Alloreactivity as therapeutic principle in the treatment of hematologic malignancies

Studies of clinical and immunologic aspects of allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning

Søren Lykke Petersen

This review has been accepted as a thesis together with six previously published papers, by the University of Copenhagen, January 12, and defended on Maj 11, 2006.

Lymphocyte Research Laboratory (now the Allo-HCT Laboratory), department of Hematology, Rigshospitalet, Copenhagen.

Correspondence: Søren Lykke Petersen, Østervang 9, 2800 Lyngby, Copenhagen, Denmark

Official opponents: Torben Barington, Hans E. Johnsen and Mogens Claësson.

Dan Med Bull 2007;54:112-39

1. INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) has changed from a treatment modality often associated with devastating complications to a standard therapy for a variety of diseases (Little & Storb, 2002). Progress in many fields of medicine has contributed to this change, including advances in tissue typing, development of new antibiotics, immunosuppressive agents and better supportive care. However, a major factor has been a better understanding of the function of the immune system and the immunologic mechanisms that are involved in HCT. This knowledge has led to the development of protocols, which focuses on reducing the toxicity while retaining the beneficial effect of the procedure in the treatment of hematologic malignancies. High-dose myeloablative radio-chemotherapy has conventionally been used of as part of the preparative regimen before HCT for two reasons: it has profound immunosuppressive effect on the host, limiting the ability to reject the graft and it has substantial anti-tumor efficacy. It has long been known that allogeneic engraftment could occur in humans without myeloablation (Storb et al, 1982). Furthermore, patients that developed Graft-versus-Host disease (GVHD) had a lower probability of leukemic relapse indicating the existence of an anti-leukemic effect of the graft and suggesting that tumor eradication in recipients of HCT was only partly due to the myeloablative conditioning regimen (Weiden et al, 1979; Weiden et al, 1981; Sullivan et al, 1989; Horowitz et al, 1990). The observation that HCT recipients with relapse of chronic myeloid leukemia could be treated with donor lymphocyte infusions (DLI) and obtain durable complete remissions further substantiated the existence of this Graft-versus-Tumor (GVT) effect (Kolb et al, 1990; Kolb et al, 1995; Slavin et al, 1995; Collins, Jr. et al, 1997; Dazzi et al, 2000b). Encouraged by these findings, several transplant teams began to develop conditioning regimens, which had reduced-intensity or were nonmyeloablative. With the use of these regimens the purpose of the conditioning changed from tumor eradication to host immunosuppression, allowing for the transplanted cells to engraft and elicit a GVT response (Giralt et al, 1997; Carella et al, 1998; Slavin et al, 1998; Childs et al, 1999; McSweeney & Storb, 1999; Sykes et al, 1999). The results of these preliminary studies were promising and led to the implementation of HCT with nonmyeloablative conditioning at the Department of Hematology, Rigshospitalet, Copenhagen, Denmark in March 2000. The regimen used was developed by Storb and colleagues at the Fred Hutchinson Cancer Research Center (FHCRC) in Seattle (McSweeney & Storb, 1999). In HCT with nonmyeloablative conditioning, the GVT effect constitutes the major therapeutic and the only curative principle of the procedure. The recipient hematopoietic tissues are not destroyed by the conditioning and the hematopoietic tissues will therefore be comprised of a mixture of recipient and donor cells, termed mixed hematopoietic chimerism, for a period of time which can last for several months after the transplant (McSweeney et al, 2001; Baron et al, 2004). It is important to monitor the development of donor hematopoietic chimerism following the transplant as this information may predict the occurrence of clinical entities such as GVHD, rejection and relapse (Antin et al, 2001; McSweeney et al, 2001; Baron et al, 2004). Graft-versus-Host disease is a major complication of allogeneic HCT following both myeloablative and nonmyeloablative conditioning and is divided into acute and chronic GVHD depending on the clinical presentation (Mielcarek et al, 2003; Couriel et al, 2004; Alyea et al, 2005). Acute GVHD has been associated with increased nonrelapse mortality and decreased progression-free survival in recipients of HCT with nonmyeloablative conditioning and strategies aiming to reduce the incidence of acute GVHD are therefore warranted (Baron et al, 2005b). One such strategy could be to estimate the risk of acute GVHD in each patient before or early after transplant with the goal of optimizing the GVT effect. In patients with a low risk of GVHD, early tapering of the immunosuppression could be done while the period of immunosuppression could be extended in patients with a high risk of GVHD. Both GVHD and the GVT effect are clinical manifestations of alloreactive responses, which occur when immunocompetent cells present in the graft encounter and react towards recipient antigens. In vitro determinations of the magnitude of alloreactive responses have shown that large variations exist between different recipient-donor pairs (Russell, 2002). When the relatively well-defined antineoplastic effect of high-dose myeloablative radio-chemotherapy is substituted with this highly variable alloreactive potential of the donor cells, the ability to monitor the level of alloreactivity following the transplant would be desirable. The aim of the work presented in this thesis was to examine clinical and immunologic aspects of HCT with nonmyeloablative conditioning and to monitor alloreactive responses following the transplant by cellular and molecular methods. The reconstitution of T, B, and NK cells (Petersen et al, 2003), hematopoietic chimerism development (Petersen et al, 2004b) and clinical outcome of HCT (Petersen et al, 2004a) following nonmyeloablative conditioning in patients with hematologic malignancies were investigated. Alloreactive responses of peripheral blood mononuclear cells were examined by use of in vitro assays measuring cytokine secretion (Petersen et al, 2002), alloreactive cell frequencies (Petersen et al, 2005) and cytokine gene expression (Petersen et al, 2006). The results showed that it is possible to use cellular or molecular methods to identify patients with increased risk of acute GVHD or relapse. We also observed that the alloreactive mechanisms responsible for a number of the complications and for the beneficial effects of this treatment modality might be inhibited by immunoregulatory cytokines and regulatory cell subsets. Finally, we found that in highly pre-treated patients who were ineligible to receive allogeneic HCT with myeloablative conditioning, HCT with nonmyeloablative conditioning is a feasible treatment option that has the potential to induce long-term disease control.

2. ALLOREACTIVITY

The major histocompatibility complex (MHC) forms the molecular basis for the ability of the adaptive immune system to distinguish between self and non-self. The MHC, which in humans is known as the human leukocyte antigen (HLA) system is a set of linked genes located on the short arm of chromosome 6 (Margulies & McCluskey, 2003; Marsh et al, 2005). The HLA-system is highly polymor-

phic, but because the HLA genes are closely linked they are generally inherited as one genetic unit. The genotype of an individual will therefore consist of a combination of the two parental haplotypes (Mickelson & Petersdorf, 2004). If two siblings have inherited the same set of haplotypes from their parents they will be HLA-identical. The MHC molecules are cell surface receptors that present antigen fragments to T cells and thereby initiate immune responses. The MHC molecules are divided into MHC class I molecules (HLA-A, HLA-B and HLA-C) that present peptides derived from intracellular proteins, and MHC class II molecules (HLA-DR, HLA-DQ and HLA-DP) that present peptides derived from extracellular proteins. The T cells of an individual will normally only respond to foreign peptides, if MHC molecules of the individual present them, known as MHC-restriction (Zinkernagel & Doherty, 1997). However, when tissues are transplanted between two individuals, who differ with respect to the MHC, a large fraction of the T cells will respond to the foreign MHC-peptide complexes. This strong alloreactive response is thought to reflect cross-reactivity of T cells carrying receptors normally specific for a variety of foreign peptides presented by self MHC molecules, but which after the transplant react to the foreign MHC-peptide complexes (Shlomchik, 2003). In HLA-identical HCT, where the MHC molecules of the donor and the recipient are identical, the main induction of alloreactivity involves presentation of minor histocompatibility antigens (mHag) by antigen presenting cells (APC) to the T cells (Goulmy et al, 1996; Mutis et al, 1999a; Dickinson et al, 2002). Minor histocompatibility antigens are peptides, that are derived from polymorphic proteins encoded by genes located outside the MHC and which are recognized as alloantigens by allogeneic T cells (Simpson et al, 2002). If the recipient of HCT is not severely immunosuppressed, the alloreactive response will lead to rejection of the transplanted tissue. One of the purposes of the conditioning regimen is to suppress this Host-versus-Graft response and thereby enable the donor cells to engraft. If the donor cells are not rejected the opposite reaction, the Graft-versus-Host reaction, can occur when recipient antigens activate the donor T cells. The clinical manifestations of this process are termed GVHD if the normal tissues of the recipient are the targets of the response and the GVT effect if the malignant cells are attacked by the response. In clinical HCT it is important to harness alloreactive responses by the use of immunosuppression as both rejection and GVHD may have potentially fatal outcome. As the GVT effect is also influenced by the immunosuppression a delicate balance exists between the desire to avoid severe GVHD and at the same time enable the donor cells to elicit a GVT response.

3. ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

3.1. SOURCE OF THE GRAFT

Bone marrow (BM) aspirated from the iliac crest has traditionally been the primary source of hematopoietic stem cells used in HCT. During the last decade peripheral blood stem cells (PBSC) harvested by leukapheresis following stimulation with granulocyte colony stimulating factor have become an alternative to BM. At present PBSC are more commonly used than BM in allogeneic HCT both overall and especially following nonmyeloablative or reduced-intensity conditioning regimens (Banna et al, 2004; Gratwohl et al, 2005). The contents of T cells and $CD34^+$ cells are higher in PBSC grafts than in BM grafts (Bensinger et al, 2001; Couban et al, 2002) and the ability of PBSC to engraft under nonmyeloablative conditions may be superior to that of BM (Maris et al, 2003b). The time to neutrophil and platelet recovery is shorter following transplantation with PBSC (Ringden et al, 2002), resulting in lower platelet transfusion requirements than following bone marrow transplantation (Blaise et al, 2000; Bensinger et al, 2001; Couban et al, 2002; Ringden et al, 2002; Schmitz et al, 2002). Another possible advantage of PBSC is a more rapid reconstitution of the $CD4^+$ T cells when compared to BM (Ottinger et al, 1996; Storek et al, 2001a; Petersen et al, 2003). Negative effects of transplantation with PBSC includes a higher risk of chronic GVHD (Cutler et al, 2001; Mohty et al, 2002; Ringden et al, 2002; Tanimoto et al, 2004; Stem Cell Trialists' Collaborative Group, 2005). The reason for this increased risk of chronic GVHD has been proposed to be related to the higher $CD34^+$ cell dose present in the PBSC grafts (Zaucha et al, 2001; Mohty et al, 2003; Lee et al, 2003b). The risk of relapse in recipients of PBSC has in some studies been reduced when compared to the risk in BM recipients, suggesting an increased GVT effect of PBSC (Powles et al, 2000; Oehler et al, 2005; Stem Cell Trialists' Collaborative Group, 2005). In the majority of studies the survival has been similar in recipients of PBSC and BM (Blaise et al, 2000; Powles et al, 2000; Mohty et al, 2002; Ringden et al, 2002; Schmitz et al, 2002; Tanimoto et al, 2004; Oehler et al, 2005) but in a recent meta-analysis of randomized trials the overall- and disease-free survival was improved in patients with late-stage disease if they were transplanted with PBSC as opposed to BM (Stem Cell Trialists' Collaborative Group, 2005). In the protocol for allogeneic HCT with nonmyeloablative conditioning employed at Rigshospitalet, only PBSC grafts are used.

