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Allogeneic stem cell transplantation after fludarabine, melphalan and thymoglobulin followed by early withdrawal of prophylactic immunosuppression in patients with acute lymphoblastic leukemia – update of single center study

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Presented are updated results of allogeneic hematopoietic stem cell transplantations (HSCTs) in 25 adult patients with acute lymphoblastic leukemia (ALL) in complete remission (CR) after a reduced intensity conditioning (RIC) combining fludarabine (150 mg/m^2) and melphalan (140 mg/m^2) with thymoglobulin (4.5 mg/kg or recently 4.0 mg/kg) followed by early initiation of reduction and withdrawal of prophylactic posttransplant immunosuppression. The median post-transplant follow-up was 32 (range, 4-87) months. Stable engraftment of donor's hematopoiesis was achieved in all patients. Acute graft versus host disease (GVHD) as well as the chronic one were equally observed in four cases (16%). Five patients (20%) relapsed with ALL in the median of 9 (range, 3-15) months after HSCT. During the above post-transplant follow-up, 4 recipients (16%) died. Disease progression and posttransplant complications were the cause of death in three (12%) and one (4%) of them, respectively. The probabilities of 2-year event-free (EFS) and overall survival (OS) were 70.3% (95% CI 51.9-88.7%) and 86.1% (95% CI 71.6-100%), respectively. Presented study confirmed our previously reported promising results and this approach may be considered as an alternative to traditional HSCTs performed in high-risk patients with ALL.

Key words: acute lymphoblastic leukemia, allogeneic hematopoietic stem cell transplantation, fludarabine, melphalan, thymoglobulin, graft-versus-host disease prophylaxis

Allogeneic HSCT still remains an important treatment modality in patients with ALL [1-3]. Transplantations after standard myeloablative conditionings may reduce the risk of post-transplant relapse but significant regimen-related toxicity (RRT) and non-relapse mortality (NRM) also negatively influence the outcome of patients. The lower cytoreductive potential of less toxic RICs results in insufficient suppression of leukemic clone [4]. Therefore standard approaches with post-transplant immunosuppressive prevention of GVHD (withdrawal is usually initiated aproximately on day +100 with the stop two or three months later) should not be used after reduced intensity HSCTs. Graft-versus-leukemia (GVL) reaction should be augmented as early as possible to achieve an immunological control of ALL and to avoid its recurrence after transplantation with RIC.

We previously reported the pilot results achieved on the small cohort of 13 patients with high-risk ALL in the first CR allografted after RIC regimen combining fludarabine (150 mg/m^2), melphalan (140 mg/m^2) and "*in vivo*" T-cell depletion with antithymocyte globulin (ATG; thymoglobulin) at a total dose of 4.5 mg/kg [5]. Any "*in vivo*" T-depletion (including alemtuzumab) reducing the risk of GVHD, potentially triggered by melphalan associated gastrointestinal regimen related toxicity (RRT) [6-8], was prospectively compensated by the early withdrawal of prophylactic immunosuppression to maintain GVL effect. Promissing results with two observed relapses and no death were obtained in that pilot study during the follow-up median of 23 (range, 10-65) months. The probability of disease-free survival (DFS) was 76.9% (95% CI 51-100%). Thus, we decided to present an update of our experience with previously published approach to the patients undergoing allogeneic HSCT for ALL in the first or subsequent CR.

Patients and methods

Patients and pre-transplant treatment. Presented study assessed the data of 25 adults with a median age at diagnosis of 34 (range, 20-58) years and available for a post-transplant follow-up of at least 3 months. Allogeneic HSCT was indicated for primary ALL in the first and second CR in 21 (84%) and 2 patients (8%), respectively. Two recipients (8%) were allografted for lymphoblastic transformation of chronic myeloid leukemia (CML). Their induction therapy was identical to the approach in primary Philadelphia chromosome positive (Ph+) ALL. Therefore they were also included in this update. Both patients achieved molecular remission of the disease after induction chemotherapy in combination with the tyrosine kinase inhibitors (TKIs). Standard induction or consolidation chemotherapy was administered in accordance with the Cancer and Leukemia Group B (CALGB) 8811 or German Multicenter Study Group for Adult ALL (GMALL) 07/2003 protocols [9, 10]. In Ph+ ALL patients, TKIs were added to their standard treatment. The characteristics of updated and enlarged study group including prognostic parameters and treatment protocols are highlighted and demonstrated in detail in Table 1 and Suppl Table 1, respectively.

