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Efficacy of CD 25 blockade as targeted adjuvant therapy in the prevention of GVHD in pediatric stem cell transplant recipients

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Project and bibliography

Prevention of Graft-versus-host disease (GVHD) in patients treated with allogeneic stem cell transplantation (SCT) can reduce morbitity and mortality. Considering the major role of activated T cells in pathophysiology of GVHD, monoclonal antibodies against interleukin-2 receptor a chain (anti-CD25) were used for reducing T cell activation and proliferation in patients after allogeneic SCT. First, we assessed the safety in 11 patients (n=11) and CD25 blockade (n=9) under treatment with chimeric or humanized anti-CD25 (ch/anti-CD25) in pediatric allogeneic SCT. Ch/anti-CD25 (1 mg/kg) was given 6 hours before SCT and on day 4 (d+4), +28, +56 and +84 after SCT and was well tolerated. 6/11 patients completed the treatment protocol. 3/6 patients showed complete CD25 blockade (<1% CD25+ T cells by FACS detected in peripheral blood) until d+100. In the other 3 patients duration of CD25 blockade was 13 ± 2.2, 16 ± 2.5 and 23 days after last antibody application. Patients suffering from chronic GVHD and showing CD25+ cells received another 2 to 5 anti-CD25 applications after d+100. The mean time of CD25 blockade in these patients ranged from 21 ± 3 days [19; 23] to 55 ± 11 days [46; 64] 95%CI (mean, SD, [95%CI]). Next, we compared the incidence of GVHD, relapse and survival in 34 patients receiving allogeneic stem cell transplants under treatment with either prophylactic ch/anti-CD25 (group A, n=11) or prophylactic murine (m/anti-CD25, group B, n=13) or without anti-CD25 (group C, n=10) after SCT. The incidence of acute GVHD grade II-IV in ch/anti-CD25 receiving patients was not lowered compared to patients receiving murine or no anti-CD25 (0.6 vs. 0.54 vs. 0.4). Moreover, a significantly higher incidence of limited but not extensive chronic GVHD was seen in group A in comparison to group B (0.75 vs. 0.22; p=.036) but not in comparison to group C. The higher incidence of limited chronic GVHD was probably caused by the higher rate of mature T cells transplanted with the peripheral blood in group A as compared to bone marrow in group B and C. Patients in group A had earlier engraftment possibly due to the same reason compared with group B and C (14 vs. 23 vs. 20.5 days; p<.015). Although probability of overall survival (0.22 vs. 0.54 vs. 0.6) and leukemia free survival (EFS, 0.11 vs. 0.54 vs. 0.6) was not significantly different between all groups, we observed a trend towards superior EFS in groups B and C. More cumulative chemotherapy in group B and C patients due to longer treatment before transplant as well as more immunosuppressive treatment due to chronic GVHD in group A may have contributed to this trend. Our findings may also suggest a role of CD25 positive T cells in the balance of achieving tolerance and leukemia control. The complex role of CD25 in regulatory and effector T cells of allo- and leukemia recognition warrants further investigation.

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Lebenslauf

Persönliche Erklärung

Danksagung

Abbreviations

A	group A patients, treated with humanized or chimeric anti-CD25
aGVHD	acute graft-versus-host disease
AICD	activation-induced cell death
AL	acute leukemia
ALL	acute lymphocytic leukemia
AML	acute myeloic leukemia
ANC	absolute neutrophil count
anti-CD25	monoclonal antibody against interleukin-2 receptor a chain
anti-CD52	monoclonal antibody against CD52 receptor, (used therapeutically to deplete T cells)
APCs	antigen presenting cells
ATG	antithymocyte globuline
B	group B patients, treated with murine anti-CD25
Bas	basiliximab, a chimeric monoclonal antibody against CD25
BFM-study	Berlin-Frankfurt-Münster-study
BM	bone marrow
BMT	bone marrow transplantation
BT563	inolimomab, a murine monoclonal antibody against CD25
Bu	busulfan
C	group C patients, without CD25 antibody treatment
c-ALL	acute lymphocytic leukemia, common type
CB	cord blood
CBSC	cord blood stem cells
CBT	cord blood transplantation
CD	clusters of differentiation
CD3	T cell receptor complex
CD3+	CD3 positive
CD25	interleukin 2 receptor a chain, Tac
CD25+	CD25 positive
CD4+CD25+	CD4 and CD25 double positive
CD122	interleukin 2 receptor ß chain
CD132	interleukin 2 receptor ? chain
CDRs	complementarity determining regions
cGVHD	chronic graft-versus-host disease
ch/anti-CD25	chimeric or humanized anti-CD25

chi-square	chi-square test
CI	confidence interval
COALL	cooperative ALL study
CR	complete remission
CSP	cyclosporine A
CTL	cytotoxic T cells
CTLA-4	high affinity receptor for costimulatory molecules on T cells
Cy	cyclophosphamide
d+x	day x from allogeneic transplantation
Dac	daclizumab, a humanized monoclonal antibody against CD25
D	donor
DOC	death of complication
DOD	death of disease
EFS	event free survival, leukemia free survival
Eto	etoposide
F	female
FAB	French-American-British classification of acute myelocytic leukemia
FACS	fluorescence-activated cell sorter
FAS	FAS receptor, member of the TNF receptor family
FITC	fluoresceinisothiocyanat
G-CSF	granulocyte-colony stimulating factor
GI	gastrointestinal
GM-CSF	granulocyte-macrophage-colony stimulating factor
GVHD	graft-versus-host disease
GVL	graft-versus-leukemia effect
Gy	gray
HLA	human leukocyte antigen
IFN	interferon
IL	interleukin
IL-2	interleukin-2
IRB	internal review board
i.v.	intravenously
IgG1	immunoglobulin G1
IL-2R	interleukin-2 receptor
IL-2Ra	interleukin-2 receptor a chain
KGF	keratinocyte growth factor
log-rank	log-rank test

LPS	lipopolysaccharide
M	male
mab	monoclonal antibody
m/anti-CD25	murine anti-CD25
M-BCR/ABL	rearrangement of t(9;22)
MDS	myelodysplastic syndrome
Me	melphalan
MHC	major histocompatibility antigens
mHC	minor histocompatibility antigens
MMF	mycophenolate-mofetil
MØ	monocyte
MTX	methotrexate
MUD	matched unrelated donor
n	number of patients
NK	natural killer cells
NR	non response
OAS	overall survival
PB	peripheral blood
PBSC	peripheral blood stem cells
PE	phycoerythrin
PPR	prednisone poor response
PRED	6-methylprednisolone
PSC	peripheral stem cells
PUVA	psoralene and ultraviolet A radiation
R	recipient
RAEB	refractory anemia with excess blasts
SCT	stem cell transplantation
SD	standard deviation
T-ALL	acute lymphocytic leukemia, T cell type
TBI	total body irradiation
TCR	T cell receptor
Th1	T helper-1 cell
TNF	tumor necrosis factor
UCB	unrelated cord blood
UPN	unique patient number
VOD	venoocclusive disease
yrs	years

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1 Introduction

1.1 Importance of graft-versus-host disease in allogeneic stem cell transplantation

Graft-versus-host disease (GVHD) is the most important complication of allogeneic stem cell transplantation (SCT) and the principal risk factor for transplant-associated morbidity and mortality. The reported incidence for acute GVHD grade II-IV in adult patients after transplantation from human leukocyte antigen (HLA)-matched related donors is 33%, in spite of immunosuppressive drugs such as cyclosporine A (CSP), methotrexate (MTX) and prednisolone (PRED) used for prevention of GVHD [Storb, R. et al. 1986]. Although younger patients tend to develop GVHD less frequently [Weisdorf, D. et al. 1991], the risk for GVHD increases with the expanded use of unrelated donors [Montagna, D. et al. 1996]. The use of allogeneic peripheral blood stem cells (PBCS) instead of bone marrow (BM) leads to a higher risk of acute and chronic GVHD [Cutler, C. et al. 2001]. However this issue has not been extensively addressed in a pediatric population.

1.2 Pathophysiology of graft-versus-host disease

GVHD is caused by donor T lymphocytes reactive against minor and major histocompatibility antigens (mHC, MHC) of the host [den Haan, J. M. et al. 1995] [Nash, R. A., Storb, R. 1996]. According to Ferrara and Antin the pathophysiology of acute GVHD can be summarized as a three-step process (**Figure 1**). First, chemotherapy and/or radiation conditioning regimen lead to tissue damage, activation of host cells and secretion of cytokines such as tumor necrosis factor alpha (TNF-a), interleukin (IL)-1, granulocyte-macrophage-colony stimulating factor (GM-CSF) and many others. The second phase of GVHD consists of donor T cell activation and proliferation of T helper 1 (Th1) T cells. T cell activation requires the T cell receptor (TCR)-peptide-MHC interaction and second (costimulatory) contact with antigen presenting cells (APCs). T cells that secrete IL-2 and interferon (IFN)-? (type 1 cytokines) are critical mediators of acute GVHD. In the third phase monocytes primed by type 1 cytokines and lipopolysaccharide (LPS) secrete IL-1 and TNF-alpha. These cytokines and IL-2 can cause direct tissue damage. TNF-alpha can also cause apoptosis via the TNFa-FAS pathway. In addition cytotoxic T cells and natural killer

(NK) cells lead to target tissue destruction [Krenger, W. et al. 1997] [Ferrara, J. L. 2000] [Hill, G. R., Ferrara, J. L. 2000] [Jacobsohn, D. A., Vogelsang, G. B. 2002].

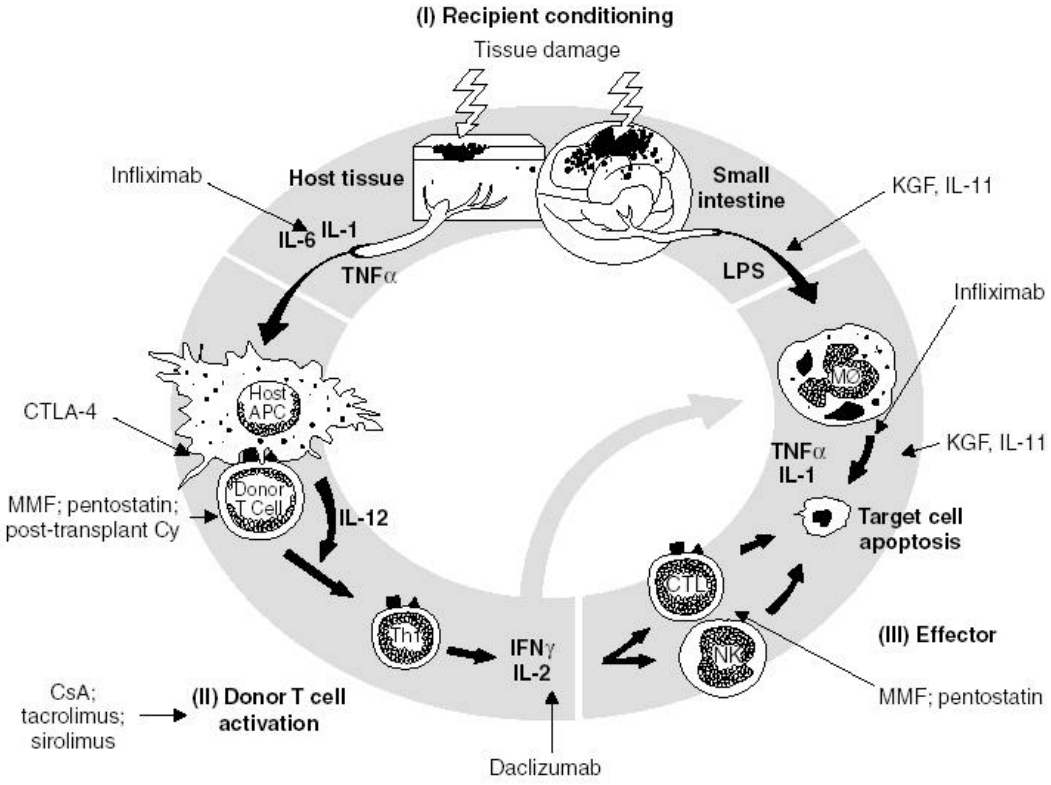

Figure 1 Acute graft-versus-host disease (GVHD): pathophysiology and pharmacotherapeutic intervention [Hill, G. R. et al. 2000]**.** The three sequential phases of GVHD (I, II, II) are detailed. Therapeutic agents are shown in relation to the phases of GVHD they disrupt.

