse/progression (Table 3). Patients beyond first complete remission at the time of HCT had a significantly increased risk for relapse after HCT compared to those in first complete remission (HR=3.9, 95% CI 1.6-9.5, *P*=0.002). For Ph– ALL (n=26), the 3-year estimated relapse rate for patients in or beyond first complete remission was 15% and 62%, respectively (Figure 2). For Ph+ ALL (n=25), the 3-year estimated relapse rate for patients in and beyond first complete remission was 32% and 67%, respectively (Figure 2). Two patients with molecular evidence of disease after transplantation were included as having disease relapse. For patients with Ph+ ALL, evi-

Table 2. Characteristics of Ph+ ALL patients treated with imatinib after HCT.

Patient #	Time from Dx to HCT (months)	Therapy pre HCT	Additional cytogenetic abn. pre-HCT	Status at HCT	MRD at HCT, assessed by	Duration of imatinib post-HCT (months)	GVHD while on imatinib	Chronic GVHD after stopping imatinib	Alive/ dead	Disease status at last follow-up/ cause of death	Overall survival since HCT (months)
1	6	HyperCVAD	no	CR1	no	11	no	n/a	dead	Rel	13
2	9	HyperCVAD	no	CR1	yes- FCM	8	yes	n/a	dead	NRM	28
3	6	Larson	no	CR1	no	50	yes	n/a	alive	CR	73
4	7	HyperCVAD	yes	CR1	no	11	yes	n/a	dead	NRM	12
5	5	HyperCVAD	no	CR1	no	24	yes	no	alive	CR	77
6	5	HyperCVAD	yes	CR1	no	6	yes	n/a	dead	Rel	8
7	10	GMALL	yes	CR1	no	4	yes	n/a	alive	CR	90
8	4	HyperCVAD	no	CR1	no	24	no	no	alive	PCR Relapse –CR on dasatinib	62
9	8	HyperCVAD	no	CR1	no	45	yes	n/a	alive	CR	62
10	9	HyperCVAD	yes	CR1	yes-FCM	3	yes	n/a	dead	Rel	5
11	5	HyperCVAD	yes	CR1	no	18	yes	n/a	alive	CR	33
12	9	Linker	no	CR1	no	12	no	yes	alive	CR	39
13	8	HyperCVAD	yes	CR1	no	3	no	yes	alive	CR	32
14	82	HyperCVAD, HCT, HyperCVAD	yes	CR2	yes-FCM	14	yes	n/a	alive	PCR Relapse – CR on nilotinib	27
15	12	HyperCVAD	no	CR2	yes-FISH	10	yes	n/a	dead	NRM	10
16	11	HyperCVAD, MEI	yes	CR2	yes-FCM	16	no	no	dead	Rel	16
17	91	SWOG 9400, HCT, HyperCVAD, HIDAC, DA	yes	CR3	no	6	yes	n/a	dead	NRM	7
18	10	HyperCVAD	no	Relapse	yes-Histo	14	yes	n/a	dead	Rel	14