The median time interval from diagnosis to HSCT was 6 months (range, 4-35 months). 17 patients (68%) in the first CR receiving transplantation from unrelated donors underwent two chemotherapy cycles in addition to successful induction therapy according to the primary protocol. Six recipients (24%) with available related donor were treated with only one consolidation therapy cycle before allografting. Except two specific cases, there was no significant difference in the interval from diagnosis to HSCT as in two patients subsequently transplanted from related available donors, consolidation chemotherapy had to be postponed due to serious infectious complications during induction therapy. Two patients (8%), allografted later from unrelated donors, refused HSCT after an achievement of the first CR with primary treatment. Nevertheless, they relapsed and were allografted in the second CR, 20 and 35 months since the ALL was diagnosed. The subsequent CRs were achieved in both cases with reinduction chemotherapy according to GMALL protocol used in the first-line treatment as well. All patients gave signed informed consent with the transplant procedure approved by the institutional review board.

Conditioning regimen. The conditioning regimen consisted of fludarabine (30 mg/m^2 on days -8, -7, -6, -5 and -4), melphalan (70 mg/m^2 on days -3 and -2) and thymoglobulin (1.5 mg/kg on days -4, -3, -2 or, recently, 2.0 mg/kg on days -3, -2 applied in 13 and 12 recipients, respectively).

HSCT. All 25 patients received donor's peripheral blood stem cells (PBSC). Stem cells from HLA-matched related donors (MRDs) and mismatched related donor (MMRD) were transplanted to 5 patients (25%) and 1 patient (4%), respectively. However, most HSCTs were from unrelated donors. Grafts from HLA-matched unrelated donors (MUDs) and

Table 1. Summarized data of presented study group

	No (%)	median (range)
recipient' gender (female / male)	12 (48) / 13 (52)	-
recipient' age at diagnosis (years)	-	34 (20 – 58)
diagnosis of ALL		
primary	23 (92)	-
secondary *	2 (8)	-
B origin	19 (76)	-
T origin	6 (24)	-
Ph+	8 (32)	-
extramedullary involvement	6 (24)	-
WBC > 25 x 10^9/L	4 (16)	-
allografting in > 1st CR of ALL	2 (8)	-
time from diagnosis to HSCT (months)	-	6 (4 – 35)
donors		
MRD	5 (20)	-
MMRD	1 (4)	-
MUD	10 (40)	-
MMUD	9 (36)	-
donation from female to male recipient and donor ABO/Rh compatible	2 (8)	-
recipient and donor CMV seronegative	7 (28)	-
	4 (16)	-
donors' age at donation (years)	-	31 (19 – 51)
graft – PBSC	25 (100)	-
infused MNC (x 10^8/kg)	-	5.98 (3.94 – 9.85)
infused CD34+ cells (x 10^6/kg)	-	4.47 (2.94 – 10.12)
post-transplant GVHD prophylaxis		
CyA	15 (60)	-
CyA + MTX	1 (4)	-
CyA + MMF	9 (36)	-
engraftment (assessable)	25 (100)	-
ANC ≥ 0.5 x 10^9/L (+day)	-	14 (12 – 18)
PLT ≥ 20 x 10^9/L (+day)	-	11 (9 – 65)
100% donors' chimerism on day +30	22 (88)	-
GVHD prophylaxis reduction and withdrawal (assessable)	21 (84)	-
start of CyA reduction	-	46 (28 – 77)
stop of CyA	-	110 (92 – 195)
acute GVHD (+day)	4 (16)	28 (21 – 32)
grade II	2 (8)	-
grade III	2 (8)	-
chronic GVHD	4 (16)	-
limited	1 (4)	-
extensive	3 (12)	-
CMV infection	13 (52)	-
asymptomatic	8 (32)	-
syndrome / disease	5 (20)	-
relapse of ALL (months after HSCT)	6 (24)	8 (3 – 16)
deaths (months after HSCT)	4 (16)	25 (12 – 32)
post-transplant complications	1 (4)	-
progression of ALL	3 (12)	-
post-transplant follow-up (months)	-	32 (4 – 87)

*** lymphoblastic crisis of CML**

mismatched unrelated donors (MMUDs) were transplanted to 10 (40%) and 9 recipients (36%), respectively. These characteristics and parameters, as well as gender of donors and recipients, their cytomegalovirus (CMV) serologic status, ABO and Rh system compatibility, the number of hematopoietic stem cells in the graft are generally summarized and fully shown in Table 1 and 2, respectively.

