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.