All patients except UPN 10 had imatinib therapy prior to HCT. UPN 16 had central nervous system (CNS) disease prior to HCT. None of the patients had CNS disease relapse after HCT. Two patients (UPN 8 and 14) had disease relapse after HCT detected by PCR and flow cytometry and are currently alive in molecular complete remission (CR). UPN 8 discontinued imatinib 24 months after HCT, had disease relapse detected by PCR for the BCR/ABL *p190 transcript at 43 months. This patient was retreated with imatinib, achieved a molecular CR within 2 months and remained PCR negative for 12 months. At 57 months after HCT, molecular relapse was again detected by BCR/ABL p190 transcript and flow cytometry of the bone marrow with 0.35% aberrant blasts, and therapy was changed to dasatinib. Molecular CR was achieved within 1 month and the patient has remained in molecular CR for 5 months. UPN14 had molecular relapse detected by PCR for* BCR/ABL *p190 transcript and flow cytometry 0.002% at 17 months after HCT. This patient was treated with nilotinib, achieved a molecular CR within 2 months and has remained in molecular CR for 8 months. Additional cytogenetic abnormalities at any time prior to allogeneic HCT: UPN4: complex (+7, +8, -11, -12, der 19), UPN6: complex (+2, +5,+10,+16,+18,+19,+21,+X), UPN7:, t(9;14), UPN10: (+8,+22), UPN11:(-7), UPN13:(-7), UPN14: complex(+2, -9,-11,-Y, inv7p13, +13p11), UPN16: complex (+5,+8,+8,+13,+14,+20), UPN17: complex (-7,-8,-13,-16,+11). Twelve patients were initiated/maintained on imatinib 600 mg daily, six patients were initiated/maintained on imatinib 400 mg daily (UPN 9, 11, 12, 13,16, 17). UPN 2, 5 and 12 had subsequent imatinib dose reductions to 200-400 mg daily. Ph+ ALL: Philadelphia chromosome positive acute lymphoblastic leukemia; HCT: hematopoietic cell transplantation; Dx: diagnosis; MRD: minimal residual disease; CNS: central nervous system; GVHD: graft* versus *host disease; HyperCVAD: hyper fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone; GMALL: German multicenter ALL regimen; MEI: mitoxantrone, etoposide, ifosfamide; DA: daunorubicin, ara-c; HIDAC: high dose ara-c; CR: complete remission; FISH: fluorescent* in situ *hybridization; FCM: flow cytometry; Histo: conventional histology. n/a: not applicable.*

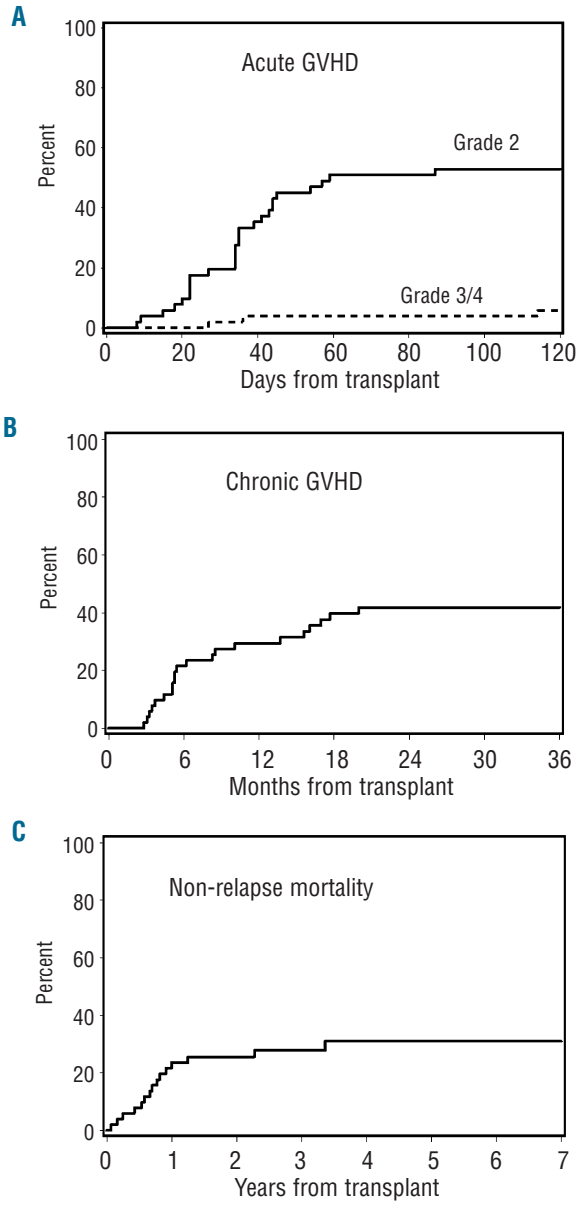

Figure 1. Cumulative incidences (n=51) of (A) acute graft-*versus*-host disease (GVHD): 53% grade II, 6% grade III-IV; (B) chronic extensive GVHD: 42% incidence at 3 years; (C) non-relapse mortality, 28% incidence at 3 years.

dence of additional cytogenetic abnormalities at diagnosis was associated, at a borderline level, with an increased risk of relapse after HCT (HR=3.4, 95% CI 0.9-13; *P*=0.06).