Post-transplant GVHD prophylaxis. As part of post-transplant GVHD prophylaxis, all patients were given cyclosporine A (CyA). Its intravenous administration was initiated on day -1 at a dose of 6 mg/kg/day which was subsequently adjusted so that the target plasma concentration ranged between 200 and 300 ng/mL. The recipients were switched to the oral form of CyA only after manifestations of gastrointestinal RRT resolved. CyA dose reduction with a gradual decrease of its plasma concentrations was planned to be started, in the absence of any possible manifestations of GVHD or ALL relapse, between days +28 and +35. The target was full withdrawal of CyA on days +90 to +110. When grafts from HLA-mismatched donors were transplanted, CyA was initially combined with methotrexate (MTX) or mycophenolate mofetil (MMF) (summarized Table 1 and fully showed in Table 2). Standard "short MTX" was administered at doses of 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6 and +11. The administration of MMF was initiated on day +1 at a dose of 30 mg/kg/day that was gradually reduced so that the drug was withdrawn on days +15 to +56. As was the case of CyA, MMF was first administered intravenously and the patients were switched to the oral form only after resolution of gastrointestinal RRT.

Anti-infectious prophylaxis. Co-trimoxazole was administered at a dose of 960 mg twice daily orally on days -8 to -1. From day 0, it was withdrawn until the absolute neutrophil count (ANC) reached ^{3} 0.5 × 10^9/L. Then, co-trimoxazole was continued at the same daily dose on two days a week. Herpes virus infections were prevented by 2× 500 mg of oral valacyclovir or 3× 250 mg of intravenous acyclovir daily, starting from day -8. Anti-infectious prophylaxis with both co-trimoxazole and valacyclovir or acyclovir was withdrawn 6 months after immunosuppression withdrawal and in the absence of any form of GVHD. Fluconazole at a daily dose of 200 mg orally or intravenously was initiated on day -8 and withdrawn at the moment of a stable ANC ^{3} 1.0 × 10^9/L, absent GVHD and if there was no need to continue with administration of broad-spectrum antibiotics or corticosteroids. Patients with a history of confirmed or suspected systemic

Table 2. Stem cell transplantations characteristics

patient No	donor type	donor age (years)	gender (R/D)	ABO/Rh (R/D)	CMV serostatus (R/D)	infused MNC (×10^8/kg)	infused CD34+ cells (×10^6/kg)	post-transplant GVHD prophylaxis	start of CyA reduction (day)	stop of CyA (day)
1	MMUD	41	F/M	B+/O+	+/-	6.37	4.46	CyA + MMF	+77	+195
2	MUD	24	M/M	AB+/O-	+/-	5.28	7.59	CyA	+46	+113
3	MUD	35	M/M	A+/A-	+/+	6.75	7.0	CyA	+50	+110
4	MUD	51	F/M	O-/O-	-/-	3.94	5.04	CyA	+46	+108
5	MMUD	29	F/M	O-/O+	-/+	9.85	10.12	CyA + MTX	+70	+92
6	MRD	41	F/F	B+/AB+	+/+	4.5	4.61	CyA	+30	+108
7	MMRD	38	M/M	AB+/ AB+	-/-	6.48	3.0	CyA + MMF	+33	+116
8	MMUD	43	M/M	O+/A+	+/+	5.58	5.05	CyA+MMF	+33	+111
9	MRD	23	M/M	A+/O+	+/-	7.56	5.56	CyA	n.a	n.a.
10	MMUD	19	F/F	A+/A+	+/+	5.28	4.01	CyA + MMF	+56	+105
11	MRD	27	M/M	B-/AB+	-/-	8.27	3.53	CyA	n.a.	n.a.
12	MMUD	20	M/F	B+/O+	+/+	4.83	2.94	CyA + MMF	+60	+120
13	MMUD	23	M/F	A+/A+	+/+	7.05	4.9	CyA + MMF	+60	+113
14	MMUD	38	M/M	A-/AB+	-/-	7.82	5.5	CyA + MMF	n.a	n.a.
15	MMUD	21	M/M	A-/A-	+/-	8.8	3.75	CyA + MMF	+42	+106
16	MRD	42	F/F	A+/O+	+/+	6.99	4.81	CyA	n.a.	n.a.
17	MUD	22	M/M	A-/O+	+/+	4.68	3.75	CyA	+30	+125
18	MUD	24	F/M	O+/O+	+/-	8.08	4.47	CyA	+32	+111
19	MUD	41	M/M	B+/O-	+/+	7.36	3.16	CyA	+28	+102
20	MMUD	40	F/M	A+/O+	-/+	5.25	4.79	CyA + MMF	+58	+116
21	MUD	26	F/M	AB+/A+	+/+	4.62	4.90	CyA	+68	+126
22	MRD	36	F/M	A+/A+	+/+	5.98	4.20	CyA	+58	+106
23	MUD	31	F/M	B-/O+	+/-	4.24	3.0	CyA	+40	+100
24	MUD	25	F/M	B+/O+	+/+	5.35	4.47	CyA	+42	+103
25	MUD	35	F/M	A+/O-	+/-	4.75	3.30	CyA	+31	+96