3.2. MYELOABLATIVE CONDITIONING

Allogeneic HCT is a potentially curative treatment for otherwise lethal hematologic malignancies (Thomas et al, 1975). The rationale for the introduction of allogeneic HCT as a treatment for hematologic malignancies was the discovery in experimental animals, that allogeneic hematopoietic cells could engraft in a lethally irradiated host and regenerate the hematopoietic tissues (Little & Storb, 2002). In humans, this allowed for intensification of the antineoplastic therapy to doses that caused lethal myeloablation and led to the development of the currently used high-dose conditioning regimens consisting of cyclophosphamide (120 mg/kg) combined with either 16 mg/kg of busulfan (Santos et al, 1983) or with 12 Gy of total body irradiation (TBI) (Clift et al, 1990; Clift et al, 1991). The intensity of the conditioning regimen affects both the anti-leukemic effect and the toxicity of the transplant procedure. Increased doses of irradiation decrease the probability of relapse in patients with myeloid leukemia, but this beneficial effect is often offset by increased transplant related mortality (TRM) (Clift et al, 1990; Clift et al, 1991). The toxicity of the currently used myeloablative conditioning regimens generally restricts the use of allogeneic HCT to patients with an age below 50 to 60 years. This age limit is in contrast to the occurrence of many hematologic malignancies where the age at diagnosis is often considerably higher. In addition, a high TRM has been observed following allogeneic HCT with myeloablative conditioning in patients with chronic lymphocytic leukemia, multiple myeloma, non-Hodgkin lymphomas and Hodgkin disease further limiting the use of this treatment option in these patients (Michallet et al, 1996; Alyea et al, 2003; Peniket et al, 2003). Besides the anti-neoplastic effect of the high-dose radio-chemotherapy it became evident that the donor cells also contributed to the curative potential of allogeneic HCT. In the early animal experiments it was suggested that the donor cells possessed an anti-leukemic effect (Barnes et al, 1956). In a series of experiments performed by Barnes and Loutit (1957), mice were inoculated with leukemia and were transplanted with hematopoietic cells from mice of the same strain (syngeneic) or from mice of another strain (allogeneic). Mice that received syngeneic grafts died of leukemia, whereas recipients of allogeneic grafts survived longer and died with symptoms of GVHD but without evidence of leukemia (Barnes & Loutit, 1957). In the clinical setting it has been shown that the risk of relapse in recipients of syngeneic grafts is higher than in recipients of allogeneic grafts indicating that some degree of genetic disparity is necessary to elicit a GVT effect (Horowitz et al, 1990; Gale et al, 1994). In addition, in a number of studies, there has been a clear association between the occurrences of acute- or chronic GVHD and a lower incidence of relapse, supporting the hypothesis that the GVT effect is part of an alloresponse (Weiden et al, 1979; Weiden et al, 1981; Sullivan et al,

1989; Horowitz et al, 1990). Further evidence for the existence of a GVT effect came with the observation that DLI administered to patients with relapse of chronic myeloid leukemia following HCT could induce complete remissions without the need for additional chemotherapy (Kolb et al, 1990; Kolb et al, 1995; Slavin et al, 1995; Collins, Jr. et al, 1997). The remissions obtained in patients with relapse of chronic myeloid leukemia in chronic phase were durable and often resulted in molecular remission, defined as no detection of BCR-ABL transcripts by reverse transcriptase polymerase chain reaction (RT-PCR) (Dazzi et al, 2000b). In patients with relapse of acute leukemia or chronic myeloid leukemia in accelerated or blastic phases chances of remission were lower and the responses most often not durable (Kolb et al, 1995; Collins, Jr. et al, 1997). Donor lymphocyte infusion is often associated with development of GVHD and responses are more likely in patients who develop GVHD (Kolb et al, 1995; Collins, Jr. et al, 1997). Because GVHD following DLI can be fatal, algorithms for DLI given in gradually increased doses, have been developed (Mackinnon et al, 1995; Dazzi et al, 2000a). GVT responses following DLI takes time and in patients with chronic myeloid leukemia the time needed to achieve molecular remission is on average 4-6 months (Kolb et al, 2004). Molecular remission is desirable after allogeneic HCT, because it is highly correlated to long-term disease control. Not only in patients with chronic myeloid leukemia but also in patients with other hematologic malignancies (Olavarria et al, 2001; Corradini et al, 2003; Ritgen et al, 2004).

3.3. NONMYELOABLATIVE AND REDUCED-INTENSITY CONDITIONING

3.3.1. Development of the conditioning regimens

Conditioning regimens that involve less radio-chemotherapy than the traditionally used myeloablative regimens have been divided in two groups: the nonmyeloablative regimens and the reduced-intensity regimens, depending on the immediate myelotoxicity induced. A truly nonmyeloablative conditioning regimen has been defined as a regimen that allowed for autologous recovery within 28 days without a transplant, did not eradicate the hematopoiesis of the host and gave rise to mixed hematopoietic chimerism upon allogeneic engraftment (Champlin et al, 2000; Baron et al, 2005a). However, this nomenclature is not strictly followed in the literature and many centers have introduced modifications to the originally described regimens further confusing the terms (Slavin, 2004). In this thesis the definitions described above have been followed which implies that the term "nonmyeloablative" refers to conditioning regimens which are mostly immunosuppressive and where complete donor hematopoietic chimerism and eradication of the malignant disease is primarily achieved by alloreactive Graft-versus-Host reactions. The term "reduced-intensity" refers to regimens, which in addition to the immunosuppressive effect have a cytoreductive element that is able to induce significant disease control and also causes severe myelosuppression.

At the FHCRC, Storb and colleagues exploited results obtained in a series of experiments using a dog model to design a TBI based nonmyeloablative conditioning regimen that could be used in the clinical setting (McSweeney & Storb, 1999). By optimizing the post-transplant immunosuppression with the combined use of cyclosporine and mycophenolate mofetil (MMF), an immunosuppressive drug more widely used in solid organ transplantation, they were able to gradually reduce the dose of TBI from 9.2 Gy to 2 Gy and still get stable engraftment of the donor cells (Storb et al, 1988; Storb et al, 1993; Storb et al, 1994; Yu et al, 1995; Storb et al, 1997; Yu et al, 1998). The majority of the dogs transplanted with this nonmyeloablative conditioning regimen became stable mixed hematopoietic chimeras and did not develop GVHD (Storb et al, 1997). The Seattle consortium, a multi-institutional group including centers in USA and Europe, carries out the human trials. The initial conditioning regimen used for HLA-identical sibling transplants consisted of 2 Gy of TBI (low-dose TBI) on day 0 combined with oral MMF 30 mg/kg/day from days 0-27 and intravenous cyclosporine 3 mg/kg/day on days –1 and 0 followed by oral cyclosporine 12.5 mg/kg/day to day 35 with taper from day 35 to day 56 (McSweeney et al, 2001). The patients transplanted with this regimen did not develop stable mixed hematopoietic chimerism but either rejected the graft and reconstituted with autologous hematopoiesis or proceeded towards complete donor hematopoietic chimerism. To reduce the rejection incidence (16% of 102 transplants performed with this regimen) the immunosuppressive purine analog fludarabine 30 mg/m^2 was added on days –4, –3 and –2 (McSweeney et al, 2001; Storb, 2003). The regimen was subsequently modified by replacing intravenous cyclosporine on day –1 and 0 with oral cyclosporine starting on day –3 (Feinstein et al, 2003). Due to a high rate of acute GVHD the cyclosporine administration was extended to day 56 and cyclosporine was then tapered to day 77 in patients with aggressive disease and to day 180 in patients with indolent disease (Georges & Storb, 2003; Maris et al, 2004b; Baron et al, 2005b). In the unrelated donor setting the dose of MMF has been increased to 45 mg/kg/day to day 40 with taper to day 96 and cyclosporine is given at full dose to day 100 with taper to day 180 (Niederwieser et al, 2003; Maris et al, 2003b; Maris et al, 2004b; Baron et al, 2005b). Future modifications will aim at a further reduction of the incidence of acute GVHD (Baron et al, 2005b).

At the M. D. Anderson Cancer Center in Houston, the rationale behind the development of nonmyeloablative or reduced-intensity conditioning regimens has been, that the drugs used in the conditioning regimens should both be immunosuppressive and have cytoreductive activity against the underlying malignancy. In acute myeloid leukemia and myelodysplastic syndrome one of the first regimens consisted of fludarabine 120 mg/m^2, cytarabine 8 g/m^2 and idarubicine 36 mg/m^2 (FAI) (Giralt et al, 1997; de Lima et al, 2004). Subsequently more intensive combinations of fludarabine and melphalan 140-180 mg/m^2 (FM) have been introduced (Giralt et al, 2001; de Lima et al, 2004). The FM conditioning regimen has also been used in patients with multiple myeloma, non-Hodgkin lymphomas and Hodgkin disease (Giralt et al, 2001; Giralt et al, 2002; Anderlini et al, 2005). Other conditioning regimens that have been developed for lymphoid malignancies include fludarabine 90-150 mg/m^2 with cyclophosphamide 900-2000 mg/m^2 (FCy) (Khouri et al, 1998; Khouri et al, 2001; Escalon et al, 2004; Khouri et al, 2004).

Slavin and collegues at Hadassah-Hebrew University Hospital in Jerusalem has introduced a reduced-intensity conditioning regimen consisting of busulfan 8 mg/kg, fludarabine 180 mg/m^2 and anti-thymocyte globulin (ATG) (Slavin et al, 1998; Or et al, 2003). This regimen, with or without modifications, has been widely used by other centers.