Survival

The estimated 3-year overall survival rate among the 51 patients was 34%. Relapse was the primary cause of death (n=20, 57% of all deaths). Univariate analysis performed for the entire cohort identified the disease status of being beyond first complete remission as the only significant factor associated with increased mortality (HR=2.7, 95% CI 1.4-5.3; *P*=0.005) (Table 3). Other factors (donor source, acute or chronic GVHD) were not significantly associated with increased mortality. The 3-year overall survival rates for patients with Ph– ALL in and beyond first complete remission were 52% and 8%, respectively, (HR=3.4, 95% CI 1.3-9.1; *P*=0.01) (Figure 3A). Excluding the six pediatric patients, the 3-year overall survival rates for patients in and beyond first complete remission were 48% and 0%, respectively (HR=4.5, 95% CI 1.4-15.1, *P*=0.01).

Table 3. Prognostic factors for relapse and mortality using univariate analysis.

	Relapse HR (95% CI)	*P*	Mortality HR (95% CI)	*P*
Entire cohort (n=51)				
Beyond CR1	3.9 (1.6-9.5)	0.002	2.7 (1.4-5.3)	0.005
Matched URD (vs. sibling)	1.1 (0.3-3.9)	0.86	0.6 (0.2-1.3)	0.16
Acute GVHD[1]	0.5 (0.2-1.2)	0.11	0.9 (0.4-1.7)	0.69
Chronic GVHD[1]	0.7 (0.2-2.3)	0.53	1.0 (0.5-2.2)	0.98
Ph+ ALL (n=26)				
Beyond CR1	2.4 (0.7-8.6)	0.20	1.8 (0.6-5.4)	0.32
Additional cytogenetic abnormalities	3.4 (0.9-13)	0.06	2.0 (0.7-5.5)	0.19
Treatment with imatinib	0.4 (0.1-1.5)	0.20	0.3 (0.1-0.9)	0.03

ALL: acute lymphoblastic leukemia, CI: confidence interval, Beyond CR1: disease stage greater than first complete remission, GVHD: graft-versus-host disease, HR: hazard ratio, Ph: Philadelphia chromosome, URD: unrelated donor. [1]Analyzed as a time-dependent covariate.

Among patients with Ph+ ALL, treatment with post-HCT imatinib was associated with significantly decreased mortality (HR=0.3, 95% CI 0.1-0.9; *P*=0.03) (Table 3). The 3-year overall survival rates for patients with Ph+ ALL who were in first complete remission or beyond first complete remission were 47% and 17%, respectively; however, this difference did not reach statistical significance (HR=1.8, 95% CI 0.6-5.4; *P*=0.32). For patients in first complete remission who received post-grafting imatinib, the 3-year overall survival rate was 62% (Figure 3B, Table 2); for the subgroup that had no evidence of minimal residual disease at HCT, the overall survival rate was 73%. Additional cytogenetic abnormalities (other than Ph+) detected at diagnosis, showed a trend to be associated with increased mortality (HR=2.0, 95% CI 0.7-5.5; *P*=0.19).

Outcomes of patients under 18 years old

Six patients with Ph– ALL were younger than 18 years at the time of HCT: these patients had a median age of 11 (range, 8-16) years. Four had relapsed after a prior allograft, one was in first complete remission and one in third complete remission. Two patients received HLA-antigen mismatched unrelated grafts. All five patients beyond first complete remission relapsed (3 of them within 6 months). All patients developed acute GVHD. The single patient in first complete remission is currently alive at 88 months post-HCT.

