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.

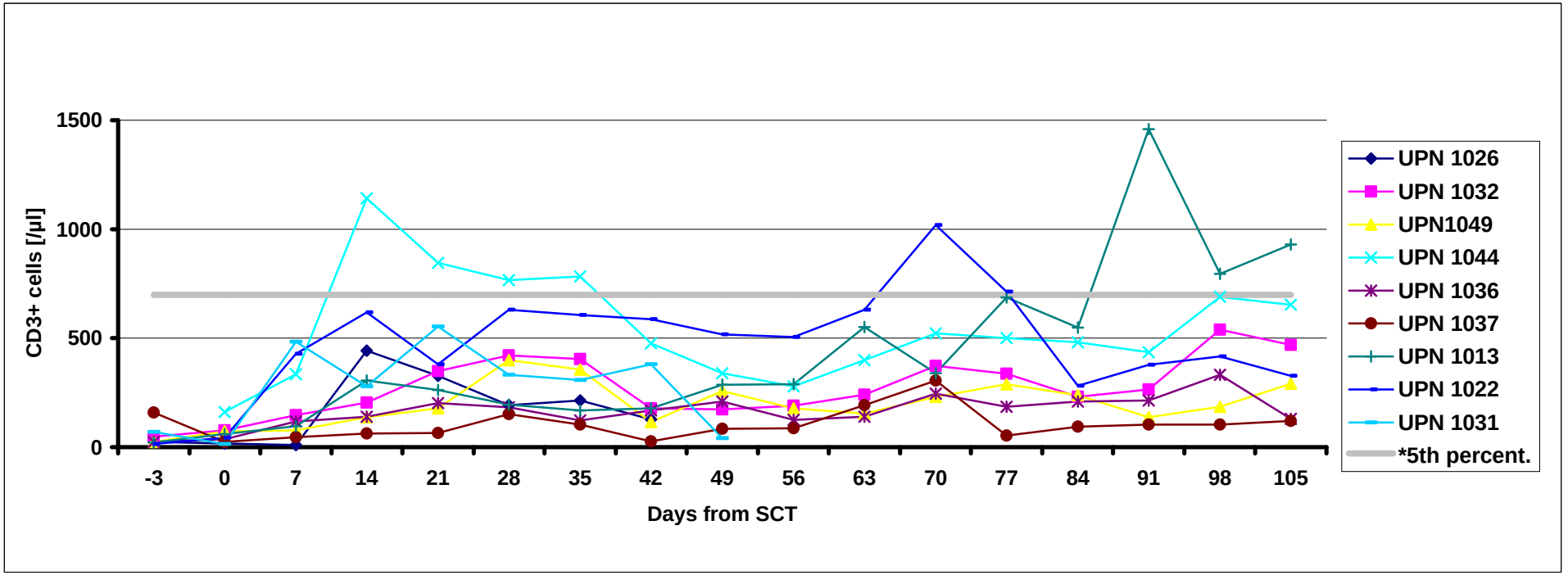

Figure 4 Absolute T cell counts (CD3+ cells) between day 0 and +100 after stem cell transplantation (SCT) of group A patients receiving chimeric or humanized CD25 antibody (ch/anti-CD25 treatment).

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.