In most of the studies performed in the United Kingdom, in-vivo T-cell depletion with the monoclonal antibody anti-CD52 (alemtuzumab) has been included in the conditioning regimen to reduce the incidence of acute GVHD (Kottaridis et al, 2000). The remaining part of the conditioning regimen has most often been FM (Kottaridis et al, 2000; Chakrabarti et al, 2002; Chakraverty et al, 2002; Morris et al, 2004; Peggs et al, 2005) but also the busulfan/fludarabine regimen (Parker et al, 2002) and the BEAM regimen (carmustine, etoposide, cytarabine and melphalan) have been combined with alemtuzumab (Cull et al, 2000; Faulkner et al, 2004). The use of alemtuzumab leads to prolonged mixed hematopoietic chimerism in many patients and DLI is often necessary to achieve complete donor chimerism or to treat residual- or progressive disease (Kottaridis et al, 2000; Marks et al, 2002; Perez-Simon et al, 2002b; Peggs et al, 2004).

Childs and colleagues at the National Institutes of Health in Bethesda developed a nonmyeloablative regimen consisting of cyclophosfamide 120 mg/kg and fludarabine 125 mg/m^2 to transplant patients with hematologic malignancies and have also ex-

plored this approach in patients with solid tumors (Childs et al, 1999; Childs et al, 2000; Gorak et al, 2005). The initial results of allogeneic HCT in renal cell carcinoma were promising with a number of patients who responded to the treatment with tumor regression or stabilization of the disease (Childs et al, 2000). The long-term results have been more disappointing and complete responses are rare (Childs & Barrett, 2004).

Based on animal models developed to achieve stable mixed hematopoietic chimerism, Sykes and colleagues at Massachusetts General Hospital/Harvard Medical School in Boston introduced a nonmyeloablative conditioning regimen consisting of cyclophosphamide 150-200 mg/kg, thymic irradiation (7Gy) and ATG (Sykes et al, 1999; Spitzer et al, 2000; Daly et al, 2003; Dey et al, 2003). Around 30% of the recipients of HCT following this regimen rejected their grafts (Daly et al, 2003; Kraus et al, 2003; Dey et al, 2005), but interestingly sustained responses were observed in some of these patients despite the loss of donor chimerism (Dey et al, 2005).

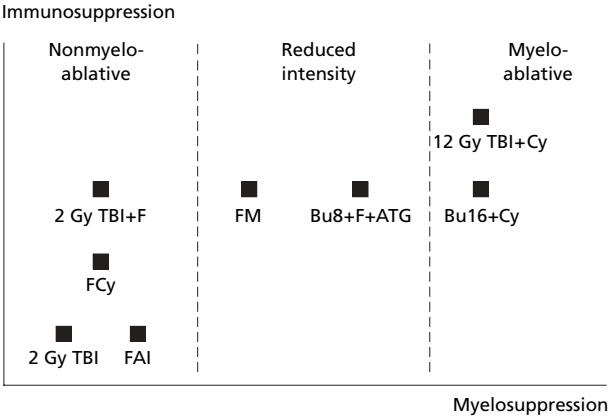
Carella and colleagues at Ospedale San Martino in Genoa pioneered an approach that includes the use of an autologous HCT to reduce the tumor burden and then proceed to an allogeneic HCT with nonmyeloablative conditioning to control and eradicate the residual malignant cells (Carella et al, 1998; Carella et al, 2000). This approach has been further explored in multiple myeloma and NHL (Kroger et al, 2002b; Maloney et al, 2003; Galimberti et al, 2005; Gutman et al, 2005).

In addition to these regimens a number of other groups have developed nonmyeloablative or reduced-intensity conditioning regimens for allogeneic HCT. In the initial phase of development of this new treatment modality, it could be seen as an advantage to explore different ways of conditioning. Today, the numerous conditioning regimens may be a disadvantage for the collection of data needed before prospective studies comparing the results of nonmyeloablative- or reduced-intensity HCT with other treatment modalities can be initiated. The degree of myelo- and immunosuppression caused by the most used conditioning regimens is summarized in Figure 1, which has been adapted from a figure made by Richard Champlin (Storb et al, 2001).

3.3.2. Engraftment and hematopoietic chimerism

One of the first goals of the newly developed nonmyeloablative or reduced-intensity conditioning regimens was to enable and document the engraftment of the transplanted donor cells (Giralt et al, 1997; Carella et al, 1998; Slavin et al, 1998; Childs et al, 1999; McSweeney & Storb, 1999; Sykes et al, 1999). In HCT with myeloablative conditioning the patients are severely pancytopenic for approximately two weeks. When the leukocyte count begins to rise it can generally be assumed that the leukocytes are of donor origin and the day of engraftment has therefore traditionally been defined as the first of 3 consecutive days with neutrophil counts above 0.5×10^9/l (Bensinger et al, 2001). With the use of nonmyeloablative conditioning the leukopenia is milder and when the peripheral counts begin to rise, it is necessary to analyze the origin of the cells, as the patient could have rejected the graft without symptoms and recovered with autologous hematopoiesis. Methods to quantify the degree of donor chimerism are therefore essential tools in HCT with nonmyeloablative- or reduced-intensity conditioning (Antin et al, 2001). Currently, the most widely used method for chimerism analysis utilize PCR to amplify minisatellite (variable number of tandem repeats, VNTR) or microsatellite (short tandem repeats, STR) regions of the human genome that differ between the recipient and the donor (Jeffreys et al, 1985; Antin et al, 2001). We used a fluorescence-based STR-PCR method to determine the level of donor chimerism in granulocytes and in $CD4^+$ and $CD8^+$ T cells in 24 recipients of PBSC grafts from their HLA-identical sibling donors following nonmyeloablative conditioning (Petersen et al, 2004b). Both the kinetics of the increase in donor chimerism and the time needed to achieve complete donor chimerism, defined as > 99% donor cells, differed between the granulocytes and the T cells. The donor granulocyte chimerism was generally low for the first two weeks and then rapidly increased and complete donor chimerism was reached at a median of 42 days (Figure 2) (Petersen et al, 2004b). The donor T-cell chimerism was initially higher than the donor granulocyte chimerism, but increased more gradually and the median time needed to achieve complete donor chimerism was 154 days in the $CD4^+$ T cells and 120 days in the $CD8^+$ T cells (Figure 2) (Petersen et al, 2004b).

The kinetics of donor hematopoietic chimerism development may potentially be influenced by several factors such as the intensity of the conditioning regimen, the post-transplant immunosuppression and the source and composition of the graft. In a study of patients transplanted with reduced-intensity conditioning regimens Pérez-Simón et al (2002a) observed that the majority of patients were complete donor chimeras in both granulocytes and in T cells within two months post-transplant. In contrast to our data Childs et al (1999) reported that complete donor T-cell chimerism occurred prior to complete myeloid chimerism. In recipients of low-dose TBI based regimens, factors that have been related to early high levels of donor T-cell chimerism includes: transplantation with PBSC grafts (Baron et al, 2004), intensive chemotherapy prior to the transplant (Baron et al, 2004), addition of fludarabine to the conditioning regimen (Panse et al, 2005) and planned autologous HCT prior to the allogeneic HCT (Panse et al, 2005). Within recipients of PBSC, high numbers of NK cells (Panse et al, 2005), $CD8^+$ T cells (Cao et al, 2005), $CD4^+$ T cells (Baron et al, 2005c) and $CD34^+$ T cells (Baron et al, 2005c) in the grafts have been related to high levels of donor T-cell chimerism following the transplant and advanced donor age has been associated with low levels of donor T-cell chimerism (Panse et al, 2005). Similarly, factors that are related to the occurrence of rejection following low-dose TBI based conditioning regimens have been identified. In recipients of HLA-identical PBSC, patients who had not received intensive chemotherapy prior to the transplant had a higher risk of rejection following conditioning with 2 Gy of TBI, leading to inclusion of fludarabine into the conditioning regimen (McSweeney et al, 2001). With the use of grafts from HLA-matched unrelated donors following conditioning with 2 Gy of TBI and fludarabine, patients who received BM grafts, patients who had not received preceding chemotherapy and patients who received low numbers of $CD8^+$ T cells or $CD34^+$ cells had an increased risk of rejection (Maris et al, 2003b; Baron et al, 2005c). Maris et al (2004a) found that an increase of the dose of MMF from 15 mg/kg twice daily to 15 mg/kg trice daily following the transplant was associated



TBI = total body irradiation; F = fludarabine; Cy = cyclophosphamide; M = melphalan; Bu8 = busulfan 8 mg/kg; Bu16 = busulfan 16 mg/kg; ATG = anti thymocyte globuli; FAI = fludarabine 120 mg/m², cytarabine 8 g/m² and idarubicine 36 mg/m².

Figure 1. Overview of the degree of immunosuppression and myelosuppression caused by the currently most used conditioning regimens. Adapted from *Storb et al,* 2001.

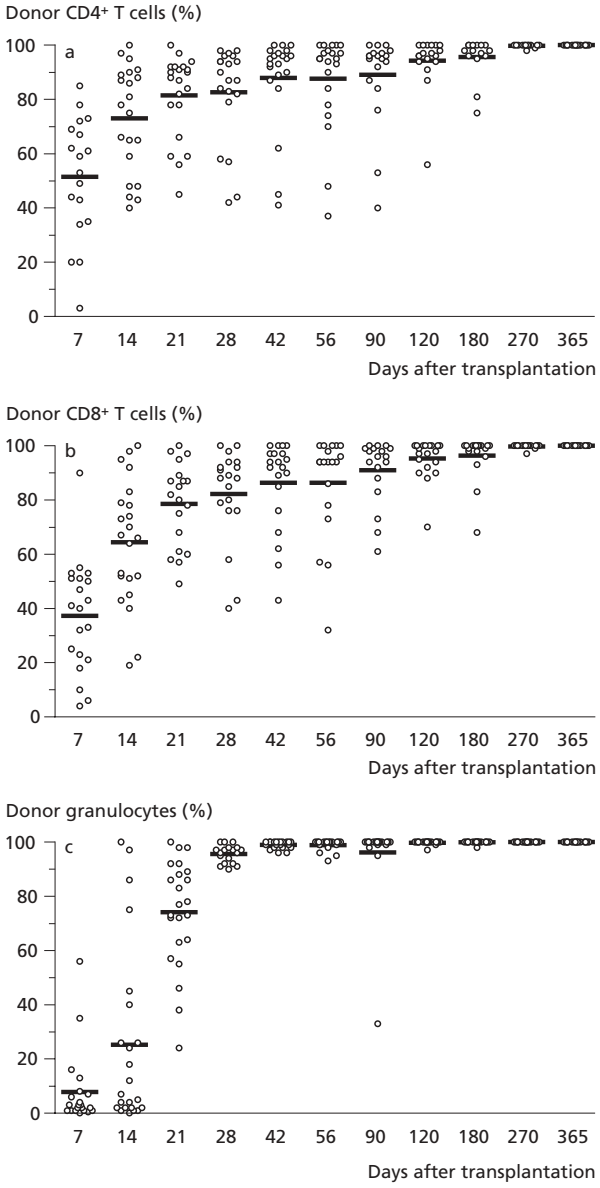


Figure 2. Degree of donor chimerism in $CD4^+$ T cells (A), $CD8^+$ T cells (B) and granulocytes (c) following nonmyeloablative HCT. The horizontal bar in each group of data-points represents the median. Adapted from *Petersen et al,* 2004b.

with a decreased risk of rejection. In summary, these findings point to the existence of a balance between host and graft factors that affects the engraftment kinetics and that this balance can be manipulated, for example by changes in the conditioning regimen or in the post transplant immunosuppression.