Outcomes of patients aged over 60 years

Sixteen patients (31%) were older than 60 years at the time of HCT: the median age of these patients was 63 (range, 61-69) years. Six of the 16 (38%) patients died from causes other than relapse and four (25%) had disease relapse. Of the nine patients with Ph– ALL, two in first complete remission are currently alive after more than 2.1 years. Among patients with Ph+ ALL (n=7), the estimated 3-year overall survival rate was 57%.

Imatinib toxicity

The median duration of post-HCT imatinib treatment was 11.5 (range, 3-50) months (Table 2). The drug was given at a daily dose ranging between 200 mg and 600 mg.

Dose modifications were made in three patients. In general, imatinib was well tolerated. Three patients (17%) discontinued imatinib because of adverse events, all of which were reversible (two cases of gastrointestinal toxicity and one of recurrent pleural effusion).

Discussion

Despite improvements in therapy, mortality from high-risk ALL has not decreased substantially in older patients. Two large prospective trials and meta-analyses summarizing the results of the previous controlled trials showed that allogeneic HCT after myeloablative conditioning improved the outcome of adult patients with high-risk ALL.[2,28-31] However, in patients with high-risk disease, a survival advantage was demonstrated only up to 35 years of age.[2,32] Developing HCT approaches for older or medically infirm patients with ALL has remained challenging. While the outcome for patients not undergoing allogeneic HCT is very poor, those who proceed with myeloablative conditioning followed by HCT have an unacceptably high rate of non-relapse mortality.[2,33] This multicenter study addressed the problem of non-relapse mortality in older and medically infirm patients by using a non-myeloablative conditioning regimen consisting of fludarabine and 2 Gy total body irradiation which depended on allogeneic graft-*versus*-leukemia effects for curing high-risk ALL.

We observed that overall survival was significantly improved for patients who underwent HCT early in the course of their disease. For Ph– ALL patients in first complete remission, the 3-year overall survival rate was 52%, while that for patients beyond first complete remission was only 8%, primarily due to increased disease relapse. Other investigators who used various reduced intensity regimens and allogeneic HCT have reported 2 to 3-year overall survival rates ranging from 20%-61%.[3,7-9,34] The heterogeneous study outcomes might have been due to differences in disease status and characteristics of the HCT donors. The positive impact of early allogeneic HCT in first complete remission was reported by Mohty *et al.* and Bachanova *et al.*[3,7] Our study confirms both this finding and the poor overall survival of patients beyond first complete remission. Furthermore, the sustained survival plateau among patients in first complete remission demonstrates that durable, long-term disease-free survival may be achieved for a majority of Ph+ ALL patients treated with imatinib as well as for Ph– ALL patients. Thus, in patients for whom allogeneic HCT is considered as post-remission therapy, it should be recommended early rather than late in the course of the disease.

The most striking finding of our study was the favorable overall survival rate of 47% at 3 years for Ph+ patients in first complete remission given imatinib after HCT. The overall survival rate was 73% for those Ph+ patients in first complete remission without minimal residual disease at HCT. Relapse was increased in Ph+ patients: (i) with additional cytogenetic abnormalities, (ii) beyond first complete remission, and (iii) in first complete remission with minimal residual disease at HCT. The latter two factors were not statistically significant probably because of the inclusion of patients before the availability of imatinib. Age did not appear to limit the feasibility of our treatment protocol. For example, Ph+ ALL patients older than 60 years had a 3-year overall survival rate of 57%.

