Chimeric and humanized antibodies have a longer circulating half-life and reduced immunogenicity [Adair, F 2002].

1.5 Objective

The first aim of this study was the evaluation of the drug safety and duration of CD25 blockade under treatment with chimeric and humanized CD25 antibodies (ch/anti-CD25) in 11 pediatric stem cell transplant recipients. Endpoints of this study were recurrence of leukemia, death or multiorgan failure.
Next, we evaluated the incidence of GVHD, relapse and survival in 34 patients receiving allogeneic stem cell transplants under treatment with either prophylactic ch/anti-CD25 (n=11) or prophylactic murine anti-CD25 (m/anti-CD25, n=13) or no CD25 antibody (n=10). Prophylactic anti-CD25 was used in addition to standard GVHD prophylaxis in 24 patients after allogeneic SCT with unrelated donors (n=22) or related donors with increased risk of GVHD (n=2) because of HLA-mismatch (1 of 2) or female donor/ male recipient pair in a PBSC transplant (1 of 2). No CD25 antibody was used in 10 children after allogeneic SCT with matched related donors and standard risk of GVHD.

2 Patients and methods

All patients and their guardians signed informed consent prior to therapy. Protocol treatment was applied after local internal review board (IRB) approval according to the precepts established by the declaration of the Helsinki Conference.

2.1 Group A: patients receiving chimeric or humanized anti-CD25 (ch/anti-CD25 treatment)

2.1.1 Patients characteristics of group A (ch/anti-CD25 treatment)

Characteristics of the 11 patients of group A are summarized in **Table 1**. Allogeneic SCT was performed between 1998 and 2002 in 6 patients with acute lymphoblastic leukemia (ALL), 1 patient with myelodysplastic syndrome (MDS) and 3 patients with acute myeloic leukemia (AML). Patients ages ranged from 1.2 to 16.6 years (median 11.3) and leukemia patients were pretreated with ALL-BFM [Schrappe, M. et al. 2000]

and AML-BFM studies [Creutzig, U. et al. 2001]. Median duration of pre-SCT induction therapy (interval between last occurrence of leukemia to SCT) was 112 days (range 12 to 323) in ALL patients and 111 days (range 15 to 346) in AML patients. 1/11 MDS patient was transplanted 204 days after diagnosis, having been treated for aplastic anemia two years before diagnosis.

All patients received myeloablative treatment with total body irradiation (TBI 6 x 2 Gy, 12 Gy total dose) and etoposide (Eto 30 mg/kg in 3/7 and 40 mg/kg in 4/7 ALL patients as a single dose) or busulfan (Bu 4 x 4 mg/kg orally, 16 mg total dose) and cyclophosphamide (Cy 2 x 60 mg/kg, 120 mg/kg total dose) in AML and MDS patients. In 1 MDS patient and the sole AML patient with resistant disease, melphalan (Me 140 mg/m^2 as a single dose) was added to busulfan and cyclophosphamide (total doses are shown in **Table 2**). All patients received peripheral stem cells (PSC): stem cell source was peripheral blood (PBSC) in 9/11 patients (2 related, 7 unrelated grafts) and unrelated cord blood (CBSC) in 2/11 patients. HLA-A, -B, -DR matching was identical (6/6 loci) in 7 patients (1 related, 5 unrelated donors), 5/6 loci in 3 patients (1 related donor with HLA-A mismatch, 2 unrelated donors with 1 HLA-B mismatch and 1 DRB1 minor mismatch) and 4/6 loci in 1 CBSC receiving patient (unrelated, HLA-A and -B mismatch). For HLA class I antigens, HLA-A and -B typing was performed serologically while class II antigens were determined using DNA typing. All patients were at high risk for GVHD, in 10/11 patients associated with unrelated or mismatch donors. In the 1/11 related HLA-identical SCT there was a sex-mismatch (female donor and male recipient), which is also associated with increased risk of GVHD [Weisdorf, D. et al. 1991]. Transplanted cell dose was 9.63 x10^6 CD34 positive cells/kg recipient (mean; range 3.05 to 19.7) in the PBSC and 0.36 x 10^6 CD34 positive cells/kg (7.7 x 10^7 nucleated cells/kg) in the CBSC receiving patients. Standard GVHD prophylaxis regimens consisted of cyclosporine A (CSP) alone (3 mg/kg i.v. beginning on day –1) or CSP and short course methotrexate (MTX 10 mg/m^2 on day +1, +3, +6 after SCT) in the PBSC transplants. CSP and 6-methylprednisolone (PRED) was given in the CBSC transplants according to the EUROCORD protocol for unrelated cord blood transplantation [Gluckman, E. et al. 2001]. As soon as oropharyngeal mucositis was resolved, oral intake of CSP was preferred (6 mg/kg orally). Dose reduction of CSP (10% per week) was started on day +180 in the absence of GVHD. Conditioning, grafts and GVHD prophylaxis is shown in **Table 2**.