R – recipient, D – donor, F – female, M – male, n.a. – not applicable because of GVHD development

fungal infection received secondary antifungal prophylaxis, namely voriconazole or posaconazole at daily doses of 200 mg and 3× 200 mg (solution) or 3x 100mg (tablets), respectively. Gastrointestinal decontamination was performed with 2× 400 mg daily of rifaximin from day -8 until a stable ANC ^{3} 0.5×10^9/L, complete resolution of mucositis and withdrawal of broad-spectrum antibiotics. CMV-seronegative recipients transplanted from CMV-seronegative donors were substituted with leukodepleted blood products (erythrocytes and platelets). CMV-seropositive patients or CMV-seronegative ones transplanted from CMV-seropositive donors were regularly screened for the presence of CMV DNA in plasma once weekly until day +100 after HSCT, so that the risk for development of CMV disease could be eliminated by early initiation of preemptive therapy. If patients developed GVHD or were treated with corticosteroids or intensive immunosuppressive therapy, this regular monitoring was continued past day +100.

RRT. Non-hematological RRT was assessed according to the National Cancer Institute Common Toxicity Criteria, version 2.0 [11].

Hematopoietic recovery, engraftment and therapeutic respons. Post-transplant hematopoietic recovery was defined as the first of three subsequent days with a stable ANC ^{3} 0.5×10^9/L and platelets (PLTs) count ^{3} 20×10^9/L in recipients' peripheral blood. Cellular chimerism was assessed in peripheral blood nucleated cells and bone marrow aspirate on days +15, +60, +270 and +30, +90, +180, +360, respectively, using amplification of microsatellite DNA short tandem repeats (STR) with fluorescently labeled primers in polymerase chain reaction and subsequent separation by capillary electrophoresis. Prior to HSCT, blood samples were collected from both recipients and donors and their STR profiles were determined. Those were used in the post-transplant period to quantify donor's chimerism [12]. Complete donor's chimerism was defined as 100% of donor's nucleated cells in peripheral blood or bone marrow. Post-transplant relapse of ALL was confirmed if following criteria had been fulfilled: > 5% of original recipient's pathological lymphoblasts in bone marrow (with or without their finding in peripheral blood, decreased hemoglobin level, ANC and PLTs) and/or proven extramedullary involvement (e.g. leukemic cells in cerebrospinal fluid or proven pathological lymphoblastic infiltration of any tissue).

GVHD diagnosis and assessment. Acute or chronic GVHD was diagnosed based on the clinical features, laboratory findings and histological results from biopsy samples of the affected tissue. Acute GVHD stages and grades were determined in accordance with standard criteria [13]. When assessing chronic GVHD, the original classification into limited and extensive forms was used based on the extent and intensity [14].

Statistical analysis. The Kaplan-Meier method was used to calculate EFS and OS. EFS was defined as the interval from HSCT to relapse of the disease or death from any cause. OS was defined as the interval from HSCT to the death from any cause. The analysis was carried out using the SPSS, version 15 software (SPSS Inc., Chicago, USA).

Results

Mucositis (stomatitis and/or gastrointestinal involvement) as the dominant manifestation of non-hematologic RRT was observed in 18 patients (72%); in only 5 cases (20%), however, grade III was noted (data not shown). Renal and liver RRT, developed in 9 (36%) and 2 recipients (8%), respectively. Nevertheless, only one patient (4%) suffered from the renal RRT grade III and liver involvement did not exceed grade I (data not shown). Post-transplant thrombotic microangiopathy (TMA) grade II developed in one recipient (4%) allografted in the second CR of ALL and completely resolved with the change of GVHD prophylaxis (CyA was switched to the combination of MMF with corticosteroids that was gradually reduced and stopped according to mentioned plan on day +102) and the treatment of confirmed human herpesvirus type 6 (HHV6) reactivation with virostatics.