APC = antigen presenting cell; CsA = cyclosporine A; CTL = cytotoxic T cells; CTLA-4 = CTLA-4 monoclonal antibody; Cy = cyclophosphamide; Daclizumab = humanized interleukin-2 receptor antibody; IL = interleukin; IFN = interferon; KGF = keratinocyte growth factor; LPS = lipopolysaccharide; MØ = monocyte; MMF = mycophenolate-mofetil; NK = natural killer cell; Th1 = T helper 1 cell; TNF = tumor necrosis factor.

1.3 Reducing T cell proliferation and activation with monoclonal antibodies against IL-2 receptor a chain

T cell depletion of the graft can dramatically reduce the incidence of GVHD but results in increased engraftment failure and higher risk of leukemia relapse [Kernan, N. A. et al. 1989]. Various investigators have attempted to achieve specific down regulation of T cell activation associated with less GVHD and immune control of leukemia.
The IL-2 receptor (IL-2R) is a heteromultimer comprised of the a (p55, CD25), ß (p75, CD122) and ? (p64, CD132) chains [Waldmann, T. A. 1986] [Takeshita, T. et al. 1992]. The IL-2Ra chain (Tac, CD25) is found predominantly on activated cytotoxic T cells and binding of monoclonal antibodies specific to IL-2Ra blocks the proliferation induced by IL-2 and provides selective immunosuppression [Uchiyama, T. et al. 1981] [Depper, J. M. et al. 1983]. In several models of solid organ transplantation murine, chimeric or humanized CD25 antibodies can prevent rejection of allografts in vivo [Nashan, B. et al. 1997] [Bumgardner, G. L. et al. 2001] [Carswell, C. I. et al. 2001]. Preliminary reports indicate that anti-CD25 may also be useful for treatment of steroid refractory GVHD [Cahn, J. Y. et al. 1995] [Anasetti, C. et al. 1994] [Basara, N. et al. 2000] [Przepiorka, D. et al. 2000] [Willenbacher, W. et al. 2001]. Monoclonal antibodies against CD25 have also been administered for GVHD prophylaxis in adult bone marrow transplant (BMT) patients with matched related donors [Ferrant, A. et al. 1995], with mismatched related [Blaise, D. et al. 1991] [Anasetti, C. et al. 1991] and unrelated BMT [Belanger, C. et al. 1993].

1.4 Differential biologic properties between rodent, chimeric and humanized antibodies

Rodent derived monoclonal antibodies (including murine BT563/ inolimomab or rat derived 33B3.1) often cause immunologically mediated acute adverse reactions against xenogenic proteins. In addition, the therapeutic effect may be shortlived because anti-idiotype antibodies are generated and neutralize the therapeutic antibody, thus promoting rapid clearance from the circulation. Chimeric antibodies (such as basiliximab) consist of human constant regions and murine heavy and light chain variable regions. They retain the binding specificity of the original murine antibody and contain fewer amino acid sequences foreign to the human immune system. In humanized antibodies (such as daclizumab) only the complementarity determining regions (CDRs) of the original murine antibody which are primarily responsible for the unique binding characteristics of the antibody are transferred into human framework.

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).

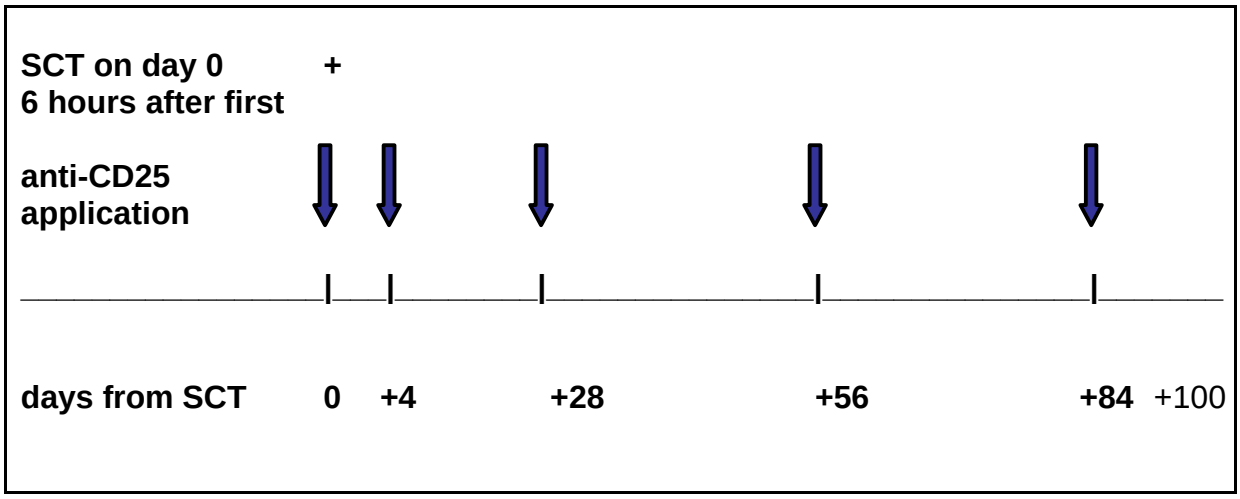

Figure 2a Administration of monoclonal chimeric or humanized interleukin-2 receptor a **antibody (ch/anti-CD25)** given 6 hours before allogeneic peripheral stem cell transplantation (SCT) and on days +4, +28, +56 and +84 after SCT in patients of group A.

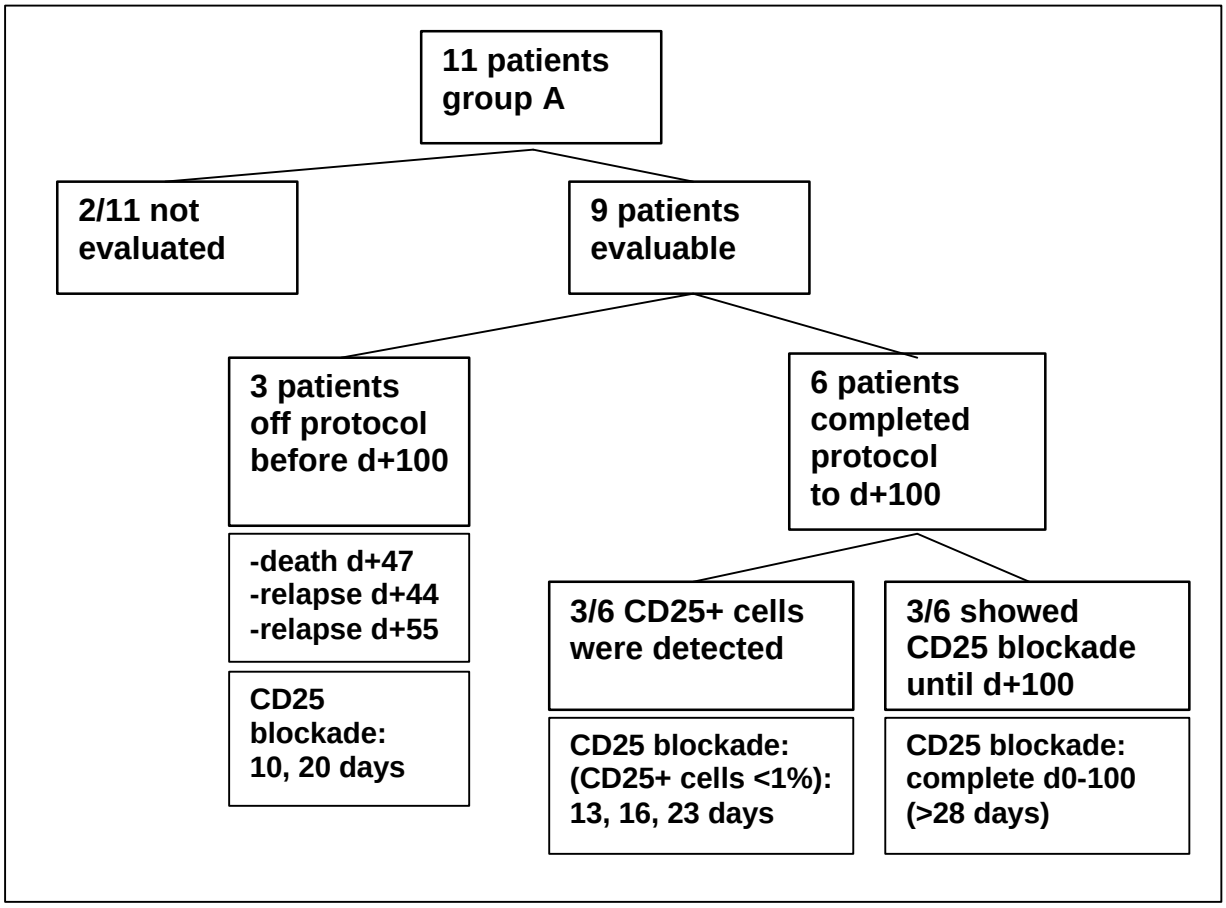

Figure 2b Patients in group A entered on protocol (ch/anti-CD25) shown in Figure 2a. CD25 Blockade was complete (CD25+ cells <1%, flow cytometry) from day 0 to +100 in 3/6 patients that completed protocol. In 3/6 patients CD25+ cells were detected.

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.

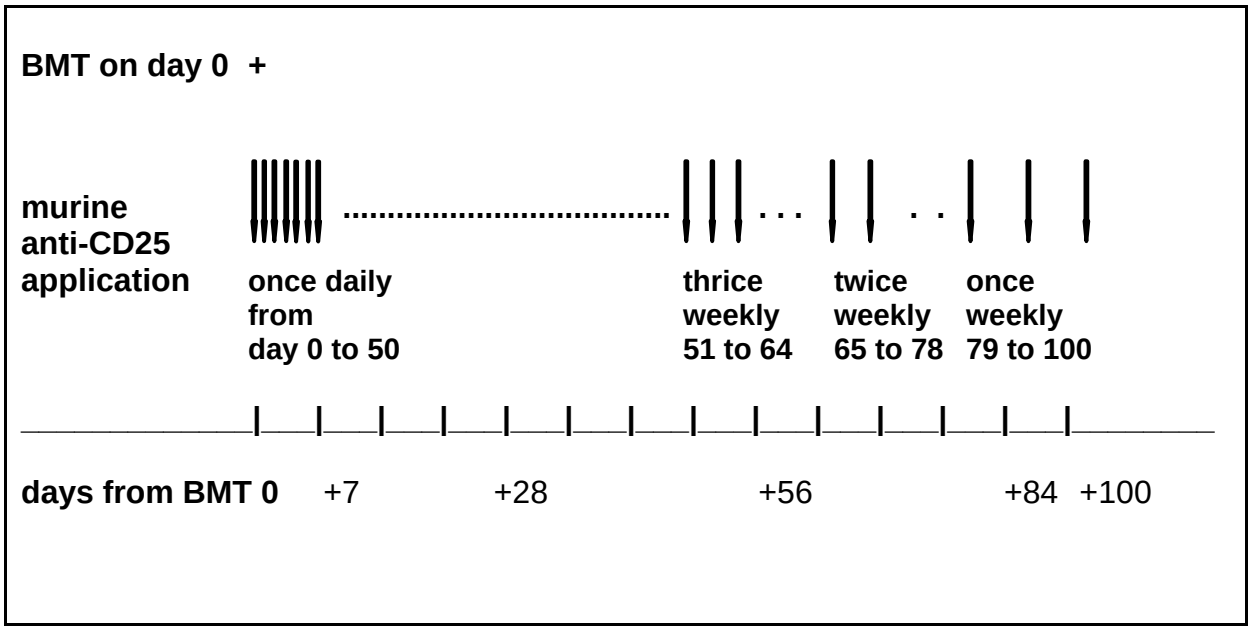

Figure 3 Administration of monoclonal murine interleukin-2 receptor a antibody (m/anti-CD25) after allogeneic bone marrow transplantation (BMT) in group B patients.
Murine anti-CD25 was given in constant doses of 0.1 mg/kg once daily from day 0 to 50 (maximal 5 mg/kg as total single dose), and was tapered 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.

