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].

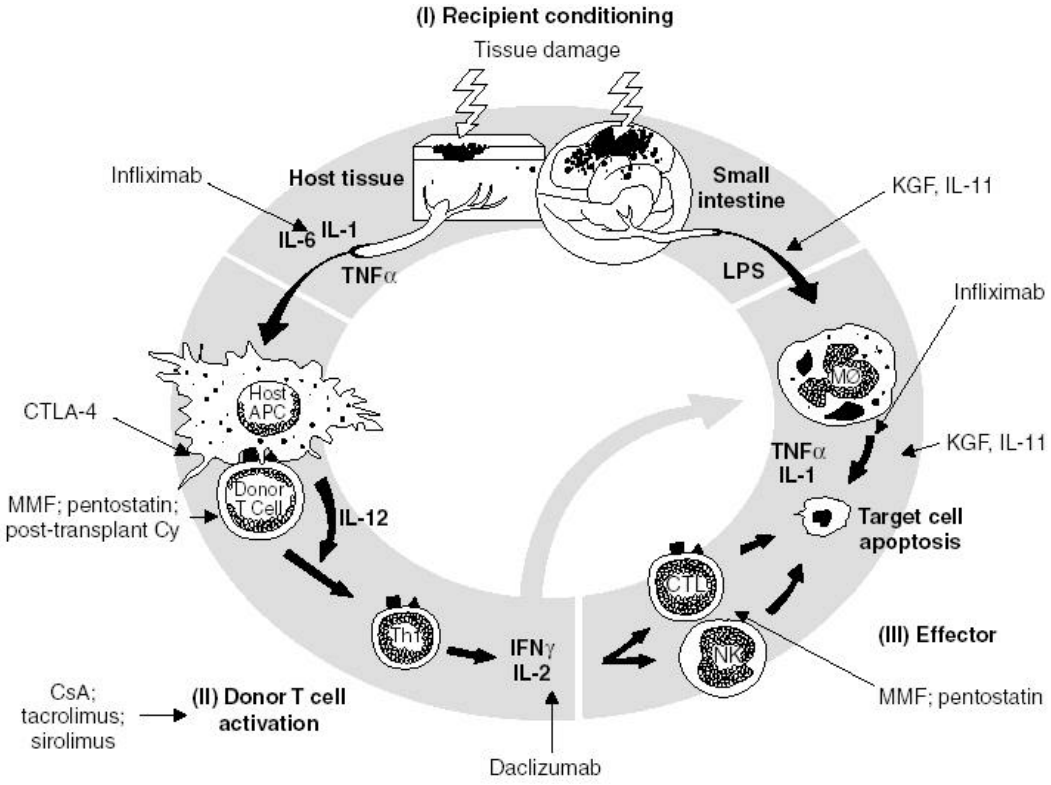

Figure 1 Acute graft-versus-host disease (GVHD): pathophysiology and pharmacotherapeutic intervention [Hill, G. R. et al. 2000]**.** The three sequential phases of GVHD (I, II, II) are detailed. Therapeutic agents are shown in relation to the phases of GVHD they disrupt.

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]