Successful engraftment was achieved in all patients. Stable ANC $\geq 0.5\times10^9$/L and PLT $\geq 20\times10^9$/L were achieved at a median of 14 (range, 12-18) and 11 (range, 9-65) days after HSCT. Complete donor's chimerism on day +30 was detected in 22 recipients (88%) (summarized in Table 1 and fully showed in Table 3).

In 21 assessable patients (84%), dose reduction and full withdrawal of CyA were within a median of 46 (range, 28-77) and 110 (range, 92-195) days after HSCT, respectively (summarized Table 1 and fully showed in Table 2). The delays were associated with the findings that could have been linked to the development of GVHD in 14 (56%) and 10 patients (40%), respectively. However, the complication was not confirmed by the subsequent clinical course and examination results including the histology of involved tissue. The delays of reduction and withdrawal of CyA in these recipients, comparing to proposed protocol, were in the median of 24 (range, 5-42) and 6 (range, 1-85) days, respectively. In four cases (16%), the early reduction and withdrawal of prophylactic immunosuppression were not carried out according to protocol due to development of acute GVHD.

The grade II and III of acute GVHD were equally observed in two (8%) of these four patients, respectively. The first clinical symptoms and/or laboratory findings of acute GVHD developed in the median of 28 (range, 21-32) post-transplant days. In presented group of 25 patients and the post-transplant follow-up median of 32 (range, 4-87) months, after HSCT with the above conditioning regimen and GVHD prophylaxis, chronic GVHD developed in four cases (16%). A limited and extensive form were observed in 1 (4%) and 3 (12%) recipients, respectively. One of them with extensive chronic GVHD died of septic shock 12 months after HSCT (summarized Table 1 and fully showed in Table 3).

Primary or reactivated CMV infections were observed in 1 (4%) and 12 (48%) patients, respectively. It was clinically

Table 3 Patients' post-transplant outcome

patient No	engraftment			acute GVHD (day, grade)	chronic GVHD (severity)	CMV reactivation	post-transplant ALL relapse (months)	death (cause)	post-transplant follow-up (months)
	ANC ≥ 0.5×10^9/L (day)	PLT ≥ 20×10^9/L (day)	donor's chimerism on day +30						
1	+13	+11	97%	no	no	yes (syndrome)	no	no	65
2	+14	+9	100%	no	no	yes (asymptomatic)	no	no	65
3	+15	+10	100%	no	no	no	no	no	46
4	+12	+12	100%	no	no	no	no	no	38
5	+17	+12	100%	no	no	yes (asymptomatic)	yes (3)	no	35
6	+17	+13	100%	no	no	no	yes (16)	yes (PD)	32
7	+16	+13	100%	no	no	no	no	no	23
8	+15	+10	100%	no	no	yes (asymptomatic)	no	no	17
9	+14	+10	100%	yes (+30, II)*	no	yes*	no	no	14
10	+16	+12	100%	no	no	yes (asymptomatic)	no	no	13
11	+15	+11	100%	yes (+26, III)	no	no	no	no	11
12	+14	+11	100%	no	no	no	no	no	10
13	+13	+11	100%	no	no	no	no	no	12
14	+14	+11	100%	yes (+32, II)	yes (extensive)	no	no	yes (sepsis)	12
15	+12	+14	100%	no	no	no	yes (8)	yes (PD)	18
16	+13	+10	100%	yes (+21, III)	yes (extensive)	yes (asymptomatic)	no	no	28
17	+13	+10	100%	no	no	yes (syndrome)	no	no	27
18	+12	+12	100%	no	yes (limited)	yes (asymptomatic)	yes (9)	yes (PD)	14
19	+12	+65	100%	no	no	no	no	no	21
20	+16	+12	100%	no	no	no	no	no	22
21	+13	+10	98%	no	yes (extensive)	yes (asymptomatic)	no	no	21
22	+13	+10	100%	no	no	no	yes (12)	no	14
23	+18	+11	100%	no	no	yes (syndrome)	no	no	11
24	+12	+9	100%	no	no	yes (syndrome)	yes (7)	no	8
25	+13	+10	99%	no	no	yes (asymptomatic)	no	no	4

PD – progression of primary disease, * histological confirmation of acute GVHD and concomitant detection of CMV DNA in gut mucosa

manifested in 5 recipients (20%). In 4 cases (16%), CMV syndrome developed. In one patient, CMV DNA was detected in both plasma and intestinal mucosa obtained by biopsy and the clinical manifestation of the infection (i.e. either CMV syndrome or gastrointestinal CMV disease) could not be reliably determined due to histologically confirmed concomitant acute GVHD (summarized Table 1 and fully showed in Table 3). Following virostatic therapy, the symptoms disappeared completely in the former patients. The latter patient's condition also improved but it was more difficult to assess due to the parallel immunosuppressive therapy for acute GVHD.