Incorporation of imatinib and newer tyrosine kinase inhibitors into the various phases of the treatment for Ph+ ALL patients has reshaped the therapeutic algorithm. Thomas *et al.* showed that the incorporation of imatinib into induction chemotherapy resulted in a 2-year overall survival of 56%, which included patients who proceeded to myeloablative allogeneic HCT.[35,36] A single center study showed that incorporation of imatinib into induction

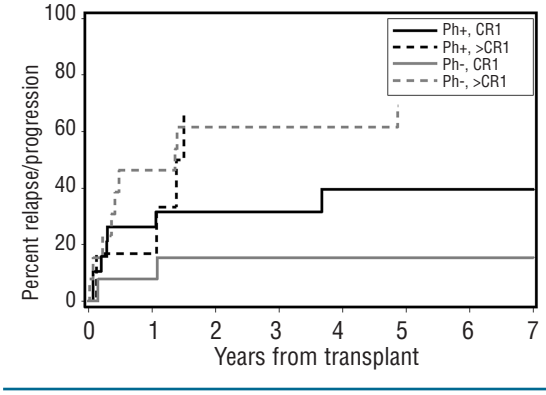

Figure 2. Cumulative relapse rate for Ph– ALL, in first complete remission (CR1) (n=13) *versus* beyond CR1 (n=13) and Ph$^+$ ALL CR1 (n=19) *versus* beyond CR1 (n=6). Molecular disease relapse (PCR or flow cytometry positive) without morphological evidence of disease was included as relapse.

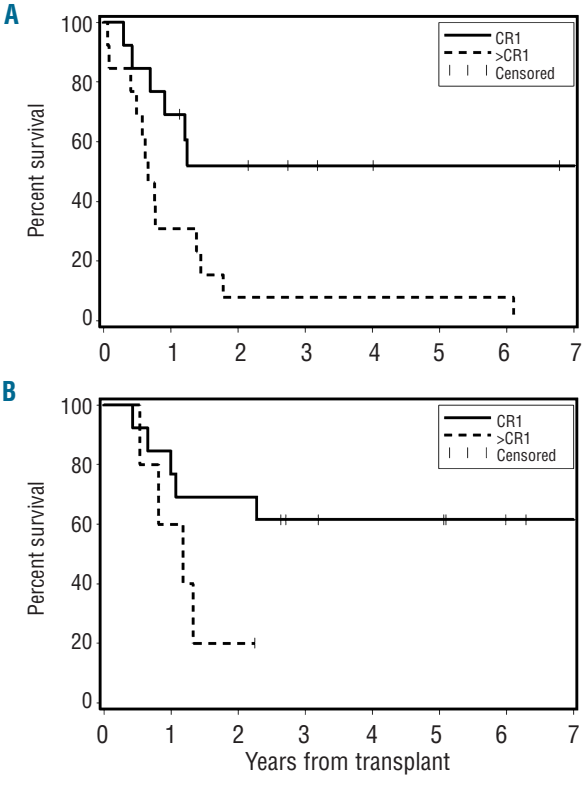

Figure 3. Overall survival for (A) Ph$^-$ ALL, in first complete remission (CR1) (n=13) *versus* beyond CR1 (n=13) and (B) Ph$^+$ ALL patients receiving imatinib after hematopoietic cell transplantation, CR1 (n=13) *versus* beyond CR1 (n=5).

chemotherapy with subsequent myeloablative conditioning and allogeneic HCT resulted in a 3-year overall survival rate of 78%.[37] Currently, data for patients treated with tyrosine kinase inhibitors and not proceeding to allogeneic HCT are scarce. A recent phase II trial showed a 2-year estimated overall survival rate of 64% for patients receiving only dasatinib in addition to induction and maintenance chemotherapy.[38] While these results are encouraging, a median follow-up of 14 months in this cohort is probably too short to enable recommendation of alternative approaches to allogeneic HCT. Thus, it is possible that the major role of tyrosine kinase inhibitors, both in medically "fit" and in medically infirm or older patients, is to allow a greater proportion of patients to receive allogeneic HCT. Consequently, particularly in the setting of non-myeloablative HCT, the use of tyrosine kinase inhibitors post-transplant appears to provide a sufficient level of disease control until the development of a graft-*versus*-leukemia effect.