Table 1 Patients characteristics of group A (ch/anti-CD25 treatment)

UPN	*Primary Disease*	*Status at SCT*	*Age (years)*	*Sex*
1026	T-ALL (PPR)	1. CR	12.2	M
1032	ALL (PPR, NR d33)	1. CR	16.6	M
1049	ALL (M-BCR/ABL)	2. CR	4.0	M
1044	c-ALL	2. CR	4.9	F
1036	T-ALL	2. CR	15.0	M
1037	T-ALL (PPR, NR d33)	NR (mediastinal[a])	5.4	M
1013	ALL	3. Relapse	11.3	M
1006	MDS	RAEB	11.7	F
1009	AML FAB[b] M5 (congenital)	1. CR	1.2	F
1022	AML FAB M2	2. CR	12.7	M
1031	AML FAB M7	Resistant disease	2.7	F

UPN = unique patient number; SCT = stem cell transplantation; ALL = acute lymphocytic leukemia; PPR = prednisone poor response on day 8 of induction therapy in ALL-BFM trial; NR d33 = non response on day 33 of induction therapy in ALL-BFM trial; MDS = myelodysplastic syndrome; RAEB = refractory anemia with excess blasts; AML = acute myeloid leukemia; M-BCR/ABL = with rearrangement of t(9;22); M = male; F = female;

[a]NR mediastinal = persistent tumor mediastinal;

[b]FAB = French-American-British classification of AML [Bennett, J. M. et al. 1985a] [Bennett, J. M. et al. 1985b]

Table 2 Graft, conditioning and graft-versus-host (GVHD) prophylaxis of group A patients (ch/anti-CD25 treatment)

UPN	Donor Relation	HLA Matching (Mismatch)	Sex R/D	Graft	CD34+ (x 10^6/kg)	Conditioning					GVHD Prophylaxis	
						TBI	Bu	Cy	Eto	Me	CSP/MTX/PRED	anti-CD25
1026	Unrelated	Identical	M/M	PBSC	7.86	12		120	30		CSP/[c]	Basiliximab
1032	Unrelated	Identical	M/F	PBSC	7.45	12		120	30		CSP/MTX[b]	Basiliximab
1049	Unrelated	Identical	M/F	PBSC	3.05	12			40		CSP/MTX	Daclizumab
1044	Sibling	5/6 loci (A)	F/F	PBSC	14.9	12			40		CSP/[c]	Daclizumab
1036	Sibling	Identical	M/F	PBSC	5.5	12			40		CSP/MTX	Daclizumab
1037	Unrelated	4/6 loci (A,B)	M/F	CBSC	0.37	12			40		CSP/PRED	Daclizumab
1013	Unrelated	5/6 loci (DR)	M/M	PBSC	11.7	12		120	30		CSP/MTX	Basiliximab
1006	Unrelated	Identical	F/M	PBSC	8.76		16	120		140	CSP/MTX[a]	Basiliximab
1009	Unrelated	5/6 loci (B)	F/F	CBSC	0.34		16	200			CSP/PRED	Basiliximab
1022	Unrelated	Identical	M/M	PBSC	7.77		16	120			CSP/[c]	Basiliximab
1031	Unrelated	Identical	F/M	PBSC	19.7		20	120		140	CSP/[c]	Basiliximab

PBSC = peripheral blood stem cells; CBSC = Cord blood stem cells; loci = HLA-A, -B, -DR loci; Identical = 6/6 loci; mismatch (A) = HLA class A mismatch; D = donor; R = recipient; CD34+ = CD34 positive cells in graft (x10^6 per kg); TBI = total body irradiation (total Gy); Bu = busulfan (total dose, mg/kg); Cy = cyclophosphamide (total dose, mg/kg); Eto = etoposide (total dose mg/m^2); Me = melphalan (total dose, mg/m^2); CSP = cyclosporine A (3 mg/kg i.v. daily); MTX = methotrexate short term (10 mg/m^2 on day +1, +3, +6); PRED = 6-methylprednisolone; anti-CD25 = antibody against interleukin-2 receptor a chain; Basiliximab = chimeric monoclonal anti-CD25; Daclizumab = humanized monoclonal anti-CD25; d+1 = day 1 from SCT;
MTX[a] = methotrexate 15 mg/m^2 on d+1 and 10 mg/m^2 on d+3, +6; MTX[b], only one dose MTX (10 mg/m^2 on d+1) administered because of acute renal failure;
CSP/[c] = only cyclosporine, no MTX was given because of toxicity.