3 Results

3.1 Clinical safety of chimeric or humanized anti-CD25 (ch/anti-CD25)

Five infusions of monoclonal IL-2 receptor antibody (anti-CD25) were planned according to protocol between day 0 and day +100 after SCT (**Figure 2a**). 7/11 patients received chimeric anti-CD25 (basiliximab 1 mg/kg, maximum 40 mg), 4/11 patients received humanized anti-CD25 (daclizumab 1 mg/kg, maximum 50 mg). 4/7 basiliximab and 1/4 daclizumab patients did not receive the full dose of study medication: 3 patients (UPN 1031, 1037, 1009) experienced leukemic relapse after three or four infusions (2 basiliximab and 1 daclizumab receiving patients), another 2 basiliximab patients (UPN 1006, 1026) had multiorgan failure after two respectively three antibody infusions due to adenovirus infection or venoocclusive disease (VOD) plus adenovirus infection (patient with VOD died on day +28). In 2/11 patients (1 basiliximab, UPN 1022; 1 daclizumab, UPN 1044) one additional infusion resulting in a total of six antibody applications was performed until day +100 because lymphocytes stained positive for CD25 (**Table 5**). 5/11 patients who suffered from chronic GVHD were treated after day +100 with another infusion at the same dose as soon as CD25+ cells were detected in the peripheral blood (**Table 6**). Thus, study patients received 2 to 10 infusions beginning with day 0 of SCT. Another patient received one single infusion daclizumab in preparation for an unrelated SCT for AML in first complete remission. She subsequently withdrew from unrelated SCT because of pancreatitis during chemotherapy.

None of the antibody infusions were followed by adverse events related to the infusion of the antibody. There were no changes in serum chemistries after treatment with ch/anti-CD25. There was no evidence of other toxicities. 1 patient inadvertently received daclizumab 2 mg/kg without suffering any infusion-related side effects.

3.2 Efficacy of CD25 blockade after application of chimeric or humanized anti-CD25 (ch/anti-CD25)

3.2.1 Analysis of receptor blockade before transplant

5 patients received a single dose of ch/anti-CD25 before stem cell transplantation to determine the duration of the CD25 blockade (defined above) and the clinical safety

without transplant. Saturation of IL-2 receptor by anti-CD25 occurred in all patients. Moreover, CD25 blockade was always observed with the first analysis after administration i.e. within the first 43 hours (median; range 24 to 96 hours) following the first application. In 4/5 patients the duration of CD25 blockade by basiliximab lasted at least 18 days (patient observation period was 18 to 26 days). In these 4 patients the next infusion of basiliximab was performed before CD25 positive cells were detected because they proceeded to allogeneic SCT. In the remaining patient receiving daclizumab, the CD25 blockade was complete until day 77 after the single infusion. No additional dose of daclizumab was given, because the patient withdrew from study. In summary, in the evaluable patients complete CD25 blockade was observed after a single dose for an observation period of 18 to 77 days.

3.2.2 Analysis of receptor blockade after transplant from day 0 to day +100

Table 5 displays the course of CD25 blockade between day 0 and day +100 in 9/11 group A patients receiving ch/anti-CD25 after SCT. As shown in **Figure 2b**, 6/11 patients completed treatment protocol. CD25 blockade was complete from day 0 to day +100 in 3/6 patients (UPN 1032, 1036, 1013) receiving anti-CD25 according to the protocol shown in **Figure 2a**.

In 3/6 patients (1 basiliximab and 2 daclizumab receiving patients) CD25+ cells could be detected between day 0 and d+100 while on protocol (UPN 1022, 1044, 1049). Duration of complete CD25 blockade in these patients was 13 ± 2.2, 16 ± 2.5 and 23 days after last antibody application. In 2/6 patients (UPN 1022, 1044) the detection of CD25+ cells prompted additional application of anti-CD25 prior to the next application according to protocol. 3 additional patients (UPN 1027, 1037, 1031) went off protocol early because of death or relapse. 2 of these were evaluable for duration of receptor blockade after early termination of antibody application. They stained CD25 negative 10 and 20 days (seven and six days before leukemia relapse was diagnosed) and at least 24 days in previous intervals. 2 additional patients failed to be evaluated periodically by CD25 flow cytometry assays (UPN 1006, 1009).

Table 5 Efficacy of CD25 blockade from day 0 to day 100 (d+100) after allogeneic pediatric stem cell transplantation

	ch/anti-CD25		*Days after SCT*	*Time of CD25 blockade* [a]	
			on which	*in PB after the last*	*Cause to finish*
	Generic	*Number of applications (n)*	*CD25+ cells are detected*	*antibody application*	*antibody therapy*
UPN	*name*	*d0 to d+100*	*between d+1 and d+100*	*days (mean, SD)*	
1026	Bas	3[b]	-		[b]DOC d+47
1032	Bas	5	-		
1049	Dac	5	d+84	23	
1044	Dac	6	d+22, +41,+65, +97	16.3 ± 2.5	
1036	Dac	5	-		
1037	Dac	4[c]	d+51[c]	20	[c]relapse d+55
1013	Bas	5	-		
1006	Bas	2[d]	n.e.	n.e.	[d]mulitorgan failure d+24
1009	Bas	4[c]	n.e.	n.e.	[c]relapse d+83
1022	Bas	6	d+16, +59, +90	13.0 ± 2.2	
1031	Bas	3[c]	d+43[c]	10	[c]relapse d+44

SCT = stem cell transplantation; ch/anti-CD25 = chimeric or humanized monoclonal antibody against interleukin-2 receptor a chain; Bas = basiliximab; Dac = daclizumab; n = number of antibody applications; SD = standard deviation; DOC = death of complication; n.e. = not evaluated; CD25+ cells = CD25 positive T cells in peripheral blood; PB = peripheral blood;

[a]CD25 blockade in PB = CD25 positive cells were not detectable in peripheral blood by flow cytometry (CD25+ <1%). Cause of finish antibody therapy: [b]death of complication; [c]relapse; [d]mulitorgan failure.

3.2.3 Analysis of receptor blockade after day +100 in group A patients (ch/anti-CD25) suffering from chronic GVHD

After d+100 ch/anti-CD25 was further given to 5 patients of group A suffering from chronic GVHD and showing increasing levels of CD25+ cells. They received another 2 to 5 infusions after d+100; the next application was given as soon as CD25+ cells were detected (**Table 6**). The mean time of CD25 blockade in these chronic GVHD patients ranged from 21 ± 3 days [19; 23] to 55 ± 11 days [46; 64] 95%CI (mean, SD, [95%CI]). The mean duration of CD25 blockade was 37.3 ± 12.8 days (mean, SD) of all antibody applications after d+100. Interpatient variability was greater than intrapatient variability. In the 2 patients (UPN 1044, 1022) in whom we had detected CD25+ cells within the first 100 days after SCT (incomplete CD25 blockade with treatment schedule), we also observed periods of CD25 blockade shorter than 28 days following antibody applications after d+100. In this low number of patients there was no correlation with body weight, body surface area, creatinine, renal clearance or other clinical parameters of the patients.

Table 6 Efficacy of CD25 blockade in group A patients with chronic GVHD

	ch/anti-CD25		*Time of CD25 blockade* [a] *in PB after antibody application*	
UPN	*Generic name*	*Number of applications (n) after d+100*	*Days (mean, SD)*	*CI 95%*
1032	Bas	3	48.7 ± 13.6	[33; 64]
1049	Dac	2	35.0 ± 9.9	[21; 49]
1044	Dac	5	21.2 ± 2.6	[19; 23]
1036	Dac	5	54.8 ± 10.5	[46; 64]
1022	Bas	3	26.7 ± 6.5	[19; 34]

ch/anti-CD25 = chimeric or humanized monoclonal antibody against interleukin-2 receptor a chain; Bas = basiliximab; Dac = daclizumab; d+100 = day +100 after SCT; PB = peripheral blood; CI = confidence interval; n = number of antibody applications;
[a]CD25 blockade in PB = CD25 positive cells were not detectable in peripheral blood by flow cytometry (CD25+ <1%).

3.3 Absolute numbers of T cells in group A patients (ch/anti-CD25 treatment)

Absolute numbers of T cells (CD3 positive cells, CD3+ cells) in group A patients from day 0 to +105 after SCT are shown in **Figure 4**. Between day 0 and +100 after SCT only 1/6 patient (UPN 1013) achieved CD3+ cell counts >700/µl, which represents the 5th percentile of the normal range [Comans-Bitter, W. M. et al. 1997]. 5/6 patients achieved CD3+ cells >500/µl by day +365. A median time to >500/µl CD3+ cells of the 6 patients was 108 days (range 31 to 480). The patient with prolonged lymphopenia (UPN 1036) suffered from extensive chronic GVHD and was treated first with CSP, prednisolone, mycophenolate-mofetil, humanized anti-CD25. After day 500 he received a T cell depleting antibody (anti-CD52, alemtuzumab, MabCampath®, Schering) and never reached normal T cell levels again before he expired on day +593. In addition, 2 patients with early relapse (UPN 1031, 1037) and 1 patient died due to VOD and adenovirus infection (UPN 1026) failed to achieve T cell counts >500/µl. 2/11 patients failed to be evaluated periodically by CD3 and CD25 flow cytometry assays as indicated before.

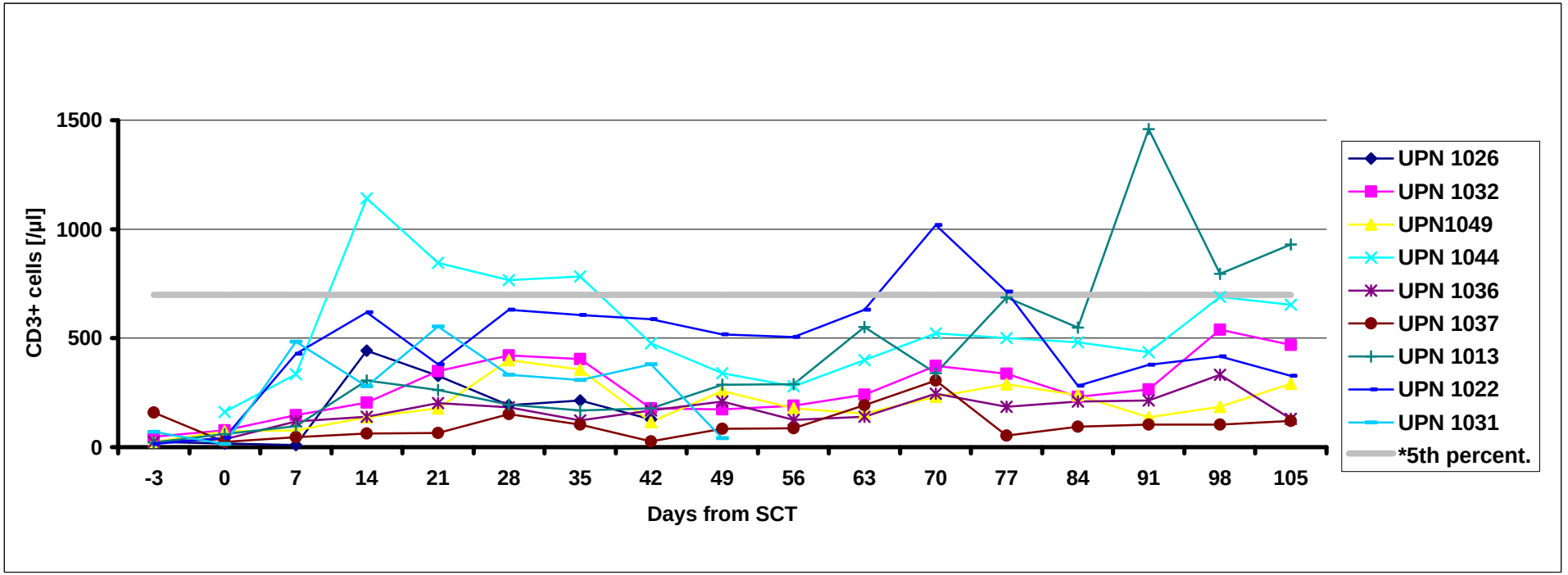

Figure 4 Absolute T cell counts (CD3+ cells) between day 0 and +100 after stem cell transplantation (SCT) of group A patients receiving chimeric or humanized CD25 antibody (ch/anti-CD25 treatment).

Only 1 patient achieved CD3+ cell counts of >700/µl (lower 5% of normal variation) until day +100.

*5th percent. = 5th percentile of the normal range for patients aged 5-10 years and adults [Comans-Bitter, W. M. et al. 1997].

Endpoints of evaluation before day +100 were death of disease on d+51 (UPN 1031) and death of complication on d+47 (UPN 1026). Another patient (UPN 1037) suffered from relapse of ALL on d+55, however he was evaluable for CD3+ counts until d+100.