In accordance with published data for imatinib after myeloablative HCT,[14] we showed that imatinib was safe in the context of non-myeloablative allogeneic HCT and was generally well tolerated. In contrast to recent publications that suggested a role for imatinib in the prevention and treatment of chronic skin GVHD,[39,40] we did not observe a significant decrease in the incidence of chronic GVHD, nor did we detect an increase in the incidence of chronic GVHD after the discontinuation of imatinib, although the number of patients in our study was limited. The optimal duration of post-HCT imatinib therapy is unknown. In our study, patients were treated with imatinib for varying periods, according to preferences of the physicians and patients. Two patients were successfully treated with a second-generation tyrosine kinase inhibitor after detection of molecular relapse. Although the follow-up for these two patients was limited, the findings suggest that close follow-up of patients is necessary and that molecular complete remission can be achieved after detection of molecular relapse. Until data from larger cohorts are available, we would cautiously recommend continuing treatment with tyrosine kinase inhibitors indefinitely unless there is evidence of either toxicity or disease relapse.

Although the incidence of acute GVHD was high, most cases were grade II and only 6% of patients developed grades III-IV acute GVHD. In accordance with previous publications, the highest incidence of acute GVHD occurred in patients receiving HLA-mismatched grafts.[24] In contrast to a previous study, we did not observe an inverse correlation between chronic GVHD and relapse.[41] In part, this could be explained by the fact that relapse in our cohort occurred early after HCT (at a median of 5 months) particularly among patients with disease beyond first complete remission.

Since ALL is relatively rare in adults, our study was limited by the relatively small number of patients and the fact that enrollment occurred over a nearly 10-year period. Nonetheless, we showed that non-myeloablative conditioning and allogeneic HCT is a very feasible and effective treatment option for high-risk ALL patients in first complete remission. For Ph+ ALL patients in first complete remission, imatinib should be given after HCT since it appears to improve overall survival. Our results suggest that non-myeloablative conditioning and allogeneic HCT is a potentially curative treatment option for older or medically infirm patients with high-risk ALL in first complete remission. For patients beyond first complete remission, post-HCT maintenance with novel agents should be explored and studies addressing the role of next generation tyrosine kinase inhibitors given after HCT are warranted.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Laport GG, Alvarnas JC, Palmer JM, Snyder DS, Slovak ML, Cherry AM, et al. Long-term remission of Philadelphia chromosome-positive acute lymphoblastic leukemia after allogeneic hematopoietic cell transplantation from matched sibling donors: a 20-year experience with the fractionated total body irradiation-etoposide regimen. Blood. 2008;112(3):903-9.
2. Goldstone AH, Richards SM, Lazarus HM, Tallman MS, Buck G, Fielding AK, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). Blood. 2008;111(4):1827-33.
3. Mohty M, Labopin M, Tabrizzi R, Theorin N, Fauser AA, Rambaldi A, et al. Reduced intensity conditioning allogeneic stem cell transplantation for adult patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. Haematologica. 2008;93(2):303-6.
4. Storb R. Can reduced-intensity allogeneic transplantation cure older adults with AML? Best Pract Res Clin Haematol. 2007; 20(1):85-90.
5. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001;97(11):3390-400.
6. Maris MB, Niederwieser D, Sandmaier BM, Storer B, Stuart M, Maloney D, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood. 2003;102(6):2021-30.
7. Bachanova V, Verneris MR, DeFor T, Brunstein CG, Weisdorf DJ. Prolonged survival in adults with acute lymphoblastic leukemia after reduced-intensity conditioning with cord blood or sibling donor transplantation. Blood. 2009;113(13):2902-5.
8. Martino R, Giralt S, Caballero MD, Mackinnon S, Corradini P, Fernandez-Aviles F, et al. Allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning in acute lymphoblastic leukemia: a feasibility study. Haematologica. 2003;88(5):555-60.
9. Shimoni A, Rand A, Hardan I, Shem-Tov N, Stanevsky A, Yerushalmi R, et al. Allogeneic hematopoietic stem-cell transplantation in adult acute lymphoblastic leukemia: better outcome in transplantation during first complete remission and with myeloablative conditioning [abstract]. Blood. 2008;112(11):1131-2, abst. #3295.
10. Yanada M, Takeuchi J, Sugiura I, Akiyama H, Usui N, Yagasaki F, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. J Clin Oncol. 2006;24(3):460-6.
11. de Labarthe A, Rousselot P, Huguet-Rigal F, Delabesse E, Witz F, Maury S, et al. Imatinib combined with induction or con-

solidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the GRAAPH-2003 study. Blood. 2007;109 (4):1408-13.
12. Ottmann O, Dombret H, Martinelli G, Simonsson B, Guilhot F, Larson RA, et al. Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a phase 2 study. Blood. 2007; 110(7):2309-15.
13. Kantarjian H, Giles F, Wunderle L, Bhalla K, O'Brien S, Wassmann B, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med. 2006;354(24):2542-51.
14. Carpenter PA, Snyder DS, Flowers MED, Sanders JE, Gooley T, Martin PJ, et al. Prophylactic administration of imatinib after hematopoietic cell transplantation for high-risk Philadelphia chromosome-positive leukemia. Blood. 2007;109(7):2791-3.
15. Hatta Y, Mizuta S, Ohtake S, Sugiura I, Ueda Y, Kanamori H, et al. Promising outcome of imatinib-combined chemotherapy followed by allogeneic hematopoietic stem cell transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the Japan Adult Leukemia study Group (JALSG) Ph+ALL202 regimen. Blood. 2009;114(22): 1201-2, abst. #3090.
16. Balduzzi A, Valsecchi MG, Uderzo C, De Lorenzo P, Klingebiel T, Peters C, et al. Chemotherapy versus allogeneic transplantation for very-high-risk childhood acute lymphoblastic leukaemia in first complete remission: comparison by genetic randomisation in an international prospective study. Lancet. 2005;366(9486):635-42.
17. Kantarjian H, Thomas D, O'Brien S, Cortes J, Giles F, Jeha S, et al. Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD), a dose-intensive regimen, in adult acute lymphocytic leukemia. Cancer. 2004;101(12):2788-801.
18. Larson RA, Dodge RK, Burns CP, Lee EJ, Stone RM, Schulman P, et al. A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. Blood. 1995;85(8): 2025-37.
19. Linker C, Damon L, Ries C, Navarro W. Intensified and shortened cyclical chemotherapy for adult acute lymphoblastic leukemia. J Clin Oncol. 2002;20(10): 2464-71.
20. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003; 21(24):4642-9.
21. Sorror M, Storer B, Sandmaier BM, Maloney DG, Chauncey TR, Langston A, et al. Hematopoietic cell transplantation-comorbidity index and Karnofsky performance status are independent predictors of morbidity and mortality after allogeneic nonmyeloablative hematopoietic cell transplantation. Cancer. 2008;112(9):1992-2001.
22. Burroughs L, Mielcarek M, Leisenring W, Sandmaier BM, Maloney DG, Baron F, et al. Extending postgrafting cyclosporine decreases the risk of severe graft-versus-host disease after nonmyeloablative hematopoietic cell transplantation. Transplantation. 2006;81(6):818-25.
23. Giaccone L, McCune JS, Maris MB, Gooley TA, Sandmaier BM, Slattery JT, et al. Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. Blood. 2005;106(13):4381-8.
24. Nakamae H, Storer BE, Storb R, Storek J, Chauncey TR, Pulsipher MA, et al. Low-dose total body irradiation and fludarabine conditioning for HLA class I-mismatched donor stem cell transplantation and immunologic recovery in patients with hematologic malignancies: a multicenter trial. Biol Blood Marrow Transplant. 2010; 16(3):384-94.
25. Maris MB, Sandmaier BM, Storer BE, Maloney DG, Shizuru JA, Agura E, et al. Unrelated donor granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell transplantation after nonmyeloablative conditioning: the effect of postgrafting mycophenolate mofetil dosing. Biol Blood Marrow Transplant. 2006; 12(4):454-65.
26. Przepiorka D, Weisdorf D, Martin P, Klingemann H-G, Beatty P, Hows J, et al. 1994 Consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15(6):825-8.