2.1.2 Treatment protocol and monitoring of group A patients (ch/anti-CD25 treatment)

In addition to standard GVHD prophylaxis, all patients received monoclonal antibody against IL-2Ra (anti-CD25). 7/11 patients received chimeric anti-CD25 (basiliximab), 4/11 patients received humanized anti-CD25 (daclizumab) as shown in **Table 2**. Basiliximab was given as a single infusion over 30 minutes at 1 mg/kg (maximal 40 mg as total single dose). Daclizumab was also administered as a single infusion over 30 minutes at 1 mg/kg (maximal 50 mg as total single dose). Antibody application was given without premedication. 1 patient received 2 mg/kg daclizumab by mistake as single dose on day 0. Vital signs were monitored every 15 minutes until 2 hours after beginning infusion. The treatment started on day 0 six hours before SCT. Additional doses were given on days +4, +28, +56 and +84 after SCT (**Figure 2a**). The infusion was repeated earlier, if CD25 positive (CD25+) cells were detected in peripheral blood by flow cytometry (see below). Patients who showed chronic GVHD after day +100 at the time when CD25+ cells were first detected after the last antibody application were eligible for retreatment once at a subsequent time with an identical dose of antibody. We discontinued therapy earlier if patients developed multiorgan failure or relapsed from their leukemia.

Patients were monitored for peripheral blood cell and platelet counts, serum chemistry, renal and liver function daily until day +50. Engraftment was defined as first of three consecutive days in which the absolute neutrophil count (ANC) exceeded 500/µl. Patients were monitored for signs of infection and infections were treated according to standard guidelines.

Supportive therapy consisted of selective antimicrobial decontamination with amphotericin, colistine and paromomycin orally and antiviral prophylaxis. Pneumocystis carinii prophylaxis with cotrimoxazole 5 mg/kg orally was performed daily until day -2 before SCT and twice weekly after discharging. Cytomegaly virus (CMV)-seronegative blood products were used. For all patients surveillance for CMV, human herpes virus 6 (HHV6) and Adenovirus was performed with blood samples every 1-2 weeks until day +180. Pre-emptive antiviral therapy with gancilovir intravenous (i.v.) was given to patients in cases of CMV reactivation. After ganciclovir induction (5 mg/kg twice daily) for 14 days, maintenance therapy followed (once daily, same dose). Patients received immunoglobulin supplementation 400 mg/kg every week from day –1 to day +100 (d+100).