3.4 Engraftment

In group A (ch/anti-CD25 treatment) 9/11 patients received unmanipulated PBSC with a transplanted cell dose of 9.63 ± 5.07 x 10^6 CD34+ cells/kg. All PBSC patients engrafted, median time from transplantation to neutrophil engraftment was 14 days (range 11 to 25). There was only one secondary graft failure on d+148 during chronic GVHD in a patient who had received the lowest cell dose (3.05 x 10^6 CD34+ cells/kg) because of circulatory arrest of his donor during apheresis [Cassens, U. et al. 2003]. Pancytopenia resolved after intensifying immunosuppression for GVHD treatment. 2/11 patients received unrelated CBSC transplants containing 0.355 ± 0.02 x 10^6 CD34+ cells/kg and 7.77 ± 0.04 x 10^7 nucleated cells/kg. There was no primary or secondary graft failure. Neutrophil engraftment was observed on d+46 and d+26 after unrelated cord blood transplantation (median 36 days).

All group B patients (m/anti-CD25 treatment) were transplanted by unmanipulated bone marrow with 6.38 ± 2.89 x 10^8 nucleated cells/kg recipient. The number of CD34+ cells was not assessed. The median time from BMT to =500/µl neutrophils was 23 days (range 17 to 44). 2/13 patients in the group B suffered from secondary graft failure, treatment with granulocyte-colony stimulating factor (G-CSF) was followed by neutrophil recovery.

All patients of group C (no anti-CD25) were transplanted with unmanipulated bone marrow of HLA-identical siblings with 4.99 ± 2.05 x 10^8 nucleated cells/kg recipient. Median time to neutrophil engraftment was 20.5 days (range 14 to 44 days). There was no primary or secondary graft failure.

There was a significant shorter time to engraftment in group A patients receiving PBSC as in group B patients (median 14 vs. 23 days; p=.010) and group C patients (14 vs. 20.5 days, p=.012; log-rank test) receiving bone marrow grafts.

3.5 Incidence of acute and chronic GVHD

Incidence of acute GVHD was observed in 7 of the evaluable 10 group A patients (ch/anti-CD25 treatment), in 6/10 (0.6) we saw GVHD grade II-IV (**Figure 5**). Severe acute GVHD (GVHD grade III+IV) was seen in 5/8 PBSC receiving patients and in 0/2 CBSC transplanted patients, yielding 5/10 patients (0.5). The median day of onset of acute GVHD was 17 days (range 9 to 26) for all group A patients and did not differ between the two monoclonal antibodies (18 days, range 11 to 24 in basiliximab and 18 days, range 9 to 26 in daclizumab receiving patients). In the patient with venoocclusive disease, adenovirus infection and multiorgan failure (UPN 1026), the severity of hepatic GVHD was not evaluable; there was a stage 1 skin involvement in this patient.

Chronic GVHD was seen in 7/8 evaluable patients of group A (**Figure 6**). Only 1 patient showed extensive disease, the others suffered from limited disease. 3/11 patients are not evaluable for chronic GVHD (death before d+90 in 2 patients, 1 patient received a second transplant). Incidence of chronic GVHD, calculated based on patients who survived beyond d+90 after the first transplant, was 7/8 (0.87) in all patients (including the CBSC receiving patient) and 7/7 (1.0) in the PBSC receiving patients; 6/8 (0.75) patients suffered from limited disease. The 1/8 patient who showed extensive GVHD (UPN 1036) died on d+593 with persistent manifestations of GVHD and after therapy of extramedullary relapse on d+484. GVHD details of group A (ch/anti-CD25) are shown in **Table 7**.

All 13 patients of the murine anti-CD25 receiving group B (m/anti-CD25 treatment) suffered from acute GVHD. As shown in **Figure 5** GVHD grade II-IV was seen in 7/13 (0.54), incidence of severe GVHD was 4/13 patients (0.31). The median onset of acute GVHD was seen on day 22 (range 17 to 40 days). Incidence of chronic GVHD calculated based on patients surviving beyond d+90 was 4/9 (0.44). 2/4 patients showed limited and 2/4 patients showed extensive disease (**Figure 6**). Both patients who suffered from extensive chronic GVHD are alive.

In group C (no anti-CD25 treatment) 4/10 (0.4) patients suffered from acute GVHD grade II-IV, only 1/10 (0.1) patient had severe GVHD. Median onset of acute GVHD was seen on day 22 (range 18 to 71 days). In group C 4/10 patients did not show any signs of acute GVHD.

3/8 patients (0.38) of group C surviving beyond day +90 suffered from chronic GVHD. One of these patients (0.13) showed extensive disease.

In summary, there was no significant difference in the incidence of acute GVHD grade II-IV (0.6 vs. 0.54 vs. 0.4) or of severe (grade III+IV) GVHD (0.5 vs. 0.31 vs. 0.1) in group A (ch/anti-CD25) compared to group B (m/anti-CD25) and group C (no anti-CD25) patients (chi-square test). However, calculated amongst patients surviving beyond day +90, there was a significantly higher incidence of limited chronic GVHD in group A patients compared to group B (0.75 vs. 0.22; p=.036; chi-square test) but not to group C patients (0.75 vs. 0.25). Overall incidence of chronic GVHD (0.87 vs. 0.44 vs. 0.38) and of extensive chronic GVHD (0.13 vs. 0.22 vs. 0.13) was not significantly different comparing group A vs. B vs. C patients.

Table 7 GVHD and outcome of group A patients (ch/anti-CD25 treatment)

UPN	*Acute GVHD Day of onset*	*Grade*	*Stage*			*Chronic GVHD*	*Outcome*	*Disease Status/ Cause of death*
		Overall	*Skin*	*Liver*	*GI*			
1026	12	n.e.[a]	1	n.e.[a]	0	n.e.[b]	[b]DOC d+47	[a]VOD, adenovirus, multiorgan failure
1032	-	0	0	0	0	limited	DOC d+352	aspergillosis, ARDS
1049	26	IV	4	1	0	limited	*alive d+381*	*CR*
1044	9	III	3	1	2	limited	DOD d+300	relapse d+275
1036	23	III	3	0	0	extensive	DOC d+593	relapse d+437/GVHD, liver failure, toxic epidermal necrolysis
1037	13	II	2	1	0	n.e.[c]	DOD d+195	relapse d+55/ DOD after 2. SCT[c]
1013	24	I	1	0	0	limited	DOD d+503	relapse d+484
1006	-	0	0	0	0	limited	*alive d+587*	*CR*
1009	-	0	0	0	0	-	*alive d+438*	*CR* (after CNS relapse d+83[d])
1022	21	III	3	2	0	limited	DOD d+568	relapse d+462
1031	11	III	3	1	2	n.e.[b]	[b]DOD d+51	relapse d+44

GI = gastrointestinal tract; DOC = death of complication; VOD = venoocclusive disease; DOD = death of disease; SCT = stem cell transplantation; CR = complete remission; ch/anti-CD25 = chimeric or humanized anti-CD25;

n.e.[a] = not evaluable because of liver failure due to VOD and adenovirus infection; n.e.[b] = not evaluable because of death before day +90; n.e.[c] = not evaluable because of second SCT; CNS relapse[d] = AML relapse oculomotor nerve.

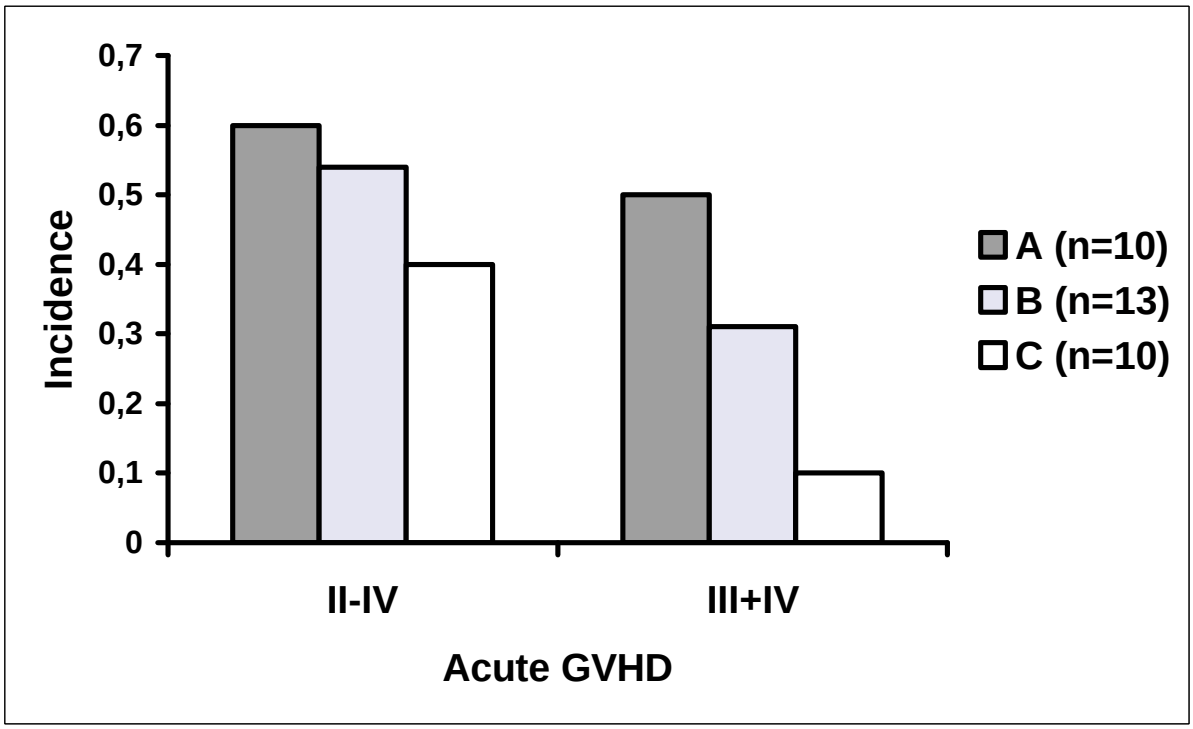

Figure 5 Incidence of acute graft-versus-host disease (acute GVHD)
in group A patients (n=10) receiving chimeric or humanized anti-CD25 compared with group B patients (n=13) receiving murine anti-CD25. No significant difference in acute GVHD grade II-IV: 6/10 (0.6) vs. 7/13 (0.54) and severe acute GVHD grade III+IV: 5/10 (0.5) vs. 4/13 (0.31) was seen. Compared with group C patients (n=10) patients transplanted with matched related donors without receiving antibody therapy incidence of severe GVHD was less, but not significantly decreased (0.5 vs. 0.31 vs. 0.1).
n = number of patients.

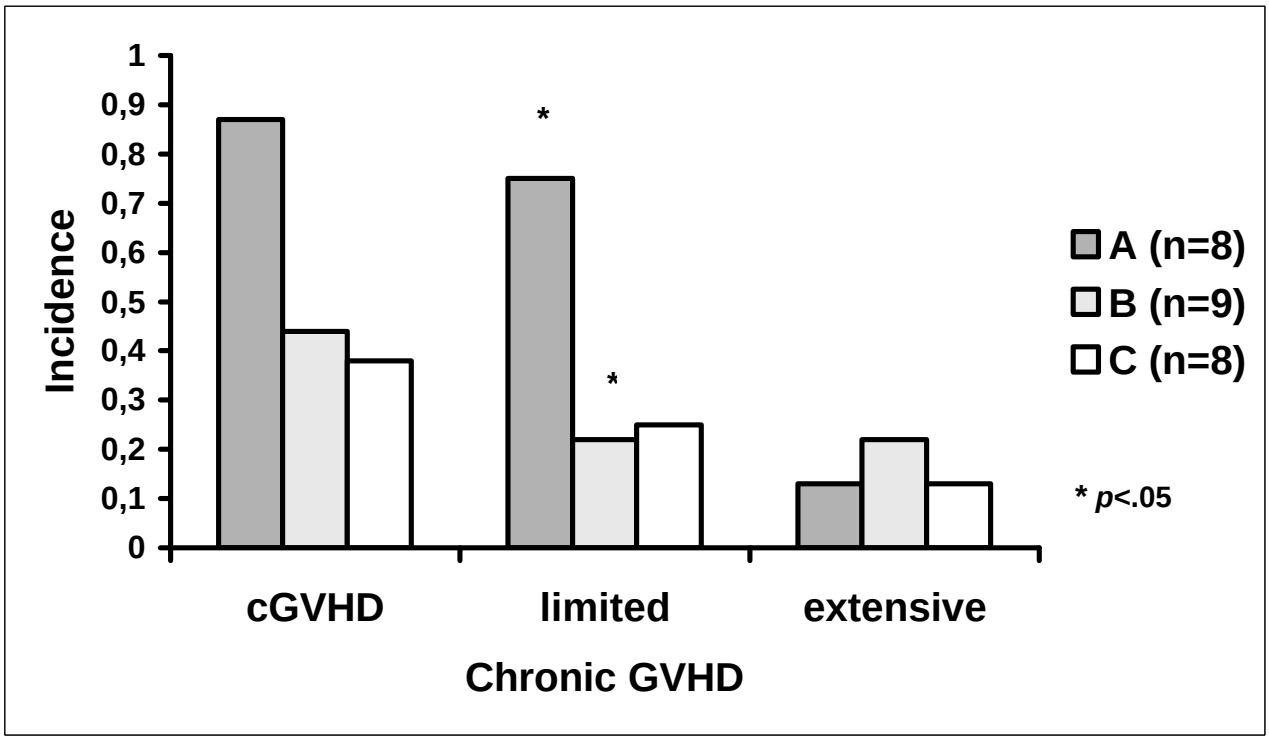

Figure 6 Incidence of chronic graft-versus-host disease (cGVHD) calculated amongst patients who survived beyond day +90 after SCT:

Limited chronic GVHD in group A patients (n=8) receiving chimeric or humanized anti-CD25 after allogeneic SCT was significantly higher as in group B patients (n=9) receiving murine anti-CD25 after allogeneic BMT (0.75 vs. 0.22; *p*=.036; chi-square test).