27. Cazzaniga G, d'Aniello E, Corral L, Biondi A. Results of minimal residual disease (MRD) evaluation and MRD-based treatment stratification in childhood ALL. Best Pract Res Clin Haematol. 2002;15(4):623-38.
28. Cornelissen JJ, van der Holt B, Verhoef GE, van't Veer MB, van Oers MH, Schouten HC, et al. Myeloablative allogeneic versus autologous stem cell transplantation in adult patients with acute lymphoblastic leukemia in first remission: a prospective sibling donor versus no-donor comparison. Blood. 2009;113(6):1375-82.
29. Fielding AK, Rowe JM, Richards SM, Buck G, Moorman AV, Durrant IJ, et al. Prospective outcome data on 267 unselected adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia confirms superiority of allogeneic transplantation over chemotherapy in the pre-imatinib era: results from the International ALL Trial MRC UKALLXII/ECOG2993. Blood. 2009;113(19): 4489-96.
30. Yanada M, Matsuo K, Suzuki T, Naoe T. Allogeneic hematopoietic stem cell transplantation as part of postremission therapy improves survival for adult patients with high-risk acute lymphoblastic leukemia: a metaanalysis. Cancer. 2006;106(12):2657-63.
31. Ram R, Gafter-Gvili A, Vidal L, Paul M, Ben-Bassat I, Shpilberg O, Raanani P. Management of adult patients with acute lymphoblastic leukemia in first complete remission: systematic review and meta-analysis. Cancer. 2010;116(14):3447-57.
32. Goldstone AH, Rowe JM. Transplantation in adult ALL. Hematology Am Soc Hematol Educ Program. 2009:593-601.
33. Pulte D, Gondos A, Brenner H. Improvement in survival in younger patients with acute lymphoblastic leukemia from the 1980s to the early 21st century. Blood. 2009;113(7):1408-11.
34. Stein AS, Palmer JM, O'Donnell MR, Kogut NM, Spielberger RT, Slovak ML, et al. Reduced-intensity conditioning followed by peripheral blood stem cell transplantation for adult patients with high-risk acute lymphoblastic leukemia. Biol Blood Marrow Transplant. 2009;15(11):1407-14.
35. Thomas DA, Faderl S, Cortes J, O'Brien S, Giles FJ, Kornblau SM, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. Blood. 2004;103 (12):4396-407.
36. Thomas DA, Kantarjian HM, Ravandi F, Faderl S, Garcia-Manero G, Wierda W, et al. Long-term follow-up after frontline therapy with the hyper-CVAD and imatinib mesylate regimen in adults with Philadelphia (Ph) positive acute lymphocytic leukemia (ALL). Blood. 2007;110(11):10a-11a, abst. #9.
37. Lee S, Kim YJ, Min CK, Kim HJ, Eom KS, Kim DW, et al. The effect of first-line imatinib interim therapy on the outcome of allogeneic stem cell transplantation in adults with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood. 2005;105(9):3449-57.
38. Ravandi F, O'Brien S, Thomas D, Faderl S, Jones D, Garris R, et al. First report of phase II study of dasatinib with hyperCVAD for the frontline treatment of patients with Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia. Blood. 2010;116(12):2070-7.
39. Magro L, Mohty M, Catteau B, Coiteux V, Chevallier P, Terriou L, et al. Imatinib mesylate as salvage therapy for refractory sclerotic chronic graft-versus-host disease. Blood. 2009;114(3):719-22.
40. Olivieri A, Locatelli F, Zecca M, Sanna A, Cimminiello M, Raimondi R, et al. Imatinib for refractory chronic graft-versus-host disease with fibrotic features. Blood. 2009;114(3):709-18.
41. Baron F, Maris MB, Sandmaier BM, Storer BE, Sorror M, Diaconescu R, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. J Clin Oncol. 2005;23(9):1993-2003.