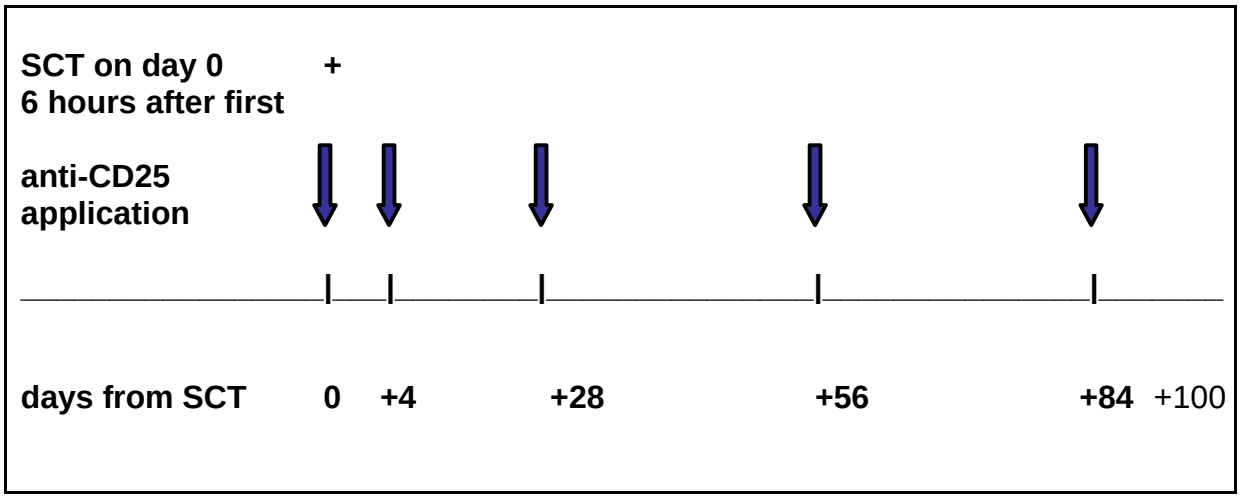

Figure 2a Administration of monoclonal chimeric or humanized interleukin-2 receptor a **antibody (ch/anti-CD25)** given 6 hours before allogeneic peripheral stem cell transplantation (SCT) and on days +4, +28, +56 and +84 after SCT in patients of group A.

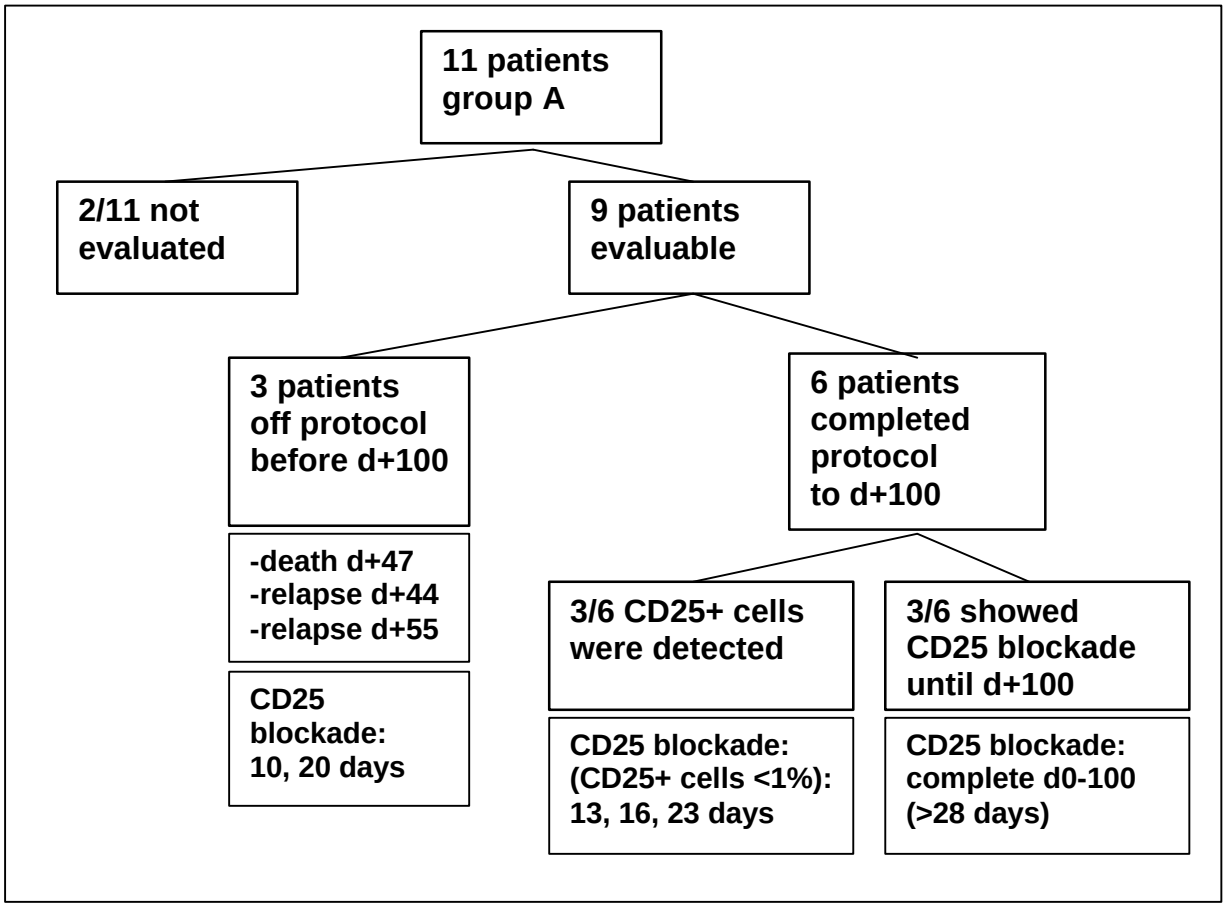

Figure 2b Patients in group A entered on protocol (ch/anti-CD25) shown in Figure 2a. CD25 Blockade was complete (CD25+ cells <1%, flow cytometry) from day 0 to +100 in 3/6 patients that completed protocol. In 3/6 patients CD25+ cells were detected.