Whether the engraftment kinetics could yield information with predictive value for clinical outcomes such as GVHD, rejection and the GVL effect has also been investigated. In the study by McSweeney et al (2001) the level of donor T-cell chimerism on day +28 predicted the subsequent occurrence of acute GVHD and rejection. In a cohort of 38 patients conditioned with 2 Gy of TBI and fludarabine, Keil et al (2003) found that a donor T-cell chimerism level of 90% or more on day 28 predicted a better progression-free survival, whereas the relation between chimerism and GVHD was not investigated. In our study of 24 patients, we were unable to document a relationship between the levels of donor T-cell chimerism early after transplant and acute GVHD, but we found a significant increased risk of acute GVHD in patients who had a donor $CD8^+$ T-cell count above the median on day +14 (Figure 3) (Petersen et al, 2004b). The data from the Seattle consortium were later extended to encompass chimerism determinations in 120 patients (Baron et al, 2004). The level of donor T-cell chimerism on day +28 predicted the occurrence of acute GVHD in this cohort and there was a suggestion that the day +14 T-cell chimerism levels could also predict the occurrence of acute GVHD (Baron et al, 2004). Patients with donor chimerism levels below 50% for the NK cells or the T cells on day +14 had a higher risk of rejection and rapid attainment of complete donor chimerism of the NK cells was predictive of improved progression-free survival (Baron et al, 2004). Recently the chimerism development of NK cells and of subsets of T cells has been investigated in 157 patients (Baron et al, 2005d). Among other findings the day +14 level of donor chimerism of the $CD3^+$, $CD4^+$ and $CD8^+$ T cells and the absolute numbers of $CD4^+$ and $CD8^+$ T cells of donor origin on day +14-42 all predicted the subsequent occurrence of acute GVHD grades II-IV (Baron et al, 2005d). In conclusion, these data indicate that it is possible to use early determinations of donor chimerism or absolute donor counts of leukocyte subsets to predict clinical outcomes related to alloreactivity.

3.3.3. Donor lymphocyte infusion

Following HCT with nonmyeloablative conditioning DLI is given to increase the level of donor chimerism and/or to treat relapse or progression of the malignant disease. The effect of DLI on the level of donor chimerism has been investigated in several studies. In a study by Bethge et al (2004) 16 patients were given DLI for low or falling donor T-cell chimerism and of these 6 responded. Ten patients rejected their grafts despite DLI. Dey et al (2003) observed that the level of donor T-cell chimerism had to be above 40%, if DLI should effectively convert the state of mixed chimerism into complete donor chimerism. In a study by Marks et al (2002) 35% of the recipients of DLI converted to complete donor chimerism and this response was significantly associated with the occurrence of acute- and chronic GVHD. Similarly, Peggs et al (2004) observed that 9 of 12 patients, who received DLI to increase the level of donor hematopoietic chimerism, converted to complete donor chimerism. Thus it appears that DLI can potentially convert mixed hematopoietic chimerism to complete donor chimerism. The responses are, however, highly variable and may depend on the level of donor T-cell chimerism pre-DLI. In patients with persistent or relapsing disease following the transplant, the immunosuppression is tapered in

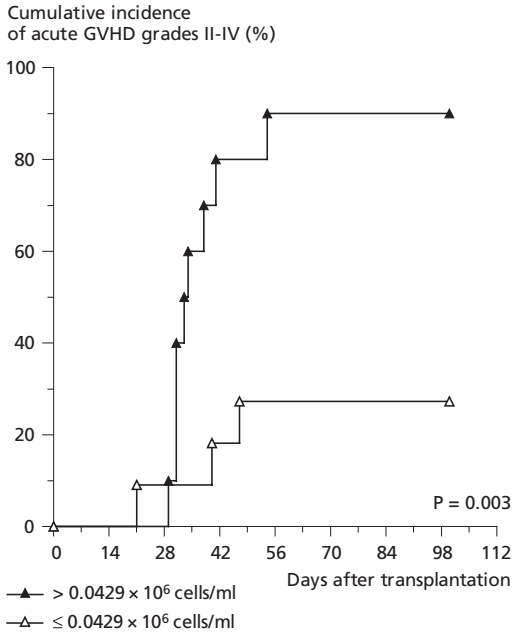


Figure 3. Cumulative incidences of acute GVHD grades II-IV in patients with a donor $CD8^+$ T-cell count ≤ or > the median on day +14. The P-value of the logrank test is shown. Adapted from *Petersen et al,* 2004b.

patients without GVHD, if GVHD does not occur DLI is routinely administered (Petersen et al, 2004a; Petersen et al, 2004b). Disease responses to DLI have been achieved in a variety of hematologic malignancies following HCT with nonmyeloablative or reduced conditioning (Badros et al, 2001; Marks et al, 2002; Dey et al, 2003; Dreger et al, 2003; Bethge et al, 2004; Peggs et al, 2004; Peggs et al, 2005; Kollgaard et al, 2005; Crawley et al, 2005a; Crawley et al, 2005b).

3.3.4. Toxicity

The non-hematologic toxicity of HCT with myeloablative conditioning is often the factor that limits the eligibility of the patients to receive this treatment. In addition, the patients are hospitalized and isolated in specialized wards to limit the effects of the profound myelosuppression associated with the procedure. A common goal for all the centers that were developing reduced-intensity or nonmyeloablative conditioning regimens was to broaden the range of patients eligible for allogeneic HCT and therefore it was necessary to reduce the non-hematologic toxicity. The Seattle team further wanted to perform the procedure in the outpatient setting and that would require a substantial reduction in the hematologic toxicity as well. The hematologic toxicity of the regimen composed of 2 Gy of TBI and fludarabine 90 mg/m^2 is relatively mild as illustrated by median neutrophil and thrombocyte nadirs of 0.33×10^9/l (range 0-1.8 $\times 10^9$/l) and 45×10^9/l (range 4-209 $\times 10^9$/l) respectively in the study of 120 patients by Baron et al (2004). In a comparison to patients who received myeloablative conditioning the neutropenia in recipients of nonmyeloablative HCT was both shorter and less severe (Junghanss et al, 2002b). The median duration of neutrophil counts $< 0.5 \times 10^9$/l has varied between studies and was 0 days in patients with multiple myeloma (Maloney et al, 2003), where the patients received only TBI, 4 days in patients with mantle cell lymphoma where all the patients received fludarabine and TBI (Maris et al, 2004b) and 11 days in patients with chronic lymphocytic leukemia where 82% of the patients received fludarabine and TBI and where 25% of the patients had neutrophil counts $< 0.5 \times 10^9$/l before the transplant (Sorror et al, 2005). Thus while low pre-transplant neutrophil counts clearly affects the duration of neutropenia, the inclusion of fludarabine in the conditioning regimen may also prolong this period. In our study of 30 patients with primarily lymphoid malignancies, where 90% of the patients received fludarabine, we found that the median neutrophil and thrombocyte nadirs were 0.2 $\times 10^9$/l (range 0-0.9 $\times 10^9$/l) and 30 $\times 10^9$/l (range 1-88 $\times 10^9$/l) respectively and that the median duration of neutrophil counts $< 0.5 \times 10^9$/l and platelet counts $< 20 \times 10^9$/l were 12 days and 0 days respectively (Figure 4) (Petersen et al, 2004a).

The neutropenia following the reduced-intensity regimens is more severe than following the nonmyeloablative regimens, but the duration is not necessarily longer than experienced in our study. In a study of patients conditioned with busulphan 8 mg/kg and cladribine 0.66 mg/kg or fludarabine 180 mg/m^2 the majority of the patients experienced a neutrophil count $< 0.1 \times 10^9$/l and the median duration of neutrophil counts $< 0.5 \times 10^9$/l was 9 days (Hori et al, 2004). In recipients of 150 mg/m^2 of fludarabine combined with either 140 mg/m^2 of melphalan or 10 mg/kg of busulfan all the patients became neutropenic and the median duration of neutrophil counts $< 0.5 \times 10^9$/l and platelet counts $< 20 \times 10^9$/l was 13 days and 4 days respectively (Martino et al, 2001b).

The transfusion requirements following HCT with nonmyeloablative conditioning are generally low (Weissinger et al, 2001). The patients in our study received a median of 0 (range 0-60) platelet transfusions and 3 (0-92) red blood cell transfusions during the first 60 days post-transplant (Petersen et al, 2004a). These figures are similar to the transfusion needs reported in other studies (McSweeney et al, 2001; Weissinger et al, 2001; Feinstein et al, 2003; Maloney et al, 2003). Beyond day +60, complications such as gastrointestinal GVHD, thrombotic thrombocytopenic purpura (TTP) and disease progression were the major causes of additional transfusion requirements (Petersen et al, 2004a).