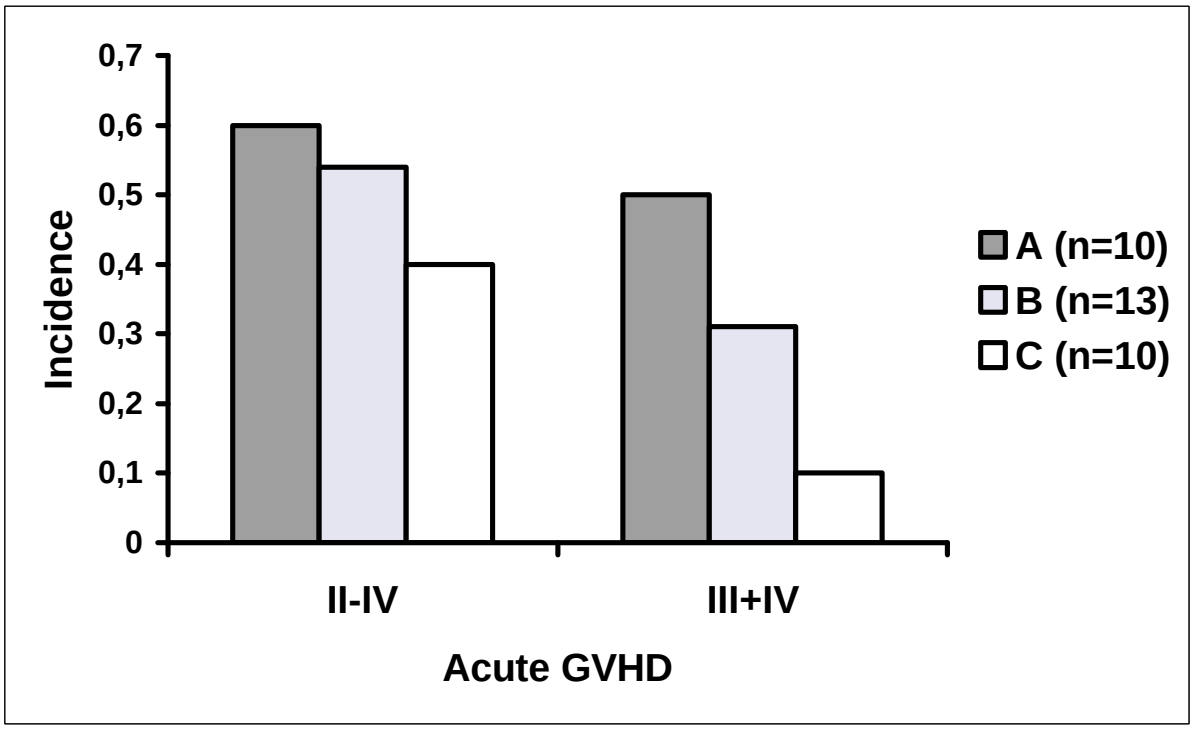

Figure 5 Incidence of acute graft-versus-host disease (acute GVHD)
in group A patients (n=10) receiving chimeric or humanized anti-CD25 compared with group B patients (n=13) receiving murine anti-CD25. No significant difference in acute GVHD grade II-IV: 6/10 (0.6) vs. 7/13 (0.54) and severe acute GVHD 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.