Incidence of overall chronic GVHD in group A vs. group B vs. group C patients was 7/8 (0.87) vs. 4/9 (0.44) vs. 3/8 (0.38) and incidence of extensive chronic GVHD was 1/8 (0.13) vs. 2/9 (0.22) vs. 1/8 (0.13), which was not significantly different.

Group C consisted of patients transplanted with matched related donors without antibody therapy.

n = number of patients

3.6 Transplant related mortality, relapse and survival

3.6.1 Transplant related mortality (DOC)

Death of complication (DOC) occurred in 3/11 (0.27) group A (ch/anti-CD25 treatment) patients (**Figure 7**). 1/3 patient (UPN 1026) died after VOD, adenovirus infection and multiorgan failure, another patient (UPN 1032) succumbed to respiratory failure due to invasive aspergillosis one year after SCT. A third patient (UPN 1036) developed an extramedullary relapse of his T-ALL on d+437 followed by extensive chronic GVHD with liver failure after withdrawal of immunosuppressive therapy. Cause of death was toxic epidermal necrolysis possibly due to GVHD (**Table 7**).
Transplant related mortality was the cause of death in 3/13 (0.23) group B patients (m/anti-CD25 treatment). 2/3 patients died with overt acute GVHD.
1/10 (0.1) patients of group C (no anti-CD25) deceased after acute respiratory distress syndrome (ARDS) with infection on day +107.

3.6.2 Relapse and death of disease (DOD)

Relapse was diagnosed in 7/11 patients (0.64) of group A (ch/anti-CD25 treatment) and incidence of relapse and DOD is shown in **Figure 7**. 3/7 patients suffering from relapse were transplanted with resistant disease or with relapse: 2 ALL patients (UPN 1037, 1013) and one AML patient (UPN 1031). 2/3 patients with persistent disease at time of SCT suffered from early relapse on day +51 and day +55 (1 AML, 1 ALL patient). The other relapsed ALL patient showed recurrence of leukemia on d+484 after SCT. 2/7 patients (UPN 1036, 1009) suffered from extramedullary relapse (orbita, oculomotor nerve) and another 2 patients (UPN 1013, 1037) showed extramedullary infiltrations (kidney, orbita, skin) before bone marrow infiltration was seen. Relapse after SCT occurred in 4/7 ALL patients and in 3/3 AML patients in this group. Median time to relapse was 179 days (range 44 to 484). Time of relapse and DOD is shown on **Table 7**. 6/11 patients (0.55) of group A died after relapse of leukemia (DOD is shown in **Figure 7**). 1/7 relapsed patients lives in a subsequent remission after CNS relapse of AML on d+83 after allogeneic unrelated CBSC transplantation [Haase, R. et al. 2002].
Incidence of relapse was 4/13 (0.31) in the group B (m/anti-CD25 treated) patients. 2/8 ALL patients and 2/4 AML patients suffered from leukemia recurrence after BMT; 1 of these ALL patients did not achieve complete remission before BMT. Relapse occurred at a median time of 55 days (range 22 to 212) after BMT.

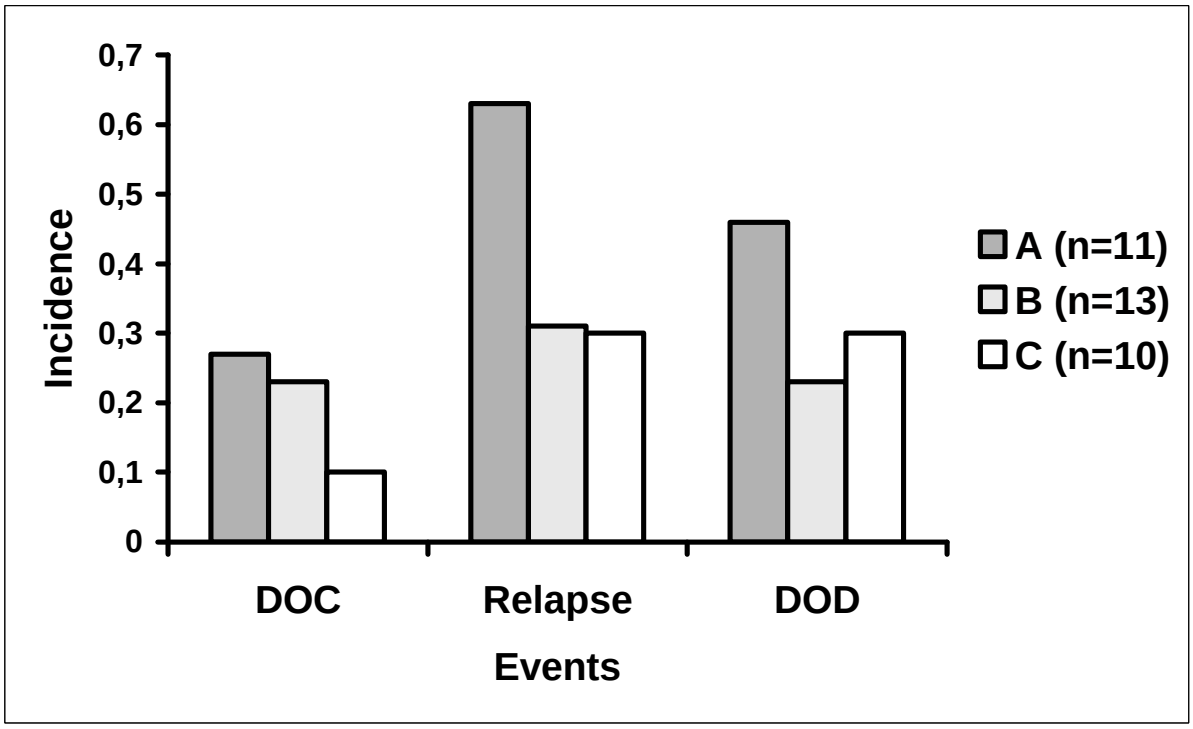

Figure 7 Incidence of relapse and cause of death

Incidence of relapse was 7/11 in group A (n=10) receiving chimeric or humanized anti-CD25 vs. 4/13 in group B patients receiving murine anti-CD25 (0.63 vs. 0.31; not significant; chi-square test). Death of disease (DOD) was 0.53 vs. 0.31 in these groups. In group C consisting of patients transplanted with matched related donors without antibody therapy 3/10 (0.3) patients suffered from relapse.

Death of complication (DOC) occurred in 3/11 group A vs. 3/13 group B vs. 1/10 group C patients (0.27 vs. 0.23 vs. 0.1; not significant).

n = number of patients.

3/4 relapsed patients (0.23) of group B died due to leukemia. The other relapsed patient died with persistent disease and multiorgan failure after withdrawal of immunosuppressive therapy.

In group C (no anti-CD25) incidence of leukemia relapse was 3/10 (0.3). Relapse was diagnosed on day +42, +56 and +217 (median 55 days). 2/3 patients (1 ALL, 1 AML) suffering from early relapse after transplantation did not achieve complete remission at time of BMT. All relapsed patients died due to leukemia (2/6 ALL patients and 1/4 AML patient). 1/3 patient showed extramedullary relapse (testis) of his ALL on day +217 after BMT, he died on day +661 after secondary bone marrow infiltration of ALL.

In summary, the rate of DOC (0.27 vs. 0.23 vs. 0.1) and DOD (0.55 vs. 0.23 vs. 0.3) was not significantly different in group A (ch/anti-CD25) as compared to the groups B (m/anti-CD25) and C (no anti-CD25). Also incidence of relapse (0.64 vs. 0.31 vs. 0.3) was not significantly different (**Figure 7**).

3.6.3 Outcome

As shown in **Figure 8** probability of overall survival (OAS) was 0.22 (3/11 patients) in group A (ch/anti-CD25 treatment) compared to 0.54 (7/13 patients) in group B (m/anti-CD25 treatment) and was not significantly different (p=.35; log-rank test). OAS in group C (no anti-CD25) was 6/10 (0.6) and was also not significantly different compared to A or B. Median time of follow up was 381 days in group A and 2927 days (8 years) in group B and 3656 days (10 years) in group C.

Leukemia free survival (EFS) as shown in **Figure 9** was 0.11 (2/11 patients) in group A vs. 0.54 (7/13 patients) in the group B vs. 0.6 (6/10) in group C. The cumulative EFS was not significantly different in the low number of patients comparing A vs. B (p=.19; log-rank test) as well as A vs. C (p=.10; log-rank test) and also B vs. C.

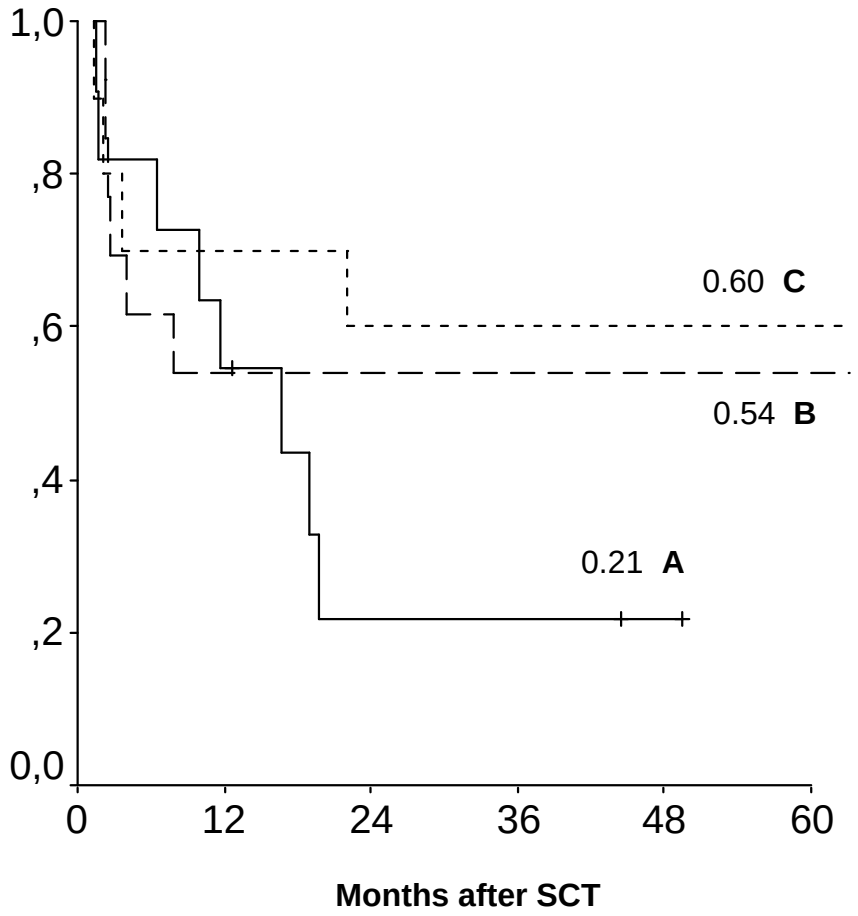

Figure 8 Probability of survival after allogeneic SCT

Kaplan-Meier estimates of overall survival in group A patients (n=11) receiving chimeric or humanized anti-CD25 compared with group B (n=13) treated with murine anti-CD25. Overall survival was 0.22 vs. 0.54 (not significant, *p*=.35, log rank test). Group C (n=10) patients were transplanted with matched sibling donors without receiving antibody therapy and overall survival was not significantly different compared with group A or B. n = number of patients.