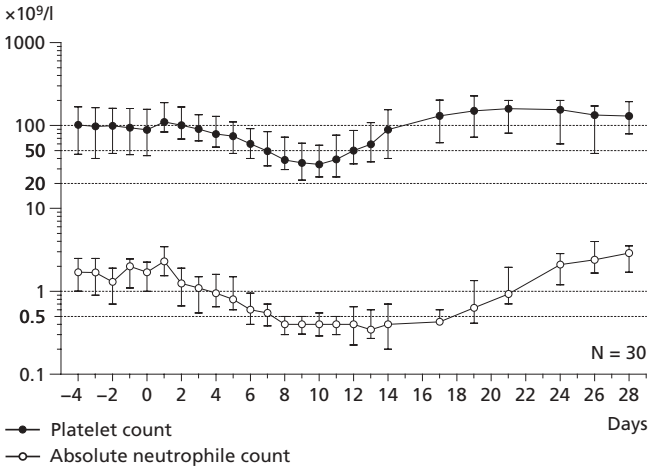


Figure 4. Median and 25 to 75 percentiles (errorbars) of the platelet counts and absolute neutrophile counts from day –4 to day +28 in 30 recipients of HLA-identical sibling PBSC grafts following nonmyeloablative conditioning with 2 Gy of TBI with (27 patients) or without (3 patients) fludarabine 90 mg/m^2.

Patients transplanted with nonmyeloablative and reduced-intensity conditioning regimens were generally considered ineligible for myeloablative conditioning. It is therefore difficult to compare the toxicity of the conditioning regimens because matching for age, disease status and co-morbidity is not possible. However, despite these factors which tends to favor the outcome for recipients of myeloablative conditioning the toxicity to especially the gastrointestinal system, the liver, the kidney and the hematopoietic system is significantly reduced in recipients of nonmyeloablative conditioning when compared to myeloablative conditioning (Weissinger et al, 2001; Diaconescu et al, 2004; Sorror et al, 2004; Parikh et al, 2005). In addition a decreased decline in pulmonary function and decreased occurrence of pulmonary complications such as idiopatic pneumonia syndrome and bronchiolitis obliterans have been observed following nonmyeloablative or reduced-intensity conditioning (Fukuda et al, 2003b; Chien et al, 2005; Yoshihara et al, 2005). The clinical impact of the reduced toxicity is illustrated by a decreased TRM following nonmyeloablative or reduced-intensity conditioning regimens when compared with myeloablative conditioning regimens (Diaconescu et al, 2004; Sorror et al, 2004; Alyea et al, 2005; Dreger et al, 2005; Kojima et al, 2005; Massenkeil et al, 2005; Aoudjhane et al, 2005).

3.3.5. Graft-versus-Host disease

Graft-versus-Host disease is defined by a spectrum of clinical manifestations that are the result of immunologic reactions caused by cells contained in the graft. Acute GVHD is a characteristic clinical syndrome, which includes various degrees of dermatitis, hepatitis and enteritis. The magnitude of these clinical features forms the basis for a clinical grading system where grade 0 represents no symptoms, grade I represents mild often self limiting GVHD, grade II represents moderate acute GVHD requiring immunosuppressive therapy, grade III represents severe multiorgan GVHD and grade IV represents life-threatening or fatal GVHD (Glucksberg et al, 1974; Sullivan, 1999). Acute GVHD usually develops before day +100 following HCT with myeloablative conditioning, but may be delayed in recipients of nonmyeloablative or reduced-intensity conditioning (Mielcarek et al, 2003; Taussig et al, 2003; Perez-Simon et al, 2005). Chronic GVHD is more heterogeneous in its manifestations and many of the symptoms resemble the symptoms occurring in autoimmune disorders. As for acute GVHD a staging system has been developed that divides chronic GVHD into limited and extensive

(Shulman et al, 1980; Lee et al, 2003b). Limited chronic GVHD has a favorable course without treatment, whereas extensive chronic GVHD often requires long-term immunosuppressive treatment (Socie et al, 1999; Sullivan, 1999). In many studies of HCT with reduced-intensity or nonmyeloablative conditioning GVHD is a significant cause of morbidity and mortality (Slavin et al, 1998; Giralt et al, 2001; Schetelig et al, 2002; Mielcarek et al, 2003; Mineishi et al, 2003; Wong et al, 2003; Petersen et al, 2004a; Flowers et al, 2005; Kojima et al, 2005; Mielcarek et al, 2005; Perez-Simon et al, 2005; Schmid et al, 2005). We found that following nonmyeloablative HCT, the probabilities of acute GVHD grades II-IV and III-IV were 57% and 17% respectively and that the 2-year cumulative incidence of extensive chronic GVHD was 80% (Figure 5) (Petersen et al, 2004a). Acute or chronic GVHD were directly responsible for 22% of the days in hospitalization and were the primary causes of death in 10% of the patients (Petersen et al, 2004a). The cumulative incidences of acute and chronic GVHD found in our study are similar to the incidences observed in the matched related setting in a study by Mielcarek et al (2003). However, in a recent study of 297 HLA-matched related transplants following low-dose TBI containing regimens, the cumulative incidences of acute GVHD grades II-IV and of extensive chronic GVHD were lower, i.e. 45% and 47%, respectively (Mielcarek et al, 2005). The incidences of acute- and chronic GVHD following nonmyeloablative or reduced-intensity conditioning varies widely between studies and in T-cell replete transplants incidences of acute GVHD grades II-IV and of chronic extensive GVHD in the range of 9-14% have been reported (Couriel et al, 2004; Miller et al, 2004).

Due to the long half-life of alemtuzumab the use of this agent as part of the conditioning regimen results in an in vivo T-cell depletion of the graft, and the recipients of these regimens therefore have very low incidences of acute and chronic GVHD following the transplant (Kottaridis et al, 2000; Chakraverty et al, 2002; Perez-Simon et al, 2002b; Faulkner et al, 2004). Donor lymphocyte infusions are frequently given following these regimens and this may increase the overall occurrence of GVHD in these patients (Marks et al, 2002; Peggs et al, 2004). Increasing age is a risk factor for development of severe acute GVHD following myeloablative conditioning (Nash et al, 1992). Because the recipients of non-myeloablative HCT are generally older than recipients of myeloablative HCT it is difficult to compare the incidences of GVHD observed following the different regimens. However, studies that were not age-matched have generally shown similar or lower incidences of acute GVHD grades II-IV in recipients of reduced-intensity or nonmyeloablative conditioning than in recipients of myeloablative conditioning and that the incidences of chronic GVHD were similar in the two groups (Mineishi et al, 2003; Sorror et al, 2004; Alyea et al, 2005; Kojima et al, 2005; Perez-Simon et al, 2005; Aoudjhane et al, 2005). In a recent age-matched study the incidence of severe (grades III-IV) GVHD was higher in recipients of reduced-intensity conditioning when compared to recipients of myeloablative conditioning, while the risk of chronic GVHD was the same (Massenkeil et al, 2005). However, DLI was more frequently given in the reduced-intensity group and was more often followed by severe acute GVHD than in the myeloablative group (Massenkeil et al, 2005). In another age-matched study the incidence of acute GVHD grades II-IV were lower in the recipients of nonmyeloablative conditioning than in recipients of myeloablative conditioning, but the incidence of chronic GVHD showed no difference based on the conditioning regimen (Mielcarek et al, 2003). In our studies we have used conventional guidelines (Sullivan, 1999) to diagnose and grade acute and chronic GVHD in order to compare the observed incidences to other studies. These conventional criteria are, however, challenged by the finding that acute GVHD can occur beyond day +100 in recipients of nonmyeloablative or reduced-intensity HCT (Mielcarek et al, 2003; Taussig et al, 2003; Perez-Simon et al, 2005). In addition, the toxicity profile caused by GVHD is different based on the conditioning regimen with more skin and gut morbidity occurring in the nonmyeloablative group than in the myeloablative group 6-12 months post transplant (Mielcarek et al, 2003). To fully compare the morbidity caused by GVHD following nonmyeloablative and myeloablative HCT new grading criteria are needed, which focus on the symptoms of GVHD rather than the time of occurrence (Mielcarek et al, 2003). The effect of GVHD on survival and disease progression has been investigated in several studies of HCT with nonmyeloablative or reduced-intensity conditioning. The occurrence of acute GVHD is generally associated with increased TRM and decreased survival. In most studies a negative effect of acute GVHD on the survival has been observed for the more severe forms, i.e. grades III-IV (Mineishi et al, 2003; Corradini et al, 2005; Schmid et al, 2005; Shimazaki et al, 2005; Crawley et al, 2005a). In a recent study of 322 patients with various hematologic malignancies it was found that acute GVHD grades II-IV negatively affected the progression-free survival and increased the non-relapse related mortality (Baron et al, 2005b). In that study extensive chronic GVHD increased the progression-free survival and decreased the risk of progression or relapse (Baron et al, 2005b). A similar beneficial effect of chronic GVHD on the risk of relapse or on the progression-free survival has also been observed in other studies (Dreger et al, 2003; Perez-Simon et al, 2003; Mohty et al, 2004; Gerull et al, 2005; Blaise et al, 2005). In a recent study the effects on survival of no chronic GVHD, limited chronic GVHD and extensive chronic GVHD were analyzed separately (Crawley et al, 2005a). Both limited and extensive chronic GVHD were associated with a better progression-free survival but the highest progression-free survival was observed in the patients with limited chronic GVHD (Crawley et al, 2005a) and similar observations have been done by others (Corradini et al, 2005; Schmid et al, 2005).

In conclusion, whereas acute GVHD and especially severe acute GVHD is most often only associated with increased toxicity follow-

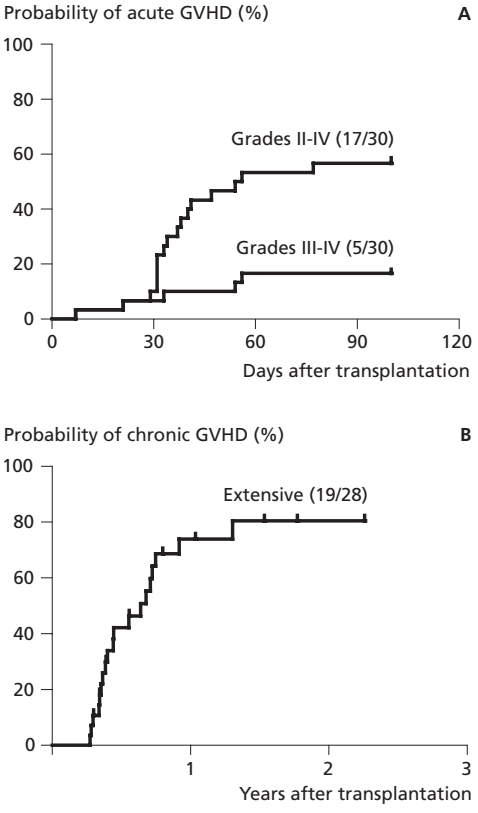


Figure 5. Kaplan-Meier plots of the probability of acute GVHD grades II-IV and III-IV (A) and extensive chronic GVHD (B). The estimated probabilities of acute GVHD grades II-IV and of grades III-IV were 57% (95% CI 39-74%) and 17% (95% CI 4-30%), respectively. The estimated probability of development of extensive chronic GVHD was 80% (95% CI 72-98%) at two years. Adapted from *Petersen et al,* 2004a.

ing nonmyeloablative or reduced-intensity HCT, the occurrence of chronic GVHD seems to be associated with a beneficial GVL effect, that can potentially override the toxic effects of this complication and increase the survival.