2.2 Group B: patients receiving murine anti-CD25 (m/anti-CD25)

2.2.1 Patients characteristics of group B (m/anti-CD25 treatment)

As a comparison cohort of patients, we identified 13 patients at the pediatric bone marrow transplantation program of the University of Düsseldorf. The principal investigator headed this program before introducing the present SCT program at the University of Halle-Wittenberg. From 1991 to 1995 patients undergoing unrelated allogeneic bone marrow transplantation (BMT) were treated with a murine monoclonal antibody against IL-Ra (m/anti-CD25, inolimomab, BT563) in addition to standard GVHD prophylaxis with CSP and short course MTX as described in group A. Patients were matched by diagnosis, stage of disease, age and HLA-matching as shown in **Table 3**. Patients age ranged from 0.55 to 29 years (median 7). ALL patients were pretreated with COALL and ALL-BFM studies in contrast to BFM studies only used in group A. AML patients were pretreated with AML-BFM studies as described for group A. Median duration of pre-SCT induction therapy was 272.5 days (range 19 to 392) in 8/13 ALL patients and 154.5 day (range 72 to 231) in 4/13 AML patients. 1/13 MDS patient (29 years old at SCT) was transplanted within 831 days after diagnosis. All group B patients received myeloablative conditioning with total body irradiation (6 x 2 Gy; 12 Gy total dose), etoposide (40 mg/kg as total single dose in 12/13 patients and 20 mg/kg in the other patient) and cyclophosphamide (2 x 60 mg/kg; 120 mg/kg total dose). All group B patients received unrelated bone marrow stem cell transplants. HLA-matching was 6/6 in 10 patients (HLA-identical donors), 5/6 in 3 patients (2 with HLA-B mismatch, 1 with DR minor mismatch). Transplanted cell dose was $6.38 \pm 2.89 \times 10^8$ nucleated cells/kg recipient. The dose of containing CD34+ cells was not evaluated. Conditioning and GVHD prophylaxis are shown in **Table 3**.

2.2.2 Treatment protocol of group B patients (m/anti-CD25 treatment)

Inolimomab was given in constant doses of 0.1 mg/kg (maximal dose 5 mg/kg as total single dose) once daily from d+1 to d+50 after BMT and tapered through d+100 in three decrements (**Figure 3**): 0.1 mg/kg given thrice weekly from d+51 to d+64; 0.1 mg/kg twice weekly from d+65 to d+78 and once weekly from d+79 to d+100 after BMT [Burdach, S. et al. 1995] [Sander, A. 1999]. Comparable standards of supportive care and monitoring were used in both groups.