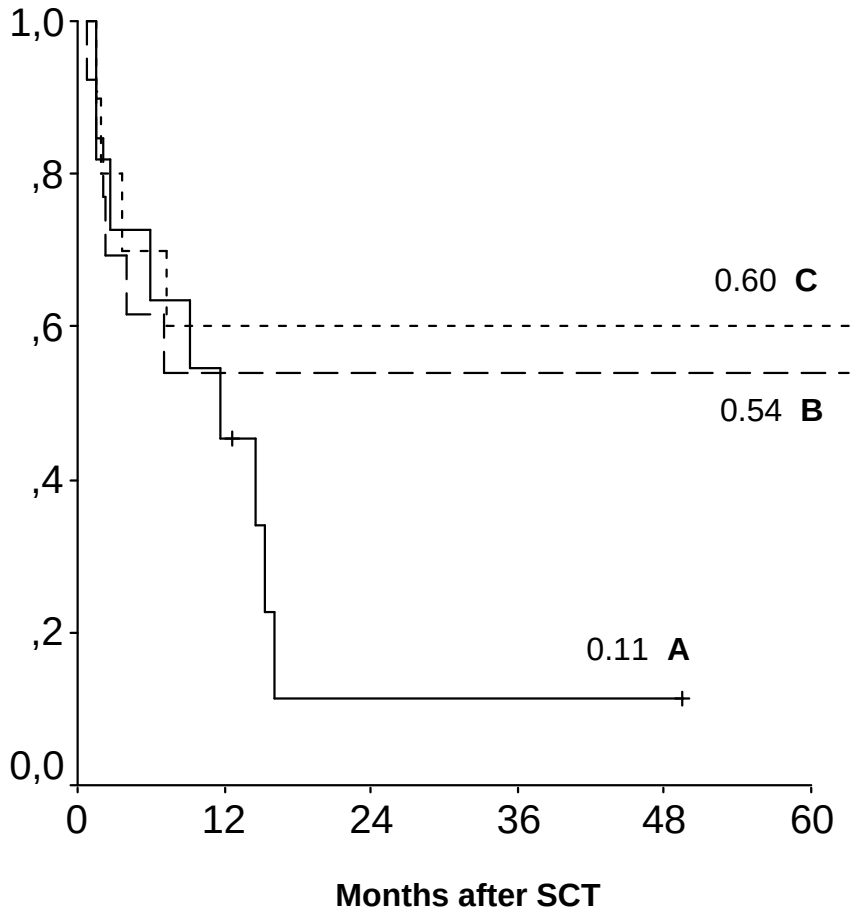

Figure 9 Probability of leukemia free survival (EFS) after allogeneic SCT

Kaplan-Meier estimates of leukemia free survival in group A patients (n=11) receiving chimeric or humanized anti-CD25 compared with group B patients (n=13) treated with murine anti-CD25. EFS was 0.11 vs. 0.54 (not significant, *p*=.19, log rank test). Group C patients (n=10) were transplanted with matched sibling donors without receiving antibody therapy. EFS of group C (0.6) was also not significantly different in comparison to group A or group B.

n = number of patients.

4 Discussion

4.1 How to prevent GVHD?

Acute GVHD is mediated by donor T cells and a major barrier to successful hematopoietic cell transplantation despite prophylaxis using the best currently available drugs [Storb, R. et al. 1986] [Storb, R. 1995]. Although histocompatibility remains the strongest predictive factor for acute GVHD, a number of other variables have been associated with an increased risk of acute GVHD. These include diagnosis, female donor/ male recipient pair, recipient age, increased dose of TBI and lower intensity of GVHD prophylaxis [Weisdorf, D. et al. 1991]. However, several investigators have shown that the occurrence of acute GVHD, even though usually associated with higher transplant related mortality, may produce a favorable effect in patients with leukemia due to the immune-mediated graft-versus-leukemia activity (GVL) [Weiden, P. L. et al. 1981] [Sullivan, K. M. et al. 1989] [Locatelli, F. et al. 2000]. There are reports suggesting no difference in survival between patients transplanted with HLA-matched sibling donors and unrelated donors [Ringden, O. et al. 1995] as well as between young adults (aged 18 to 30 years) with matched unrelated and with partially mismatched unrelated donors [Beatty, P. G. et al. 1993].

Acute GVHD is known as a risk factor for the development of chronic GVHD [Przepiorka, D. et al. 2001]. Extensive chronic GVHD can have severe consequences on the quality of life of long-term survivors and, more importantly has an adverse impact on survival [Sullivan, K. M. et al. 1989] [Sullivan, K. M. et al. 1991] [Przepiorka, D. et al. 2001]. At least in selected subgroups chronic GVHD can have a protective role against leukemia relapse by exerting a GVL effect [Zikos, P. et al. 1998].

The choice of a specific protocol for prophylaxis of acute GVHD in children should take into account not only the risk factors for the development of acute GVHD but also the clinical and prognostic characteristics of the disease being treated. For example, patients with acute leukemia or advanced disease status may benefit from a less intensive or shorter immunosuppressive treatment [Zecca, M., Locatelli, F. 2000]. To date, the optimal immunosuppressive treatment for prevention of acute or chronic GVHD has not yet been identified [Peters, C. et al. 2000].

Currently, prophylaxis of GVHD is based mainly on in vivo post-grafting immunosuppressive therapy, CSP alone or in combination with MTX being the most frequently used drugs [Storb, R. et al. 1986] [Simpson, D. 2000] [Locatelli, F. et al. 2000]. Adverse effects of this regimen like mucositis, delayed engraftment and liver

toxicity (due to MTX), hypertension, renal failure and microangiopathic hemolysis (caused by CSP), limit their use [Nash, R. A. et al. 1992]. Increasing knowledge of the pathophysiology of acute GVHD will hopefully lead to the development of new therapeutic agents or an efficient drug combination disrupting the GVHD cascade [Ferrara, J. L. 2000]. One approach is to achieve immunosuppression by blocking of IL-2 induced T cell proliferation.

It has been shown that murine monoclonal antibodies against IL-2Ra have immunosuppressive activity in patients with acute GVHD resistant to treatment with glucocorticoids [Cahn, J. Y. et al. 1995] [Hertenstein, B. et al. 1994] [Herbelin, C. et al. 1994]. Since antibodies of murine origin have the disadvantage of short half-life and restricted effectiveness related to human anti-murine immune responses, chimeric and humanized anti-CD25 antibodies have been designed. A single dose humanized anti-CD25 (daclizumab) achieved a response rate of 40% in patients with steroid-refractory acute GVHD. However, survival was only 20% at 180 days and 10% at 587 days from application [Anasetti, C. et al. 1994]. Other authors reported a response rate of 47% to a five dose treatment schedule with daclizumab 1 mg/kg on day 1, 4, 8, 15, 22 and a survival of 53% on day 120 for treatment of patients with advanced or steroid-resistant acute GVHD, but 40% of these patients subsequently required additional antithymocyte globulin [Przepiorka, D. et al. 2000]. Some studies have demonstrated sufficient CD25 blockade after application of CD25 antibodies for prevention of GVHD [Burdach, S. et al. 1995] [Anasetti, C. et al. 1991], but this effect was not accompanied by a significant decrease in GVHD incidence. Moreover, germ cell line targeting (knockout) models have surprisingly shown CD25 may also promote survival of activated T cells [Willerford, D. M. et al. 1995] suggesting that the IL-2-CD25 interaction may be obligatory for maintaining IL-2 dependent regulatory T cells and T cell homeostasis [Schimpl, A. et al. 2002].

4.2 Safety and efficacy of CD25 blockade

In our study, we applied monoclonal chimeric or humanized anti-CD25 (ch/anti-CD25, group A) in addition to standard GVHD prophylaxis in children with high risk of GVHD after allogeneic stem cell transplantation on day 0, +4, +28, +56 and +84. The tolerability and safety on the use of these antibodies in pediatric patients undergoing allogeneic peripheral stem cell transplantation was evaluated. As described in adult patients and in children after solid organ transplantation, basiliximab [Nashan, B. et al. 1997] [Offner, G. et al. 2002] and daclizumab [Niemeyer, G. et al. 2002] [Leonard, P. A.

et al. 2002] [Sarwal, M. M. et al. 2001] were well tolerated, even without premedication before antibody application.

Observation period of complete receptor blockade after a single dose of 1 mg/kg anti-CD25 ranged from 18 to 77 days in patients evaluated before SCT. Our results are in accordance with Kovarik [Kovarik, J. et al. 1997], who reported in adult recipients of renal allografts receiving a single dose basiliximab 40 mg/ 60 mg, that concentrations of antibody remained 26 ± 8 days (range 16 to 46)/ 32 ± 11 days (range 22 to 51) above levels sufficient to saturate CD25 receptor. Other authors showed in adult renal transplant patients a median CD25 blockade of 42 days after one dose daclizumab (2 mg/kg, d0) [Vincenti, F. et al. 2003].

In our patients, efficacy of CD25 blockade between day 0 and 100 after SCT was investigated by assessment of CD25+ cells three times a week. In 3 of 6 patients who completed the protocol schedule, there was a complete CD25 blockade until day +100, defined as no detection of CD25+ cells (no staining of CD25 =1% by FACS) in all collected blood samples.

In 3/6 patients to whom CD25+ cells could be detected between day 0 and d+100 while on protocol, the duration of complete CD25 blockade was 13 ± 2.2, 16 ± 2.5 and 23 days after last antibody application. As other authors showed for children after kidney transplantation given a two dose regimen of basiliximab on day 0 and 4 (10 mg/dose in patients <40 kg, 20 mg/dose in patients >40 kg), the duration of CD25 blockade varied from one patient to another and was sometimes shorter than three weeks (range 14 to 21 days) or longer than six weeks (range 70 to 85 days) [Sterkers, G. et al. 2000] and the average duration of CD25 blockade was 5 weeks in adult and pediatric patients after two applications [Kovarik, J. M. et al. 2002]. Although a five dose regimen of daclizumab 1 mg/kg on day 0, 14, 28, 42, 56 in adults was sufficient to accomplish a CD25 blockade lasting up to 120 days after renal transplantation [Vincenti, F. et al. 1998], only 3/6 of our pediatric allogeneic stem cell patients remained CD25 negative until day +100. To achieve a complete CD25 blockade in all pediatric allogeneic stem cell patients, a shorter interval between applications of antibody seems to be necessary.

Duration of CD25 blockade after a single antibody application in 5 of our patients with chronic GVHD after d+100 was 21 ± 2.6 days [19; 23] 95%CI to 55 ± 10.5 days [46; 64] 95%CI (mean, SD, [95%CI]). The mean duration of CD25 blockade was 37.3 ± 12.8 days. Interpatient variability was greater than intrapatient variability. We could not find a relationship with age, body weight or body surface area as described before in children after renal transplantation [Sterkers, G. et al. 2000]. We also could not find a

relationship of CD25 blockade in the first 100 days with transplanted cell dose/kg recipient.

Whether the duration of the CD25 blockade correlates with clinical response, is an open question. Freedom from rejection after renal transplantation was not associated with the duration of CD25 blockade [Kovarik, J. M. et al. 2002]. The clinical effectiveness of the two-dose daclizumab regimen was demonstrated as well as effectiveness of the five-dose regimen [ter Meulen, C. G. et al. 2001] [Vincenti, F. et al. 2003]. A two-dose regimen with a total dose of 1.5 mg/kg daclizumab was sufficient to prevent acute rejection after liver transplantation effectively, although CD25+ cells were detected at low levels [Koch, M. et al. 2002]. In group A patients (ch/anti-CD25 treatment) we could not find a correlation between duration of CD25 blockade and prevention or severity of GVHD.

4.3 Incidence of GVHD

To assess the efficacy of chimeric and humanized CD25 antibodies in prevention of GVHD we compared the data of the group A (ch/anti-CD25 treatment) to data from group B treated with murine anti-CD25 (m/anti-CD25 treatment) after unrelated BMT. Therefore patients were matched by disease and disease status, age and HLA-matching. We compared all results to a group C consisting of patients with matched related BMT without additional antibody therapy (no anti-CD25 treatment); patients were again matched by disease and disease status. Group B and C patients received the same supportive care as group A patients. All patients had myeloablative conditioning. Differences consisted in using of bone marrow in group B and C vs. peripheral stem cells in group A. GVHD prophylaxis consisted in all groups predominantly of a standard two-dose regimen (CSP and MTX or CSP and PRED); anti-CD25 was added as study medication in group A (ch/anti-CD25) and B (m/anti-CD25).