3.3.6. Immune reconstitution

Myeloablative conditioning induces a period of approximately two weeks of severe granulocytopenia, where the patients are at risk of bacterial infections. In addition, functional defects of the recovering granulocytes increase the risk of bacterial infections for several months (Zimmerli et al, 1991). While NK cells recover to normal values within one to two months both T- and B-cell recovery may be delayed for several months or even years (Petersen et al, 2003; Auletta & Lazarus, 2005). Low counts of $CD4^+$ T cells and of B cells following allogeneic HCT are associated with an increased risk of infections and failure to mount cytomegalovirus (CMV) specific T-cell responses after the transplant is associated with decreased survival (Storek et al, 1997; Storek et al, 2000; Boeckh et al, 2003). Studies of long-term survivors of allogeneic HCT indicate that the function of the thymus is critical for the T-cell reconstitution and different strategies to enhance the thymopoiesis have therefore been explored (Storek et al, 2001b; van den Brink et al, 2004). As radiation has a negative effect on the thymopoiesis (Chung et al, 2001), it has been speculated that a reduction in the doses of radio-chemotherapy included in the conditioning regimen would limit the thymic damage and allow for a more rapid reconstitution of naïve T cells following nonmyeloablative or reduced-intensity HCT than after myeloablative HCT. Data to support this hypothesis has been presented by several groups (Friedman et al, 2001; Chao et al, 2002; Jimenez et al, 2005), but other groups have found that T-cell reconstitution following nonmyeloablative HCT occurs primarily by peripheral expansion with limited contribution of recent thymic emigrants (Bahceci et al, 2003; Larosa et al, 2005). The toxicity to the thymus induced by the conditioning regimen is not the only factor that influences the T-cell reconstitution following HCT. The use of alemtuzumab or ATG as part of the conditioning regimen has been associated with delayed T-cell reconstitution because of the in vivo T-cell depletion induced by these antibodies (Chakrabarti et al, 2002; Fallen et al, 2003; Saito et al, 2003; Dodero et al, 2005). In addition to the immediate role of the conditioning regimen, factors such as age and GVHD and its treatment may affect both T and B cell reconstitution (Weinberg et al, 2001; Storek et al, 2001c; Storek et al, 2002; Fallen et al, 2003; Petersen et al, 2003; Omazic et al, 2005). We studied the numbers of T, B and NK cells in the peripheral blood of 15 recipients of nonmyeloablative conditioning and transplantation with PBSC (Petersen et al, 2003). The NK cells and $CD8^+$ T cells reached normal values quite rapidly, whereas the levels of $CD4^+$ T cells and of B cells were reduced for 9-12 months (Figure 6 and Figure 7) (Petersen et al, 2003). These observations are similar to the findings in other studies of patients transplanted with low-dose TBI containing regimens (Baron et al, 2003; Busca et al, 2003; Maris et al, 2003a) and indicate that the patients have severe immune deficiencies for up to one year post transplant. When we compared the lymphocyte subset counts at four months and at one year to the counts in 13 recipients PBSC following myeloablative conditioning we observed no difference in the NK- or T-cell counts between the two groups and lower B-cell counts at four months but not at 12 months in the nonmyeloablative group (Petersen et al, 2003). Others have also observed that most of the lymphocyte subsets reconstituted at similar rates in recipients of nonmyeloablative and myeloablative conditioning (Busca et al, 2003; Maris et al, 2003a). Maris et al (2003a) found that recipient T cells that survive the conditioning regimen are likely to contribute to the protection against CMV in the early period post transplant. However, it was also found that the naïve T-cell counts were reduced in the nonmyeloablative transplant recipients at one year post-transplant (Maris et al, 2003a). It is thus clear that the reconstitution of B and T cells following HCT is a dynamic process that is dependent on many factors, and firm conclusions on whether nonmyeloablative or reduced-intensity conditioning enhance the reconstitution of the adaptive immune system must await further research.

3.3.7. Infection and other complications

The management of infections is an important issue in patients undergoing allogeneic HCT with nonmyeloablative or reduced-intensity conditioning. During the first year following nonmyeloablative HCT we observed that 17% of the hospitalization days were due to known infections and 14% were due to fever/pneumonia of unknown origin (Petersen et al, 2004a). Early after transplant the reduced severity of the neutropenia in recipients of nonmyeloablative conditioning has translated into a lower rate of infections when compared to myeloablative conditioning (Junghanss et al, 2002b; Maris et al, 2003a; Diaconescu et al, 2004; Sorror et al, 2004). However when the observation period is extended infectious complications continue to occur (Mohty et al, 2000; Martino et al, 2001a; Daly et al, 2003; Maris et al, 2003a) and the incidence of invasive

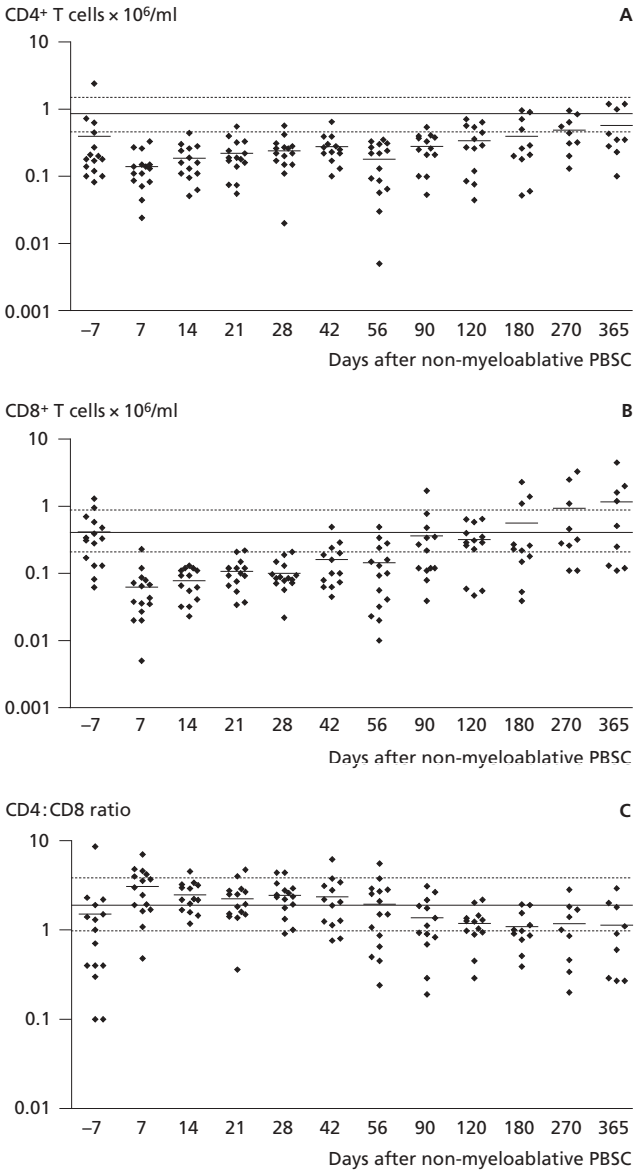


Figure 6. T-cell reconstitution in recipients of peripheral blood stem cells (PBSC) following non-myeloablative conditioning. Absolute numbers of $CD4^+$ T cells (A), $CD8^+$ T cells (B) and CD4:CD8 ratio (C) in 15 patients. The median absolute cell number (solid line) and the 5% and 95% percentiles (dotted lines) of 51 adult sibling donors are shown. The short solid line in each group of datapoints represents the median of the group. Adapted from *Petersen et al,* 2003.

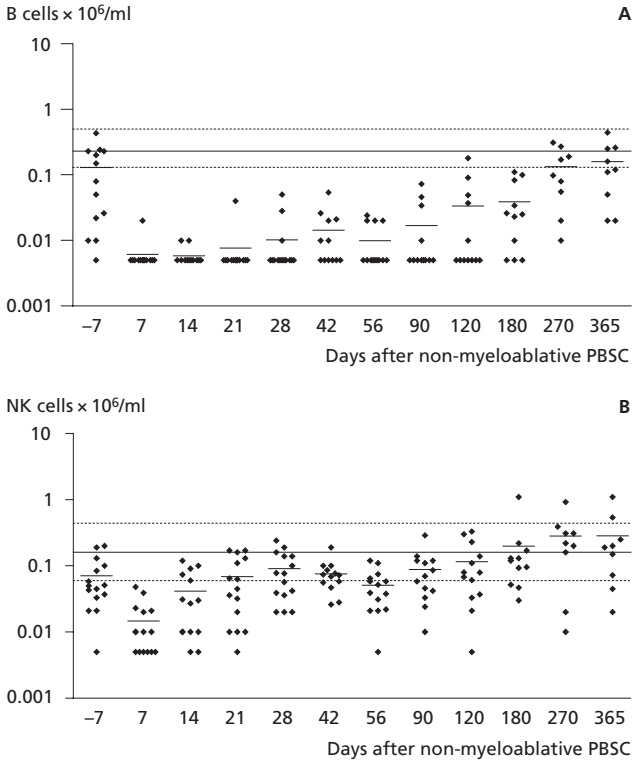


Figure 7. B and NK cell reconstitution in recipients of peripheral blood stem cells (PBSC) following non-myeloablative conditioning. Absolute numbers of B cells (A) and NK cells (B) in 15 patients. The median absolute cell number (solid line) and the 5% and 95% percentiles (dotted lines) of 51 adult sibling donors are shown. The short solid line in each group of datapoints represents the median of the group. Adapted from *Petersen et al,* 2003.