In 6 ALL cases (24%), the underlying disease relapsed at the median of 8 (range, 3-16) months after HSCT (Table 3). Three (12%) cases (patients No.6, No.15 and No.18) were isolated extramedullary relapses (two with CNS involvement and one with an infiltration of soft tissues and bones arround the right hip joint) without the diffuse bone marrow involvement. One (4%) recipient (patient No.5) developed systemic relapse of ALL with bone marrow as well as CNS involvement. The bone marrow relapse without any evidence of extramedullary involvement were observed in the rest two (8%) patients (patients No.22 and No.24). Significantly delayed start of CyA reduction was noted in two (33.3%) relapsed recipients (on days +58 and +70). However, there was almost no delay in the withdrawal of immunosuppression comparing to protocol (range, 92-111 post-transplant days). One relapsed patient (16.7%) was allografted in the second CR of ALL, other three ones (50%) had poor prognostic cytogenetic findings, t(9;22)(q34;q11) and t(4;11)(q21;q23) in one (16.7%) and two cases (33.3%), respectively. All but one relapsed patients were without the evidence of any GVHD form. Only one developed limited chronic GVHD without the need of any systemic immunosuppressive treatment.

Five of relapsed patients (83.3%) achieved another CR after the second transplantation with cryopreserved hematopoietic cells obtained from the original donors. Intravenous high-dose MTX (3 g/m^2) and intrathecal treatment (MTX 15 mg, cytarabine 40 mg, dexamethason 4 mg) were used in three recipients to reduce CNS leukemic involvement at relapse. All five patients were allografted after non-myeloablative conditioning regimen FLAG-Ida (fludarabine 120 mg/m^2, cytarabine 8g/m^2, idarubicin 36 mg/m^2) and CyA was the only immunosup-

pressant in GVHD prophylaxis, regardless of recipient/donor HLA compatibility. Moreover, to enhance GVL effect, CyA was tapered since day +14 and withdrawn on day +42. Two patients with isolated CNS involvement relapsed again in the same location and died 41 and 18 months after the first HSCT, respectively. The sixth patient was commended to palliative treatment and died of disease progression 14 months after allografting. The last three subsequently allografted recipients have survived 57, 14 and 8 months after the first transplantation, respectively. One of these patients developed extensive chronic GVHD that regressed with combined immunosuppressive therapy; at 53 months after the second HSCT, she still remains in CR of ALL. The other two patients have also achieved the CR but their actual post-transplant follow-up is too short, equally 2 months in both cases.

At a median post-transplant follow-up of 32 (range, 4-87) months, NRM and overall mortality in the group were 4% and 16%, respectively. The probabilities of 2-year EFS and OS were 70.3% (95% CI 51.9-88.7%) and 86.1% (95% CI 71.6-100%), respectively (Figure 1).

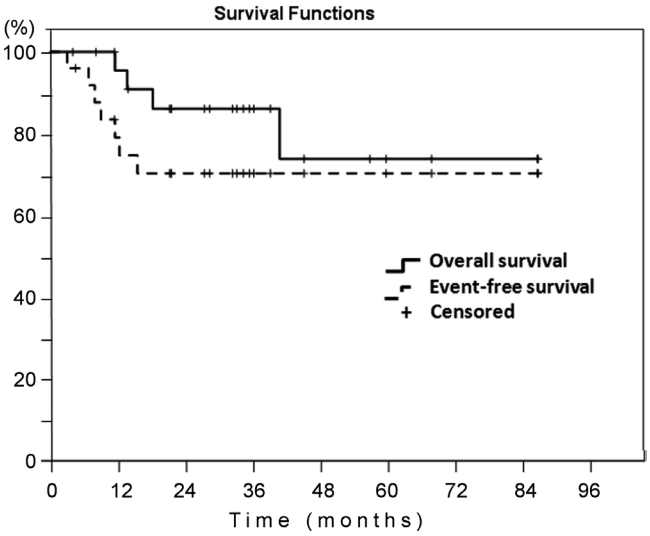

Figure 1. Estimated event-free and overall survival Kaplan-Meier's curves of presented cohort

Discussion

Some recent studies proved no significant differences in general outcome between the patients with ALL allografted after myeloablative conditioning (MAC) and RIC [15]. Our updated results confirmed an efficacy of allogeneic HSCT following RIC in adult patients with high-risk ALL (including lymphoblastic crises of CML) in the CR. Nevertheless, several proposals had played an integral role in the design of reported transplant approach.