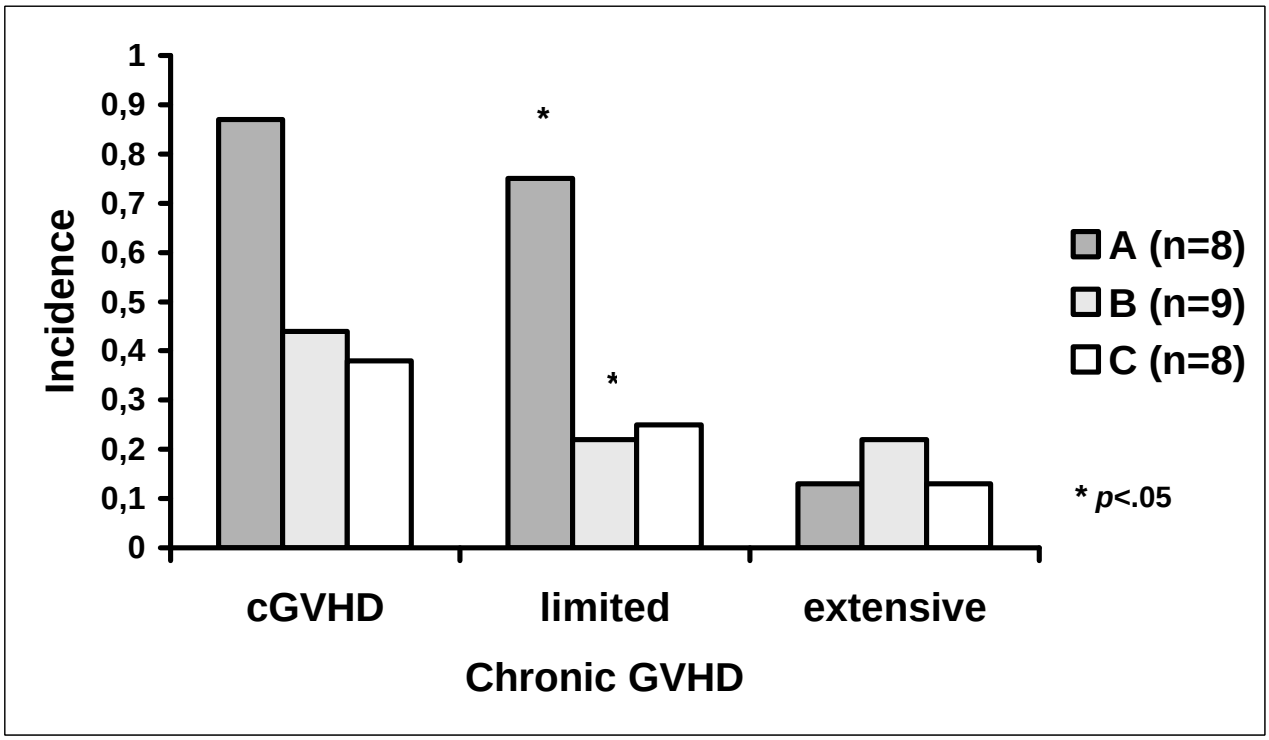

Figure 6 Incidence of chronic graft-versus-host disease (cGVHD) calculated amongst patients who survived beyond day +90 after SCT:

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.

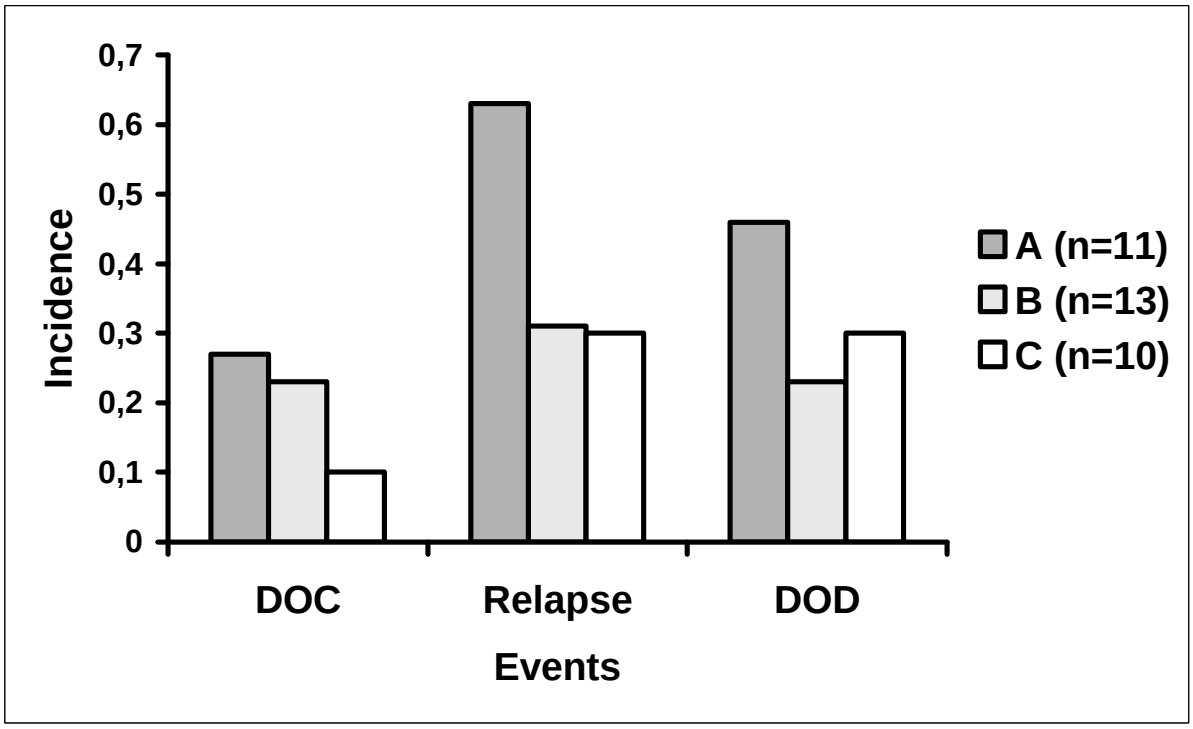

Figure 7 Incidence of relapse and cause of death

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.