fungal infections may be similar or even higher in recipients of nonmyeloablative or reduced-intensity HCT (Martino et al, 2001a; Junghanss et al, 2002b; Fukuda et al, 2003a; Kojima et al, 2004). Conflicting results exist concerning the risk of CMV activation following different conditioning regimens. Martino et al (2001a) observed a reduction in the risk of both CMV reactivation and overt CMV disease in recipients of reduced-intensity conditioning when compared to the risk following myeloablative conditioning whereas others did not find such a difference (Saito et al, 2003; Schetelig et al, 2003a). In a study by Junghanss et al (2002a) it was observed that though the incidence of CMV disease was initially very low after nonmyeloablative HCT, the onset of CMV disease was delayed when compared to myeloablative HCT leading to similar 1-year incidences with the two types of conditioning. Finally, the use of alemtuzumab as part of the conditioning regimen appears to be associated with a high incidence of CMV reactivation perhaps due to a delay in T-cell reconstitution (Chakrabarti et al, 2002; Dodero et al, 2005). Besides infections and GVHD, engraftment syndrome which was diagnosed in three patients and TTP which developed in seven patients, were some of the more serious complications encountered in the first 30 recipients of HLA-identical nonmyeloablative HCT at Rigshospitalet (Petersen et al, 2004a). Engraftment syndrome is characterized clinically by fever, erythrodermatous rash and hypoxia with or without pulmonary infiltrates and hepatic- and renal dysfunction may also accompany the syndrome (Spitzer, 2001). Engraftment syndrome is related to the neutrophil recovery, involves release of inflammatory cytokines and has been observed following both autologous and allogeneic HCT (Spitzer, 2001; Gorak et al, 2005). Others have also observed engraftment syndrome in recipients of nonmyeloablative HCT, and though the patients usually respond to treatment with steroids, the syndrome has been associated with increased TRM and a shorter overall survival (Spitzer et al, 2000; Gorak et al, 2005). Thrombotic thrombocytopenic purpura is a syndrome consisting of microangiopathic hemolysis, thrombocytopenia and microvascular thrombosis (Sadler et al, 2004). The clinical symptoms are related to the tissue ischemia or infarction caused by the microvascular thrombi and includes neurologic abnormalities and abdominal or chest pain often accompanied by renal insufficiency (Sadler et al, 2004; Ho et al, 2005). In TTP after allogeneic HSCT there is generally no severe von Willebrand factor-cleaving protease (ADAMTS 13) deficiency (van der Plas et al, 1999; Elliott et al, 2003; Vesely et al, 2003). Plasma exchange, which is the treatment of choice in idiopathic TTP (Sadler et al, 2004) is rarely successful in transplant associated TTP and therefore not recommended (Ho et al, 2005). The pathophysiology of transplant associated TTP is poorly understood but endothelial damage caused by the conditioning regimen (Hahn et al, 2004) or as a result of Graft-versus-Host reactions (Biedermann et al, 2002; Ganster et al, 2004; Martinez et al, 2005) may be involved. The mortality following this complication is often considerable (Fuge et al, 2001; Ruutu et al, 2002; Shimoni et al, 2004; Martinez et al, 2005). Both the diagnostic criteria for TTP and the reported incidences of this complication have varied between studies (George et al, 2004; Ho et al, 2005). We used the criteria published by Ruutu et al (2002) i.e. the simultaneous occurrence of all of the following: (1) red blood cell fragmentation, (2) hemolysis, (3) the need for red blood cell transfusions, (4) de novo or prolonged thrombocytopenia (5) negative, or at most, marginally positive laboratory tests for disseminated intravascular coagulation. Ruutu et al (2002) found a 2-year cumulative incidence of TTP of 6.7% in recipients of myeloablative HCT whereas we observed a 1-year cumulative incidence of 26% (Petersen et al, 2004a). Other groups have described the occurrence of TTP following HCT with nonmyeloablative- or reduced-intensity (Corradini et al, 2002; Elliott et al, 2003; Shimoni et al, 2004; Kornacker et al, 2005). In the study by Shimoni et al (2004) the cumulative incidence of TTP was 23% in recipients of reduced conditioning HCT and was not significantly higher than the incidence in recipients of myeloablative conditioning (16%). The factors that have been found to increase the risk of TTP includes acute GVHD, age, transplantation with a matched unrelated donor and female gender (Fuge et al, 2001; Daly et al, 2002; Ruutu et al, 2002; Elliott et al, 2003; Hahn et al, 2004; Shimoni et al, 2004; Petersen et al, 2004a; Martinez et al, 2005).

In conclusion TTP is a serious complication following allogeneic HCT and the occurrence may be related to GVHD or to the same risk factors as known for GVHD (Elliott et al, 2003; Martinez et al, 2005). The development of TTP may thus depend both on the kind of conditioning regimen, the GVHD prophylaxis and the patient population and further research is needed to clarify whether the incidence of TTP is higher following nonmyeloablative or reduced-intensity HCT than following myeloablative HCT.

3.3.8. Hospitalization

McSweeney et al (2001) showed that allogeneic HCT with nonmyeloablative conditioning could be performed as an outpatient procedure as 53% of the eligible patients did not require hospitalization during the first 2 months post-transplant and the remaining patients were hospitalized for a median of 8 days (range 1-35 days). At Rigshospitalet we have also attempted to perform the transplants in the outpatient setting (Petersen et al, 2004a). We found that 22% of the eligible patients were treated entirely as outpatients during the first 2 months and that the median hospitalization requirements for the remaining patients were 8 days (range 1-61 days) (Petersen et al, 2004a). However, the patients continued to be admitted beyond two months post transplant. In the 17 patients who had a follow-up of more than 1 year the median time spend in hospital was 44 days (range 4-151 days) and in addition they had a median of 52 (range 23-73) outpatient visits (Petersen et al, 2004a). This observation illustrates that though the transplant itself can be performed as an outpatient procedure, both the patients and the treating institution must be prepared on the possibibily that complications may result in a considerable number of admissions. In line with this result, the

1-year cost of the total transplant procedure did not differ between recipients of nonmyeloablative and of myeloablative HCT as treatment for acute myeloid leukemia (Cordonnier et al, 2005). Though the patients in the nonmyeloablative group had a significantly shorter duration of the initial hospitalization period, the total number of hospitalization days was not different and the costs in the period from 6-12 months post transplant were higher in the nonmyeloablative group than in the myeloablative group (Cordonnier et al, 2005).

3.3.9. Survival and disease control

When the survival following allogeneic HCT with nonmyeloablative or reduced-intensity conditioning is evaluated it is important to remember that the patients transplanted were ineligible for conventional myeloablative HCT due to age or co-morbidity. In addition many of the patients were heavily pretreated. The initial studies were conducted as feasibility studies and often included patients with various hematologic malignancies. In the study we performed, the overall survival at two years was 68% with a progression-free survival of 43%, a TRM of 22% and a relapse related mortality of 13% (Figure 8A) (Petersen et al, 2004a). These figures are comparable with the results of a 305 HLA-identical transplants after nonmyeloablative conditioning reported by the Seattle consortium (Sandmaier et al, 2003). The patients in our study had received a median

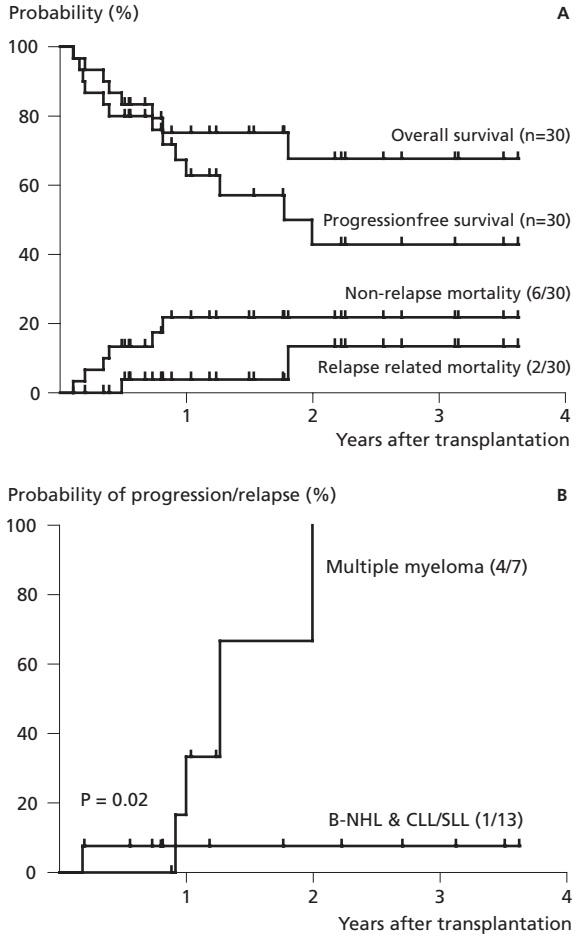


Figure 8. Outcome of non-myeloablative conditioning and transplantation with HLA-identical PBSC in 30 patients (A), and the probability of progression/relapse in patients with mature B-cell malignancies (B). The two-year Kaplan-Meier estimates were 68% (95% CI 48-88%) for overall survival, 43% (95% CI 20-66%) for progression-free survival, 22% (95% CI 6-38%) for non-relapse mortality and 13% (95% CI 0-32%) for relapse related mortality (A). The probabilities of progression/relapse in 7 patients with multiple myeloma and in 13 patients with B-cell non-Hodgkin lymphomas or chronic lymphocytic leukemia/small lymphocytic lymphoma were compared with the log-rank test and the P-value is shown (B). Adapted from *Petersen et al,* 2004a.

of 4 (range 1-10) chemotherapy regimens prior to the transplant, 50% had received an autologous HCT and only 20% were in complete remission at the time of transplant (Petersen et al, 2004a). The results thus indicate that nonmyeloablative HCT represents a feasible treatment option for patients with advanced hematologic malignancies that are ineligible to receive HCT with myeloablative conditioning.