RIC contained fludarabine and melphalan has been associated with acceptable RRT. Mucositis and gastrointestinal involvement represented the major features of organ toxicity. However, gastrointestinal RRT did usually not overcome grade II/III [6, 7] as also observed in our previous [5] and recent study. Other forms of RRT were mild and less frequent. In spite of relatively low general non-hematological toxicity, reduced combination of fludarabine and "high-dose" melphalan had significant myelosuppressive and immunosuppressive potential that allowed to achieve a stable engraftment of donor hematopoietic and immunocompetent cells responsible for GVL effect [7, 16].

On the other hand, regardless of the grade, mucositis as the major form of RRT and early engraftment after RIC combined fludarabine with melphalan may trigger some immunopathophysiological mechanisms responsible for GVHD development [17, 18]. Therefore we decided to include *"in vivo"* T-depletion with thymoglobulin as a part of conditioning regimen to suppress the early activation of immunopathophysiological cascade resulted in GVHD clinical manifestation. And indeed, acute GVHD was observed and histologically confirmed in 4 recipients (16%) only. These results support an efficacy of *"in vivo"* T-depletion to prevent early post-transplant serious immunological complications.

There might be also some arguments against the use of ATG, particularly in the cases allografted from HLA-mathed donors, because of the possible suppression of required GVL reaction. Therefore the integral parts of treatment strategy remained early reduction and withdrawal of post-transplant immunosuppressive GVHD prophylaxis as previously established in our pilot study [5]. Despite some delays of CyA reduction and withdrawal in 14 (56%) and 10 (40%) of assessable patients, respectively, the administration of post-transplant prophylactic immunosuppression was still significantly shorter than in published studies [19, 20]. Nevertheless, after the first transplantation, chronic GVHD and its extensive form developed only in 4 (16%) and 3 (12%) patients, respectively. Earlier initiation of reduction and withdrawal of GVHD prophylaxis was also reported by Cho et al. in patients with high-risk ALL transplanted in the first or second CR after RIC combining fludarabine and melphalan (FM) [21]. The 3-year disease-free survival (DFS) and OS rates were 62.6% and 64.1%, respectively. However, the apparent antileukemic effectiveness was accompanied by higher rates of both GVHD and NRM. Thymoglobulin was administered less frequent (only in 6 recipients transplanted from MMUDs) and in lower total doses (2.5 mg/kg). Similar results were reported by Stein et al. in ALL patients transplanted from MRDs or MUDs after the same FM regimen [22]. *"In vivo"* T-cell depletion was performed in only one patient. GVHD was prevented by administration of tacrolimus and sirolimus or CyA and MMF; in 14 patients, MTX was added to these combinations. The time schedule of reduction and complete withdrawal of calcineurin inhibitors was not stated. The probability of 2-year DFS and OS was 61.5%. However, the development of acute and chronic GVHD was observed in 75% and 86% of patients, respectively, with 2-year NRM of 21.5%. On the other hand, there was observed

just one death (4%) caused by post-transplant complications in our cohort during presented follow-up period.

This study presents updated results of specific approach to the allografting in adult patients with probably otherwise incurable lymphoblastic neoplasias. *"In vivo"* T-cell depletion with thymoglobulin as a standard part of RIC was confirmed to influence the occurrence of GVHD and NRM regardless of relatively high proportion of patients transplanted from HLA-mismatched donors (40%). Early reduction and withdrawal of post-transplant GVHD prophylaxis did not lead to the increase of serious immunological complications. However, despite the high-risk primary disease, antileukemic control with GVL reaction has been apparent in most treated patients.

Supplementary information is available in the online version of the paper.

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Suppl Table 1 Patients' characteristics of primary disease and pre-transplant treatment