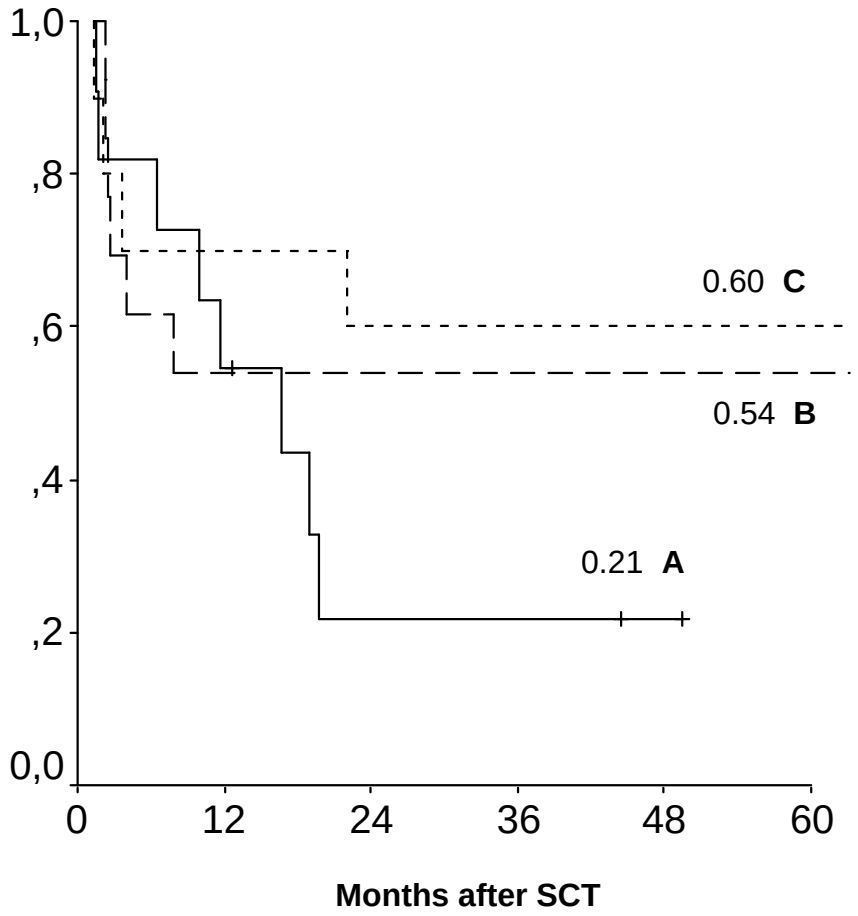

Figure 8 Probability of survival after allogeneic SCT

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.

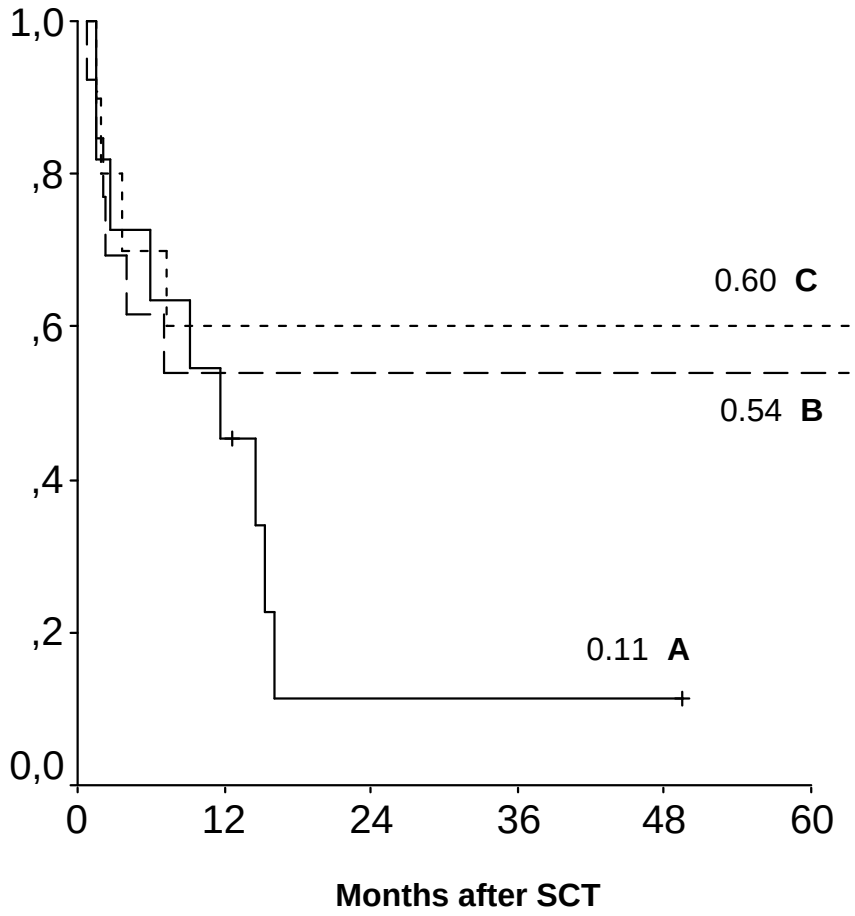

Figure 9 Probability of leukemia free survival (EFS) after allogeneic SCT

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.