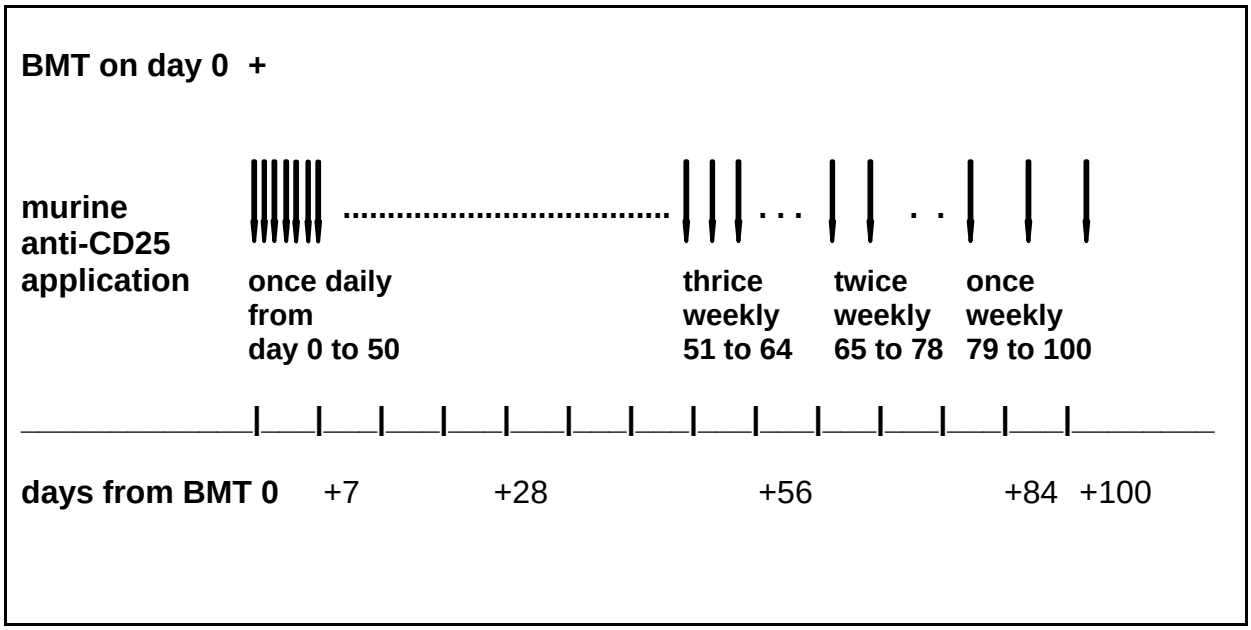

Figure 3 Administration of monoclonal murine interleukin-2 receptor a antibody (m/anti-CD25) after allogeneic bone marrow transplantation (BMT) in group B patients.
Murine anti-CD25 was given in constant doses of 0.1 mg/kg once daily from day 0 to 50 (maximal 5 mg/kg as total single dose), and was tapered through day +100 in 3 decrements: 0.1 mg/kg given thrice weekly from day +51 to +64; 0.1 mg/kg twice weekly from day +65 to +78 and once weekly from day +79 to +100 after BMT.

Table 3 Characteristics and matching of group A (ch/anti-CD25 treatment), group B (m/anti-CD25 treatment) and group C patients (no anti-CD25)

Patients characteristics		*Group **A***		*Group **B***		*Group **C***	
	number of patients	*n = 11*		*n = 13*		*n = 10*	
Age,	mean, yrs, SD	8.9 ± 5.3		8.7 ± 8.3		12.5 ± 3.9	
	median, yrs (range)	11.3 (1.2–16.6)		7 (0.75-29)		12.2 (7-17)	
Sex (female/male), n		4/7		6/7		5/5	
Disease, n (%)							
	MDS	1	(0.09)	1	(0.08)	0	
	AL in 1. CR	3	(0.27)	3	(0.23)	3	(0.3)
	AL in 2. CR	4	(0.36)	5	(0.38)	4	(0.4)
	AL in 3. CR or relapse	3	(0.27)	4	(0.31)	3	(0.3)
Donor[a], n							
	related	2		0		10	
	unrelated	9		13		0	
	HLA-identical (6/6 loci)	7		10		10	
	5/6 loci	3		3		0	
	4/6 loci	1		0		0	
Stem cell source, n							
	BM	0		13		10	
	PBSC	9		0		0	
	CBSC	2		0		0	
Duration of the pre-SCT induction therapy[b]							
to SCT, median days (range)							
	ALL	112 (12-323)		272.5 (19-392)		195 (12-481)	
	AML	111 (15-346)		154.5 (72-231)		64.5 (9-166)	
	MDS	204		841			

Table 3 Characteristics and matching of group A (ch/anti-CD25 treatment), group B (m/anti-CD25 treatment) and group C patients (no anti-CD25)

Patients characteristics	*Group **A***	*Group **B***	*Group **C***
number of patients	*n = 11*	*n = 13*	*n = 10*
Conditioning, n			
TBI + Cy	0	0	0
TBI + Eto	4	0	6
TBI + Cy + Eto	2	13	2
Bu + Cy	2	0	2
Bu + Cy + Me	2	0	0
Immunosuppression, n			
CSP	4	0	0
MTX	0	0	3
CSP + MTX	5	13	7
CSP + PRED	2	0	0
anti-CD25	11	13	0
Inolimomab (BT563)	0	13	0
Basiliximab	7	0	0
Daclizumab	4	0	0