patient No	gender	age at diagnosis (years)	type of ALL	cytogenetic findings at diagnosis*	WBC at diagnosis ($x10^9$/L)	extramedullary involvement at diagnosis	primary therapeutic protocol	time from diagnosis to HSCT (months)
1	female	24	common B	46,XX,t(12;22)(p?12;q?13)	8.5	none	CALGB	6
2	male	50	common B	standard cytogenetic methods failed, BCR-ABL1fusion with breakpoint in minor region was confirmed by the method of molecular genetics	2.4	none	CALGB + imatinib	5
3	male	57	common B	52,XY,+X,+10,der(14)t(8;14)(q?12;q32.3),+17,+20,+21,+21	5.37	none	CALGB	5
4	female	34	pre-B	46, XX,der(20)t(1;20)(q?21;?)	2.39	none	GMALL	7
5	female	25	pre-B	46,XX,t(X;6)(p?21;p?23),del(9)(p21)	34.3	none	GMALL	6
6	female	34	pre-B	47,XX,t(4;11)(q21;q23),+der(4)t(4;11)(q21;q23)/ 48,XX,+der(X)?del(X)(q?24),t(4;11)(q21;q23), +der(4)t(4;11)(q21;q23)	14.19	none	GMALL	4
7	male	39	common B	46,XY	5.92	CNS	GMALL	5
8	male	28	pre-T	46,XY	10.76	retroperitoneal lymph nodes***	GMALL	5
9	male	25	medullary T	46,XY,del(9)(p21)3/47,XY,+6,del(9)(p21)	11.95	pericardium***	GMALL	6
10	female	25	pre-T	47,XX,+4	1.23	none	GMALL	6
11	male	25	common B	69,XY,+X,+Y,+2,+4,+5,+5,+6,+8,t(9;22)(q34;q11.2),+10,+10, +11,+13,+14,+14,+15,+16,+17,+18,+19,+20,+20,+21,+der(22) t(9;22)(q34;q11.2)	5.42	none	GMALL + imatinib	6
12	male	42	common B	48,X,-Y,+X,+2,+6,-7,-9,t(9;22)(q34;q11.2), +14,+der(22) t(9;22)(q34;q11.2)/ 49,X,-Y,+X,+2,+6,-7,-9,t(9;22)(q34;q11.2),+14,+14,+der(22) t(9;22)(q34;q11.2)	22.6	none	GMALL + imatinib	7
13	male	40	common B**	51,XY,+Y,+2,+5,+6,-7, t(9;22)(q34;q11) inv(9)(p13;q34.3),+10,+der(22)t(9;22)(q34;q11)	15.63	none	GMALL + dasatinib	5
14	male	54	common B**	46,XY, t(9;22)(q34;q11)	38.22	none	CALGB + imatinib	8
15	male	22	pre-B	46,XY,?del(2)(q?),ins(16;2)(q?12;q?)	3.5	CNS	GMALL	20
16	female	43	pre-B	46,XX	1.7	none	GMALL	5
17	male	46	common B	46,XY,t(9;22)(q34;q11.2)/47,XY,+21	2.05	none	GMALL + imatinib	6
18	female	53	pre-B	60-63,XXX,?+1,-3,-4,-5,-11,-12,-13,-16,-17,-18,+21,+1-2mar	17.19	none	GMALL	5

19	male	22	pre-T	46,XY,del(9)(p21)	10.59	CNS	GMALL	35
20	female	29	pre-T	46,XX	18.58	pericardium***	GMALL	6
21	female	27	pre-T	46,XX	1.79	none	GMALL	5
22	female	35	common B	47,XX,t(9;22)(q34;q11),del(9)(p21),+der(22)t(9;22)(q34q11)/ 46,XX,t(9;22)(q34;q11),del(9)(p21)	6.71	none	CALGB + imatinib	5
23	female	58	pre-B	47,XX,der(1)t(1;18)(p?33;q?16)t(6;18)(q?21;?p?),der(4)t(4;15) (p?6;?q?),der(6)t(6;8)(q?15;?p?),+der(10),del(10)(q?22;qter), der(18)t(1;18)(?q?;p?11)/47,XX,der(1)t(1;18)(p?33;q?16)t(6;18) (q?21;?p?),der(4)t(4;15)(p?16;?q?),der(6)t(6;8)(q?15;?p?),+10, der(18)t(1;18)(?q;p?11)	1.91	none	GMALL	6
24	female	34	pre-B	47,XX,?del(X)(q?23),+X,t(4;11)(q21;q23),der(9)(del(9)(p21) del(9)(q22)/ 46,XX,t(4;11)(q21;q23),der(9)(del(9)(p21)del(9)(q22)	51.45	none	GMALL	4
25	female	52	common B	50,XX,+2,+5,del(9)(p21),t(9;22)(q34;q11),+10,+21/50,XX,+2, t(5;9)(q?34;p?12),t(9;22)(q34;q11),der(9)del(9)(p21),+5,+10,+21/ 46,XX,t(9;22)(q34;q11)	61.21	none	GMALL + imatinib	6

* evaluated by the methods of classic cytogenetics and fluorescence in situ hybridization (FISH)
** lymphoblastic crisis of CML
*** manifestation of mediastinal mass at diagnosis