The incidence of acute GVHD grade II-IV and also severe acute GVHD (grade III+IV) in patients treated with chimeric or humanized anti-CD25 (group A) compared with patients treated with murine anti-CD25 (group B) was not different (0.6 vs. 0.54 GVHD grade II-IV and 0.5 vs. 0.31 GVHD grade III+IV). Incidence of severe GVHD was higher, but not significantly higher as in group C patients (0.5 vs. 0.31 vs. 0.1). The literature on GVHD in pediatric unrelated SCT is scarce. In adult patients, an incidence of severe GVHD after unrelated BMT of 36% in matched and 51% in mismatched transplants was reported [Beatty, P. G. et al. 1993]. Other authors showed an 47% incidence of severe GVHD for a cohort of patients with 2/3 serological matched

unrelated and 1/3 mismatched unrelated donors (1/3 <18 years old) [Kernan, N. A. et al. 1993]. In a pediatric study, acute GVHD grade III+IV occurred in 37% matched and 62% mismatched recipients after unrelated BMT [Balduzzi, A. et al. 1995]. Various authors showed similar risk of acute GVHD in PBSC and BM receiving adult patients [Lickliter, J. D. et al. 2000] [Remberger M et al. 2001]. There is very limited reported experience using allogeneic PBSC in pediatric patients with related donors [Diaz, M. A. et al. 1997] [Li, C. K. et al. 1998] [Levine, J. E. et al. 2000] and even less with unrelated donors. Overall, the incidence of acute severe GVHD in group A and the group B patients, transplanted with matched and mismatched unrelated peripheral and marrow grafts, compared to the results in the literature for patients after unrelated marrow transplants with diverse prophylaxis regimens.

A significantly higher incidence of limited chronic GVHD was seen in group A patients (ch/anti-CD25) compared to the group B (m/anti-CD25) patients (0.75 vs. 0.22; p=.035; chi-square test) calculated amongst patients surviving beyond day +90. Overall incidence of chronic GVHD (limited and extensive disease) was 0.88 in group A vs. 0.44 in group B after unrelated SCT and 0.38 in group C patients (no anti-CD25) after related BMT; incidence was higher but not significantly higher in group A as in group B and C. The use of peripheral blood stem cells in group A patients vs. bone marrow in group B and C patients could be the reason for this difference. Chronic GVHD was seen in 7/8 (0.88) patients of group A; all of our PBSC receiving patients surviving beyond day +90 suffered from chronic GVHD (1.0). Again literature on chronic GVHD in pediatric unrelated SCT is scant. Other authors have also shown in adults, that the use of PBSC increase the risk of chronic GVHD [Storek, J. et al. 1997] [Blaise, D. et al. 2000], with chronic GVHD occurring 1.5 times as often after allogeneic peripheral blood SCT when compared with BMT. The increased T cell fraction transferred with the graft could account for the differences seen in chronic GVHD incidence [Cutler, C. et al. 2001]. Reported rates of chronic GVHD after allogeneic peripheral blood stem cell transplantation varied from 38% to 95% [Sullivan, K. M. et al. 1991] [Storek, J. et al. 1997] [Korbling, M., Anderlini, P. 2001] [Przepiorka, D. et al. 2001] [Mielcarek, M. et al. 2003]. In a study involving 24 pediatric related PBSC transplants an incidence of chronic GVHD of 75% was found at one year [Levine, J. E. et al. 2000]. Japanese authors reported a low incidence of 22% in related and unrelated pediatric peripheral blood SCT [Kondo, M. et al. 2001], possibly due to insular immunogenetics and similar to incidence reported in children after allogeneic BMT with HLA-identical sibling donors [Locatelli, F. et al. 1993]. Although there was a significantly higher incidence of limited chronic GVHD in the group A, incidence of extensive disease was low in all groups (1/8 vs. 2/9 vs. 1/8 patients).

Nevertheless in our study chimeric or humanized anti-CD25 used in addition to standard GVHD prophylaxis did not decrease acute or chronic GVHD as compared to murine anti-CD25. These results are in agreement to other studies using rodent anti-CD25 for GVHD prevention in adult patients [Belanger, C. et al. 1993] [Blaise, D. et al. 1995]. Only a delayed occurrence but not a complete prevention of acute GVHD was observed compared with control patients without anti-CD25 prophylaxis [Anasetti, C. et al. 1991] [Sander, A. 1999]. In the GVHD treatment study with humanized anti-CD25 loss of CD25 mediated activation-induced cell death (AICD) was discussed as one possible reason of treatment failure [Przepiorka, D. et al. 2000]. Deletion of activated T cells through AICD seems to be necessary to achieve peripheral tolerance [Lenardo, M. J. 1991] [Van Parijs, L., Abbas, A. K. 1998] [Wells, A. D. et al. 1999]. In human IL-2Ra deficiency, apoptosis in the thymus is markedly reduced, resulting in expansion of autoreactive T cell clones in multiple tissues [Roifman, C. M. 2000]. IL-2Ra knockout mice had a propensity to develop autoimmune disorders, they showed increased B and T cell populations as the result of inefficient AICD [Willerford, D. M. et al. 1995]. CD4+CD25+ cells are essential for the induction and maintenance of self-tolerance and the prevention of autoimmunity and play a vital role in T cell homeostasis [Malek, T. R. et al. 2002] [Bluestone, J. A., Abbas, A. K. 2003]. Blocking of CD25 may predispose patients to recurrence of GVHD because of lack of clonal deletion. This could be another reason for maintenance of GVHD in our patients, receiving chimeric or humanized anti-CD25.

4.4 Outcome

Death from complication was similar in both groups receiving anti-CD25 (3/11 vs. 3/13; 0.27 vs. 0.23) and mainly due to mutliorgan failure with infections. While the incidence of severe GVHD was 5/10 in group A (ch/anti-CD25) vs. 4/13 in group B (m/anti-CD25) (0.5 vs. 0.31), the rate of GVHD related deaths (1/11 vs. 2/13) was lower than reported from other groups. GVHD was the primary or secondary cause of death in 33% of all deaths after unrelated BMT [Kernan, N. A. et al. 1993]. In our patients of group A and B, GVHD-related death occurred only after withdrawal of immunosuppression followed by exacerbation of GVHD because patients had suffered from leukemia relapse before. Withdrawal of CSP has been reported to induce a GVL reaction with a risk of exacerbation of acute or chronic GVHD [Elmaagacli, A. H. et al. 1999]. In group C patients (no anti-CD25 treatment) after related transplants only 1/10 (0.1) patients died due to infection; rate of DOC reached no significant difference if compared with group A or B.

Despite of the considerable incidence of acute and chronic GVHD in group A patients (ch/anti-CD25), 7/11 (0.63) patients suffered from leukemia relapse. 3/7 patients transplanted with resistant or recurred disease relapsed at days +44 (AML), +55 (ALL) and +484 (ALL). Another patient developed CNS relapse on day +83 after primary treatment of CNS positive AML before getting cranial radiotherapy. This patient had received an unrelated cord blood without developing acute GVHD. Relapse of leukemia after day +100 was seen in 4/7 patients, who were treated because of chronic GVHD (1 AML, 3 ALL). Patients with chronic GVHD were treated with methylprednisolone and CSP, which was tapered after response and replaced if there was grade IV toxicity of liver or kidney defined by common toxicity criteria. We continued anti-CD25 therapy until GVHD was controlled. At the time of relapse, complete response of chronic GVHD was seen in 3/4 patients, the other patient who suffered from extensive disease showed a partial response to therapy.

Event free survival for the group A (ch/anti-CD25 treatment) was 0.11 vs. 0.54 in group B (m/anti-CD25 treatment) and 0.6 in group C (no anti-CD25). Event free survival in group A was lower, but not significantly lower, than in group B and C patients (p=.19 and p=.10; log-rank test). Again, the overall survival was not different (0.22 vs. 0.54 vs. 0.6). The study could not be designed to detect a difference of the frequency of relapse (0.63 in A vs. 0.31 in B vs. 0.3 in C; not significant) because of the number of pediatric patients is too low; however, the main cause of death in group A patients was loss of disease control.

Chronic GVHD was not a favorable prognostic factor for leukemia free survival in group A as described in other cohorts by other authors. A significantly reduced probability of relapse at day +150 was described in ALL patients transplanted in relapsed or advanced disease status with matched sibling donors after suffering from acute or chronic GVHD [Sullivan, K. M. et al. 1989]. Children with chronic GVHD had reduced relapse probability and better EFS compared to children without chronic GVHD, mainly observed in patients with ALL. The authors suggested findings were due to increased GVL effect associated with chronic GVHD [Zecca, M. et al. 2002] [Gustafsson Jernberg, A. et al. 2003]. In our patients, intensified and also prolonged immunosuppressive therapy for treatment of chronic GVHD might be associated with a higher rate of leukemia relapse. Intensive and prolonged immunosuppressive therapy is known as an adverse factor for survival [Locatelli, F. et al. 2000].

Whether the activity of anti-CD25 might impair the GVL effect of allogeneic transplants is of great interest. In a murine model, monoclonal rodent anti-CD25 antibodies directed predominantly against murine NK cells, lessened GVL effect induced by the allograft and resulted in development of leukemia relapse [Weiss, L. et al. 1995]. In

addition Blaise et al. [Blaise, D. et al. 1995] reported a higher relapse rate followed by significant lower EFS in allogeneic BMT patients given 33B3.1, a rat monoclonal IgG2a anti-CD25 antibody, for prevention of GVHD compared to control patients. In our study with murine anti-CD25 (BT563) in addition to standard GVHD prophylaxis, relapse and EFS in children (group B patients) and adults (not shown) with a high risk of GVHD was not different to EFS observed in patients transplanted with matched related donors (group C patients) receiving standard prophylaxis regimen. Experiments in non-human primates have suggested that humanized anti-CD25 might be more immunosuppressive than murine antibodies directed towards the same specificity [Brown , PS. Jr et al. 1991].

Whether chimeric or humanized anti-CD25 destroys CD25+ cells could not be determined by our study because of the lack of negative control. However, only 1/6 our group A patients reached T cells counts greater 700/µl in the first 100 days after SCT. 5/6 patients achieved CD3+ cells >500/µl until day 365 after SCT. The median time to >500/µl CD3+ cells was 108 days (range 31 to 480). In T-cell-depleted unrelated bone marrow transplants a shorter time to >500/µl CD3+ cells (median 42 days, range 9 to 152) was reported [Small, T. N. et al. 1999]. Other authors also reported a small decrease in the number of circulating T cells after administering humanized anti-CD25 [Anasetti, C. et al. 1994]. However, this decrease of T cells was suggested to be due to antithymocyte globuline (ATG) in patients showing progressive acute GVHD or non response of GVHD after application of humanized anti-CD25 [Przepiorka, D. et al. 2000]. In addition to depletion of effector T cells immunosuppression by humanized anti-CD25 might cause leukemia recurrence. Prolonged duration of CD25 blockade beyond day +100 after allogeneic SCT could possibly hinder generating or proliferation of GVL effector cells. In group B patients treated with murine anti-CD25 we detected significantly decreased CD25+ cells from day 0 to day +64. Tapering of antibody therapy was followed by an increase of CD25+ cells to normal levels after day +64 [Sander, A. 1999]. Possibly, a shorter duration of CD25 blockade in group B (m/anti-CD25) as compared to group A (ch/anti-CD25) might be favorable for reaching leukemia control.

In addition, high levels of minimal residual disease in patients of group A as compared to groups B and C might be contributing to leukemia recurrence [Bader, P. et al. 2002]. The median therapeutic interval between last occurrence of leukemia before SCT and day of SCT was 112 days (range 12 to 323) in group A patients with ALL vs. 272.5 days (range 19 to 392) in group B ALL patients vs. 195 days (range 12 to 481) in ALL patients of group C. Although the differences were not significant (log-rank test) as well as the incidence of relapse in our groups, we cannot exclude, that patients with ALL

might benefit from a longer and higher cumulative pre-transplant chemotherapy as was actually given to group A patients.