n = number of patients; yrs = years; SD = standard deviation; MDS = myelodysplastic syndrom; AL = acute leukemia; CR = complete remission; HLA = human lymphocytic antigen system; loci = HLA-A, -B, -DR loci; Identical = 6/6 loci; BM = bone marrow; PBSC = peripheral blood stem cells; CBSC = cord blood stem cells; TBI = total body irradiation; Bu = busulfan; Cy = cyclophosphamide; Eto = etopside; Me = melphalan; CSP = cyclosporine A; MTX = methotrexate; PRED = 6-methylprednisolone; anti-CD25 = monoclonal antibody against interleukin-2 receptor a chain; Inolimomab (BT563) = murine anti-CD25; Basiliximab = chimeric anti-CD25; Daclizumab = humanized anti-CD25; ch/anti-CD25 = chimeric or humanized anti-CD25; m/anti-CD25 = murine anti-CD25; SCT = stem cell transplantation;

[a]HLA-matched; [b]Pre-SCT induction therapy = induction therapy of the last relapse or leukemia occurence before SCT.

2.3 Group C: patients without anti-CD25 therapy (no anti-CD25)

Another cohort of patients was treated from 1991 to 1995 under identical supportive care guidelines. These 10 patients (group C) received bone marrow (BM) from HLA-identical siblings with 4.99 ± 2.05 x 10^8 nucleated cells/kg recipient. The dose of containing CD34+ cells was not evaluated. GVHD prophylaxis consisted of CSP and short course MTX in 7/10 patients as shown above and long course MTX (10 mg/m^2 on day +1, +3, +6, +11 and MTX 10 mg/m^2 once weekly until day +100 thereafter) in 3/10 patients without additional antibody therapy. Matching of age, disease and stage of disease is shown in **Table 3**. All patients (6 ALL, 4 AML) were pretreated according to COALL or BFM studies. Median duration of pre-SCT induction therapy was 195 days (range 12 to 481) in 6/10 ALL patients and 64.5 days (range 9 to 166) in 4/10 AML patients. Median age was 12.2 years (range 7 to 17). All group C patients received myeloablative conditioning with TBI (12 Gy total dose). 8/10 patients received etoposide (40 mg/kg as single dose), in 2 of these patients cyclophosphamide (2 x 60 mg/kg; 120 mg/kg total dose) was given additionally. The other 2/10 patients received busulfan (4 x 4 mg/kg orally; 16 mg/kg total dose) and cyclosphosphamide (2 x 60 mg/kg; 120 mg/kg total dose). Conditioning and GVHD prophylaxis is also shown in **Table 3**.

2.4 Diagnosis and treatment of acute and chronic GVHD

Acute GVHD severity was graded according to clinical criteria as illustrated in **Table 4a** [Przepiorka, D. et al. 1995]. Initial treatment of GVHD grade II, III and IV usually consisted of 6-methylprednisolone (PRED) 2 mg/kg/day in three divided doses. Diagnosis of chronic GVHD was performed according to established Seattle criteria [Sullivan, K. M. et al. 1991]. Clinical criteria for limited and extensive disease are shown in **Table 4b**. Chronic GVHD was treated with either CSP (6 mg/kg orally) or PRED (starting dose 2 mg/kg orally in three divided doses) as first line treatment. A dose reduction or tapering of the dose was recommended with improvement or resolution of chronic GVHD. In patients unresponsive to first-line therapy, tacrolimus, azathioprine, mycophenolate-mofetil or psoralene and ultraviolet A (PUVA) radiation were used.

Table 4a Staging and grading of acute GVHD [Przepiorka, D. et al. 1995]

	Extent of organ involvement		
	Skin	*Liver*	*Gut (modification for pediatric patients)*
Stage			
1	Rash on < 25% of skin	Bilirubin[a] 34 – 51 µmol/l	Diarrhea > 500 ml/day (> 30 ml/kg or 500 ml/day) or persitent nausea[b]
2	Rash on 25 to 50% of skin	Bilirubin 52 – 103 µmol/l	Diarrhea > 1000 ml/day (> 60 ml/kg or 1000 ml/day)
3	Rash on > 50% of skin	Bilirubin 104 – 257 µmol/l	Diarrhea > 1500 ml/day (> 90 ml/kg or 1500 ml/day)
4	Generalized erythroderma with bullous formation	Bilirubin > 257 µmol/l	Severe abdominal pain with or without ileus
Grade			
I	Stage 1 - 2	None	None
II	Stage 1 - 3	Stage 1 and/or	Stage 1
III	Stage 2 - 3	Stage 2 - 3 and/or	Stage 2 - 3
IV	Stage 2 - 3	Stage 4 and/or	Stage 2 - 4

[a]Range given as total bilirubin, converted to SI units;

[b]Persistent nausea with histologic evidence of GVHD or stomac or duodenum.

Table 4b Clinicopathological classification of chronic GVHD

[Sullivan, K. M. et al. 1991]

Limited *Chronic GVHD*
Either or both: 1. Localized skin involvement 2. Hepatic dysfunction due to chronic GVHD
Extensive *Chronic GVHD*
Either: 1. Generalized skin involvement, or 2. Localized skin involvement and/or hepatic dysfunction due to chronic GVHD *Plus:* 3a. Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis, or 3b. involvement of eye (Schirmer test with <5 mm wetting), or 3c. involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or 3d. involvement of any other target organ

2.5 Treatment of relapse after SCT

In patients who did not have GVHD and in whom relapse, progression of malignancy, or increasing host chimerism (increasing quantities of autologous hematopoietic cells) was diagnosed, immunosuppressive therapy was rapidly tapered to induce graft-versus-leukemia (GVL) effects [Bader, P. et al. 1997]. Therefore chimerism analysis in peripheral blood was performed weekly until day +100 and monthly until day +365 (only in group A patients) [Bader, P. et al. 1996] [Beck, J. F. et al. 2002]. Chimerism analysis in bone marrow was perfomed between day 28 and 35 after SCT and also d+100, d+180 and d+365. Survival was a secondary endpoint.

2.6 Monoclonal IL-2R antibodies (anti-CD25)

Inolimomab (BT563, Leucotac®, Biotest) is a murine monoclonal antibody (mab) against CD25 of the immunoglobulin G1 (IgG1) isotype. It was shown to inhibit the proliferation of activated IL-2 dependent T cells [Herve, P. et al. 1990].
Basiliximab (Simulect®, Novartis) is a gentically engineered chimeric (human and murine) mab of the Immunglobulin G1 (IgG1) isotype consisting of human constant and mouse heavy and light chain variable regions [Onrust, S. V., Wiseman, L. R. 1999]. The murine sequence confers specificity against human CD25. It inhibits IL-2 stimulated T lymphocyte proliferation [Amlot, P. L. et al. 1995].
Daclizumab (Zenapax®, Roche) is a genetically engineered humanized monoclonal IgG1 anti-CD25 mab retaining only the CDR from the mouse. It competitively antagonises IL-2 dependent T cell proliferation. The affinity of the humanized anti-CD25 is lower than that of the parent mouse antibody. However, the humanized antibody has the ability to mediate antibody-dependent cell-mediated cytotoxicity of CD25+ cells in vitro. Daclizumab does not activate complement-dependent lysis in vitro [Junghans, R. P. et al. 1990]. It was found to be less immunogenic, to have more favorable pharmacokinetics, and to be more immunosuppressive than native murine antibody [Brown, PS. Jr et al. 1991] [Hakimi, J. et al. 1991] [Carswell, C. I. et al. 2001].

2.7 Flow cytometry and CD25 blockade

Blood samples were obtained just before treatment and thrice weekly up to day +120 after SCT. Cell surface phenotype was evaluated by flow cytometry (FACS). CD3 positive (CD3+) cells were enumerated by multiparameter flow cytometry with the use of fluoresceinisothiocyanat (FITC)-conjugated anti-CD3, clone SK7 (Becton Dickinson), which reacts with the e chain of the TCR complex. Phycoerythrin (PE)-conjugated anti-CD25, clone 2A3 (different from BT563), which binds to the low-affinity interleukin-2 receptor was used in double labelling assays together with anti-CD3 after gating on lymphocytes (Becton Dickinson). Stained cells were analyzed on a FACSCalibur. Data were processed using CellQuest Pro software, version 4.0.2 (Becton Dickinson). Absolute numbers of CD3+ cells in peripheral blood were also determined once a week.
Absence of CD3+CD25+ double positive (CD25+) cells by FACS (staining of CD25 <1%) was defined as complete CD25 blockade and implies complete functional blockade of the IL-2Ra by the monoclonal antibody.

2.8 Statistical analysis

Demographic factors were summarized using percentages or median and range values. Categorical factors were compared using chi-square tests. Survival curves were evaluated using the method of Kaplan and Meier and compared using a log-rank test. Time-to-event outcomes were compared using a log-rank test. Incidence of chronic GVHD was calculated only amongst patients surviving beyond day +90.