5 Conclusion

In conclusion, the use of monoclonal chimeric or humanized anti-CD25 in pediatric allogeneic stem cell transplantation is efficacious in receptor blockade and safe with regard to drug toxicity. In addition, CD25 antibodies did not impair engraftment.
The incidence of acute and chronic GVHD in chimeric or humanized anti-CD25 receiving patients (group A) was not lowered compared to patients receiving murine (group B) or no anti-CD25 (group C) after allogeneic transplants. Moreover, a significantly higher incidence of limited chronic GVHD was seen in chimeric or humanized anti-CD25 receiving patients in comparison to patients receiving murine anti-CD25 but not in comparison to patients without anti-CD25 at all. The higher incidence of limited chronic GVHD was probably caused by the higher rate of mature T cells transplanted with the peripheral blood in chimeric or humanized anti-CD25 receiving patients as compared to bone marrow in patients receiving murine anti-CD25. However, overall incidence of chronic GVHD and of extensive chronic GVHD was not significantly higher in the peripheral stem cell receiving group A as compared to the bone marrow transplanted groups B or C.
Although overall survival and leukemia free survival was not significantly different between all three groups, we observed a trend towards superior EFS in groups B and C. More cumulative chemotherapy in group B and C due to longer treatment before transplant as well as more immunosuppressive treatment due to chronic GVHD in group A may have contributed to this trend.

Our findings may suggest an importance of CD25 positive T cells in the balance of achieving tolerance and leukemia control. The complex role of CD25 in regulatory and effector T cells of allo-recognition and leukemia recognition warrants further investigation.

6 [Zusammenfassung]

Das Überleben von Patienten nach allogener hämatopoietischer Stammzell-transplantation (SCT) wird wesentlich durch das Auftreten einer Spender-gegen-Empfänger Krankheit (GVHD) beeinflusst. Pathogenetisch spielen hierbei alloreaktive, Interleukin-2 (IL-2)-abhängige, aktivierte T-Lymphozyten eine wichtige Rolle. Monoklonale Antikörper gegen die a-Kette des IL-2 Rezeptors (CD25 Antikörper) können die IL-2 vermittelte Aktivierung und Vermehrung von T-Lymphozyten selektiv hemmen und werden als Bestandteil der immunsuppressiven Therapie zur Vermeidung einer Abstoßung nach Organtransplantationen erfolgreich angewandt sowie in Studien zur Behandlung oder Prophylaxe der GVHD eingesetzt.

Die Verträglichkeit einer Behandlung mit chimären oder humanisiertem CD25 Antikörper (Basiliximab oder Daclizumab) sowie die Effizienz der Rezeptorblockade in den ersten 100 Tagen nach SCT wurde in einer Gruppe von Kindern nach allogener peripherer SCT (Gruppe A, n=11) untersucht. Alle verabreichten Antikörpergaben wurden ohne Prämedikation und ohne Auftreten von infusionsbedingten Nebenwirkungen vertragen. Die Infusion des CD25 Antikörpers in einer Dosis von 1 mg/kg Körpergewicht sechs Stunden vor der SCT und an den Tagen +4, +28, +56 und +84 nach SCT führte bei 3/6 Patienten zu einer vollständigen Rezeptorblockade in den ersten 100 Tagen, d.h. es konnten zu keinem der Untersuchungszeitpunkte zwischen Tag 0 und 100 CD25 positive T-Zellen (CD25+ =1% in der FACS-Analyse) im peripheren Blut der Patienten nachgewiesen werden. Bei den anderen 3 Patienten, die das Behandlungsprotokoll vollständig erhielten (n=6), dauerte die vollständige CD25 Blockade 13 ± 2,2; 16 ± 2,5 und 23 Tage nach der zuletzt verabreichten anti-CD25 Gabe an.

Patienten, welche eine chronische GVHD entwickelten und zum Zeitpunkt des Auftretens einen Anstieg der CD25+ Zellen im Blut (inkomplette CD25 Blockade) zeigten, qualifizierten sich für weitere Antikörpergaben. Die Dauer der CD25 Blockade bei diesen Patienten, welche 2 bis 5 zusätzliche Antikörpergaben erhielten, reichte von 21 ± 3 Tagen [19; 23] bis 55 ± 11 Tagen [46; 64] 95%CI (Mittelwert, Standardabweichung, [95% Konfidenzintervall]).

Die Behandlung mit chimären und humanisiertem CD25 Antikörper führte bei unseren Patienten zu einer effizienten Rezeptorblockade.

Verglichen mit Patienten einer nach Alter, Erkrankung, Erkrankungsstadien und HLA-Differenzen passenden Patientengruppe (Gruppe B, n=13), welche einen Mausantikörper gegen CD25 (Inolimomab) zur GVHD Prophylaxe nach allogener

Knochenmarktransplantation (KMT) bekommen hatte, konnten wir bei den mit chimären und humanisiertem CD25 Antikörpern behandelten Patienten (Gruppe A) keine verminderte GVHD Inzidenz nachweisen. Auch im Vergleich mit einer weiteren Patientengruppe ohne Antikörpertherapie (Gruppe C, n=10), bestehend aus Patienten nach einer KMT von HLA-identischen Geschwistern, ergab sich kein signifikanter Unterschied (GVHD Grad II-IV: 0,6 vs. 0,54 vs. 0,4 und GVHD Grad III+IV: 0,5 vs. 0,31 vs. 0,1). Die Rate der limitierten chronischen GVHD lag in der Gruppe A jedoch signifikant höher (0,75 vs. 0,22; p=0,036) verglichen mit den Gruppe B Patienten, was vermutlich mit dem höheren Anteil an reifen T-Zellen in den peripheren Stammzellen, transplantiert in der Gruppe A, im Vergleich zum Knochenmark, transplantiert in den Gruppen B und C, zusammenhängt. Die Inzidenz der chronischen GVHD insgesamt wie auch die Rate der extensiven chronischen GVHD war nicht signifikant unterschiedlich im Vergleich aller drei Patientengruppen (0,87 vs. 0,44 vs. 0,38 bzw. 0,13 vs. 0,22 vs. 0,13).

Obwohl keine signifikanten Unterschiede im kumulativen Gesamt-Überleben (OAS: 0,22 vs. 0,54 vs. 0,6) und ereignisfreiem Überleben (EFS: 0,11 vs. 0,54 vs. 0,6) für alle untersuchten Patientengruppen (A vs. B vs. C) nachgewiesen wurden, ergab sich der Eindruck eines Vorteils im EFS für die Gruppen B und C. Wahrscheinlicher als antikörperbedingte Ursachen, wie eine mögliche stärkere immunsuppressive Wirkung des chimären oder humanisierten CD25 Antikörper (Gruppe A) im Vergleich zum Mausantikörper (Gruppe B), erscheint uns die, durch die hohe Inzidenz der chronischen GVHD in der Gruppe A bedingte, intensivere und länger durchgeführte immunsuppressive Behandlung verglichen mit den anderen Patientengruppen. Auch die vergleichsweise geringere chemotherapeutische Behandlung bzw. niedrigere kumulative Chemotherapiedosis der Gruppe A Patienten vor der Transplantation im Unterschied zu Gruppe B und C könnte für den Trend mit verantwortlich sein.

Die dargestellten Ergebnisse illustrieren eine mögliche Bedeutung der CD25 positiven (CD25+) T-Lymphozyten in der Behandlung durch allogene Stammzelltransplantation. CD25+ T-Lymphozyten spielen sowohl bei der Toleranzinduktion als auch bei der Kontrolle der Leukämie eine wesentliche Rolle. Weitere Erkenntnisse zum Verständnis der komplexen Rolle des CD25 Rezeptors auf regulatorischen T-Zellen und Effektor-T-Zellen bei der Erkennung von Allo-Antigenen und von leukämiespezifischen Antigenen sind notwendig, um die Wirkungen von CD25 Antikörpern bei Patienten nach allogener SCT besser zu verstehen und zu bewerten.

7 References

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8 [Thesen]

1. Die Behandlung mit chimären oder humanisiertem CD25 Antikörper (ch/anti-CD25: Basiliximab oder Daclizumab) bei Kindern nach allogener peripherer Stammzelltransplantation (SCT) wurde ohne Prämedikation und ohne Auftreten von durch die Antikörperinfusion bedingten Nebenwirkungen vertragen.

2. Die Infusion des chimären oder humanisierten CD25 Antikörpers in einer Dosis von 1 mg/kg Körpergewicht sechs Stunden vor der SCT und an den Tagen +4, +28, +56 und +84 nach SCT führte zu einer effizienten Rezeptorblockade in den ersten 100 Tagen.

3. In der mit ch/anti-CD25 behandelten Gruppe von Kindern (Gruppe A) kam es in keinem Fall zu einer Transplantatabstoßung.

4. Die Transplantation von peripheren Blutstammzellen bei Patienten der Gruppe A (ch/anti-CD25 Behandlung) führte zu einem beschleunigten Anwachsen des Transplantates im Vergleich zu den Patienten der Gruppe B, (14 vs. 23 Tage, p=0,010) und der Gruppe C (14 vs. 20,5 Tage, p=0,012), welche Knochenmarkstammzellen erhielten. Dieser Effekt wurde durch die Behandlung mit ch/anti-CD25 (in Gruppe A) im Unterschied zu einer Behandlung mit einem Mausantikörper gegen CD25 in Gruppe B (m/anti-CD25) bzw. keiner Antikörperbehandlung in Gruppe C nicht beeinflusst.

5. Die Transplantation von peripheren Stammzellen in Gruppe A führte zu einer signifikant höheren Rate an limitierter chronischer Spender-gegen-Empfänger Krankheit (GVHD) verglichen mit den Patienten in der Gruppe B, welche eine Knochenmarktransplantation erhielten (0,75 vs. 0,22; p=0,036). Dieser Effekt wurde durch die prophylaktische Gabe von chimärem oder humanisiertem CD25 Antikörper in Gruppe A im Vergleich zu Mausantikörper gegen CD25 (Inolimomab) in Gruppe B nicht kompensiert.

6. Bei einem Vergleich mit einer nach Alter, Erkrankung, Erkrankungsstadien und HLA-Differenzen angepassten Patientengruppe (Gruppe B), welche einen Mausantikörper gegen CD25 zur GVHD Prophylaxe nach allogener Knochenmarktransplantation bekommen hatte und auch im Vergleich mit einer

Patientengruppe ohne Antikörpertherapie (Gruppe C), bestehend aus Patienten nach einer Knochenmarktransplantation von HLA-identischen Geschwistern, sahen wir bei der Gruppe A eine leicht erhöhte, jedoch nicht signifikant erhöhte Rate an schwerer GVHD (GVDH Grad III+IV 0,5 vs. 0,31 vs. 0,1). Diese konnte folglich durch die prophylaktische Behandlung mit ch/anti-CD25 in Gruppe A nicht verhindert werden.

7. Die Inzidenz der chronischen GVHD insgesamt wie auch die Rate der extensiven chronischen GVHD unterschied sich bei allen Patientengruppen nicht signifikant (0,87 vs. 0,44 vs. 0,38 bzw. 0,13 vs. 0,22 vs. 0,13).

8. Obwohl keine signifikanten Unterschiede im kumulativen Gesamt-Überleben (OAS: 0,22 vs. 0,54 vs. 0,6) und leukämiefreien Überleben (EFS: 0,11 vs. 0,54 vs. 0,6) für alle untersuchten Patientengruppen (A vs. B vs. C) nachgewiesen wurden, ergab sich der Trend eines Vorteils im EFS für die Gruppen B und C.

9. Als Ursache für den Vorteil des EFS in den Gruppen B und C erscheint uns die, durch die hohe Inzidenz der chronischen GVHD in der Gruppe A bedingte, intensivere und länger durchgeführte immunsuppressive Behandlung der Patienten der Gruppe A verglichen mit den Patienten der anderen Gruppen (B und C) wahrscheinlicher, als antikörperbedingte Ursachen.

10. Auch die vergleichsweise geringere chemotherapeutische Behandlung bzw. niedrigere kumulative Chemotherapiedosis der Gruppe A Patienten vor der Transplantation im Unterschied zur Gruppe B und C könnte für den Trend zu mehr Rezidiven bzw. zu einem geringeren EFS in der Gruppe A mit verantwortlich sein.

11. CD25 positive T-Lymphozyten spielen sowohl bei der Toleranzinduktion als auch bei der Kontrolle der Leukämie eine wesentliche Rolle. Weitere Erkenntnisse zur komplexen Rolle des CD25 Rezeptors auf regulatorischen T-Zellen und Effektor-T-Zellen bei der Erkennung von Allo-Antigenen und von leukämiespezifischen Antigenen sind notwendig, um die Wirkungen von CD25 Antikörpern bei Patienten nach allogener SCT besser verstehen und bewerten zu können.

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Selbstständigkeitserklärung

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Erklärung über frühere Promotionsversuche

Ich erkläre, dass ich das Zulassungsgesuch zum Promotionsverfahren erstmalig an die Medizinische Fakultät der Martin-Luther-Universität Halle-Wittenberg stelle. Ich versichere, dass keine früheren Promotionsversuche mit derselben oder einer anderen Dissertation erfolgt sind.

Halle, 29.08.03

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