During the last few years a number of studies have been published with disease specific survival data available (Tables 1-8). It is difficult to compare the results of these studies because of differences in the patient populations and differences in the conditioning regimens. Some of the larger studies (Robinson et al, 2002; Dreger et al, 2003; Robinson et al, 2004; Aoudjhane et al, 2005; Crawley et al, 2005a; Crawley et al, 2005b) performed by the European Group for Blood and Marrow Transplantation (EBMT) includes patients previously reported in studies from single institutions. The general tendency in the studies summarized in Table 1-8 is that disease control can be achieved, but the follow up in most studies is rather short. The TRM is generally low but it is not negligible. Traditionally the day +100 TRM has been used to compare different studies of HCT with myeloablative conditioning. Because of the substantial number of complications observed during the first year post transplant after nonmyeloablative or reduced-intensity HCT, the day +100 TRM may not fully estimate the toxicity related to the procedure, and the longest period for which TRM has been reported is therefore included in Tables 1-8. Though the number of patients in our study was small we found that the number of relapses were higher in patients with multiple myeloma than in patients with other mature B-cell malignancies (Figure 8B) (Petersen et al, 2004a). When examining Tables 1-5 rather high incidences of relapse have been observed in both multiple myeloma and non-Hodgkin lymphomas, whereas the relapse rate in chronic lymphocytic leukemia generally appears lower. Whether this is due to differences in the susceptibility of the diseases to the GVT effect is to early to conclude. However, the 3-year progression-free survival of 21% in 229 patients with multiple myeloma reported recently (Crawley et al, 2005a) indicates that further progress in the treatment of this disease is warranted. In the study by Crawley et al (2005a) chemoresistant disease negatively affected both the overall- and progression-free survival, a finding which is in line with the finding by Kröger et al (2004a) that relapse after a prior autologous HCT, predicted for an increased relapse rate and decreased overall- and progression-free survival. It has also been observed that patients who had received many cycles of chemotherapy had a lover progression-free survival than the less heavily pretreated patients (Gerull et al, 2005).

In summary these data indicate that the disease control in multiple myeloma following nonmyeloablative or reduced-intensity HCT is limited in patients with advanced disease. Trials of the tandem approach where an autologous HCT is closely followed by an allogeneic HCT are ongoing and will hopefully elucidate whether the initial promising results of this schedule (Kroger et al, 2002b; Maloney et al, 2003; Galimberti et al, 2005) will lead to durable remissions.

From the studies summarized in Tables 1-8 it is not possible to determine whether one specific conditioning regimen compares favorably to other regimens with respect to the clinical outcome. It is also difficult to determine whether the outcome of these studies is better or worse than the outcome following conventional chemotherapy or following myeloablative HCT, because no randomized studies exist. Retrospectively, the role of the conditioning regimen for the clinical outcome has been examined. In patients with acute myeloid leukemia and myelodysplastic syndrome a similar overall survival was observed in recipients of the nonmyeloablative FAI regimen and in recipients of the reduced-intensity FM regimen, Table 7 (de Lima et al, 2004). The number of relapses were higher in the FAI group while the TRM was higher in the FM group (de Lima et al, 2004) indicating that both the toxicity and the anti-neoplastic

Table 1. Multiple myeloma.

Study	Number of patients	TRM, cumulative incidences, %	Disease progression cumulative incidences, %	OS, %	PFS, %
Crawley et al, 2005a	229	26 (2 y)	50 (3 y)	41 (3 y)	21 (3 y)
Kroger et al, 2004a	120	18 (1 y)	43 (2 y)	59 (2 y)	39 (2 y)
Kroger et al, 2004b	68	18-24 (1 y)	60 (2 y)	47 (2 y)	33 (2 y)
Maloney et al, 2003	52	15[1]	–	78[1]	55 (2 y)
Gerull et al, 2005	52	17[2]	56[2]	41 (1½ y)	29 (1½ y)
Shimazaki et al, 2005	45	9 (100 d)	–	39 (3 y)	19 (3 y)
Lee et al, 2003a	45	38[3]	42 (3 y)	36 (3 y)	13 (3 y)
Mohty et al, 2004	41	17[4]	51[4]	62 (2 y)	41 (2 y)
Perez-Simon et al, 2003	29	21[5]	–	60 (2 y)	33 (2 y)
Giralt et al, 2002	22	40 (1 y)	–	30 (2 y)	19 (2 y)
Einsele et al, 2003	22	23[6]	50[6]	26 (2 y)	22 (2 y)
Corradini et al, 2005	22	13 (5 y)	–	70 (5 y)	30 (5 y)
Kroger et al, 2002a	21	26 (1 y)	–	74 (2 y)	53 (2 y)
Galimberti et al, 2005	20	20[7]	–	58 (2 y)	51 (2 y)
Kroger et al, 2002b	17	11 (100 d)	–	74 (2 y)	56 (2 y)

TRM = transplant related mortality; OS = overall survival; PFS = progression free survival; y = years; d = days. The median follow-up was: 1) 550 days, 2) 479 days, 3) 15 months, 4) 389 days, 5) 366 days, 6) 20 months and 7) 16 months.

Table 2. Low grade Non Hodgkin lymphoma.

Study	Number of patients	TRM, cumulative incidences, %	Disease progression cumulative incidences, %	OS, %	PFS, %
Corradini et al, 2005	53[2]	18 (5 y)	–	66 (5 y)	73 (5 y)
Robinson et al, 2002	52[1]	31 (2 y)	21 (2 y)	65 (2 y)	54 (2 y)
Morris et al, 2004	41[3]	11 (3 y)	44 (3 y)	73 (3 y)	65 (3 y)
Kusumi et al, 2005	44[4]	–	–	81 (3 y)	–
Khouri et al, 2001	20[5]	–	0	84 (2 y)	84 (2 y)

TRM = transplant related mortality; OS = overall survival; PFS = progression free survival; y = years. Includes: 1) Low-grade lymphomas classified by the international working formulation, 2) follicular lymphoma, mantle cell lymphoma and small lymphocytic lymphoma, 3) follicular lymphoma (70%), lymphoplasmacytoid lymphoma, chronic lymphocytic leukemia and prolymphocytic leukemia, 4) follicular lymphoma (100%) and 5) folliular lymphoma (90%).

Table 3. High grade Non Hodgkin lymphoma.

Study	Number of patients	TRM, cumulative incidences, %	Disease progression cumulative incidences, %	OS, %	PFS, %
Robinson et al, 2002	62[1]	37 (2 y)	79 (2 y)	47 (2 y)	13 (2 y)
Corradini et al, 2005	52[2]	12 (5 y)	–	72 (5 y)	59 (5 y)
Morris et al, 2004	37[3]	38 (3 y)	52 (3 y)	34 (3 y)	34 (3 y)
Kusumi et al, 2005	27[4]	–	–	31 (3 y)	–

TRM = transplant related mortality; OS = overall survival; PFS = progression free survival; y = years. Includes: 1) High-grade lymphomas classified by the international working formulation, 2) Diffuse large B-cell lymphoma, peripheral T-cell lymphoma, 3) Diffuse large B-cell lymphoma, peripheral T-cell lymphoma and transformed low-grade NHL and 4) Diffuse large B-cell lymphoma.

Table 4. Mantle cell lymphoma.

Study	Number of patients	TRM, cumulative incidences, %	Disease progression cumulative incidences, %	OS, %	PFS, %
Robinson et al, 2004	144	50 (2 y)	57 (2 y)	31 (2 y)	26 (2 y)
Maris et al, 2004b	33	24 (2 y)	16 (2 y)	64 (2 y)	60 (2 y)
Khouri et al, 1999	16	31[1]	–	55 (3 y)	55 (3 y)
Morris et al, 2004	10	20 (3 y)	50 (3 y)	60 (3 y)	50 (3 y)
Kusumi et al, 2005	8	–	–	75 (3 y)	–

TRM = transplant related mortality; OS = overall survival; PFS = progression free survival; y = years. 1) Median follow-up 24 months.

effect of the conditioning regimen may affect the clinical outcome. In a study by Aoudjhane et al (2005) 315 patients with acute myeloid leukemia who had received different nonmyeloablative- or reduced-intensity conditioning were matched to 407 recipients of myeloablative conditioning. There was no difference in the overall and progression-free survival between the two groups. Again the TRM was reduced in recipients of nonmyeloablative or reduced-intensity conditioning but this advantage was offset by a higher rate of relapse (Aoudjhane et al, 2005). In contrast, Scott et al (2006) found no difference in relapse rate or progression-free survival between

Table 5. Chronic lymphocytic leukemia.

Study	Number of patients	TRM, cumulative incidences, %	Disease progression cumulative incidences, %	OS, %	PFS, %
Dreger et al, 2003	77	18 (1 y)	31 (2 y)	72 (2 y)	56 (2 y)
Sorror et al, 2005	64	22 (2 y)	26 (2 y)	60 (2 y)	52 (2 y)
Delgado et al, 2006	41	26 (2 y)	29 (2 y)	51 (2 y)	45 (2 y)
Schetelig et al, 2003b	30	15 (2 y)	16 (2 y)[1]	72 (2 y)	67 (2 y)
Khouri et al, 2004	17	22 (2 y)	18[2]	80 (2 y)	60 (2 y)

TRM = transplant related mortality; OS = overall survival; PFS = progression free survival; y = years.
1) Relapse mortality and 2) Median follow-up 21 months.

Table 6. Hodgkin's disease.

Study	Number of patients	TRM, cumulative incidences, %	Disease progression cumulative incidences, %	OS, %	PFS, %
Robinson et al, 2002	52	17 (2 y)	46 (2 y)	56 (2 y)	42 (2 y)
Peggs et al, 2005	49	16 (2 y)	43[1]	56 (4 y)	39 (4 y)
Anderlini et al, 2005	40	22 (1½ y)	55 % (1½ y)	61 (1½ y)	32 (1½ y)

TRM = transplant related mortality; OS = overall survival; PFS = progression free survival; y = years.
1) Median follow-up 967 days.

Table 7. Acute myeloid leukemia and myelodysplastic syndrome.

Study	Number of patients	TRM, cumulative incidences, %	Disease progression cumulative incidences, %	OS, %	PFS, %
Aoudjhane et al, 2005	315	18 (2 y)	41 (2 y)	47 (2 y)	40 (2 y)
Sayer et al, 2003	113	53 (2 y)	26[1]	32 (2 y)	29 (2 y)
de Lima et al, 2004	62 (FM)	39 (3 y)	30[2]	35 (3 y)	32 (3 y)
	32 (FAI)	16 (3 y)	61[2]	30 (3 y)	19 (3 y)
Schmid et al, 2005	75	33 (1 y)	20[3]	42 (2 y)	40 (2 y)
Ho et al, 2004	62	15 (1 y)	–	74 (1 y)	62 (1 y)
van Besien et al, 2005	52	33 (2 y)	40 (2 y)	39 (2 y)	31 (2 y)
Scott et al, 2006	38	41 (3 y)	31 (3 y)	28 (3 y)	27 (3 y)
Martino et al, 2002	37	5 (1 y)	28 (1 y)	–	66 (1 y)
Blaise et al, 2005	33	9 (1 y)	18 (2 y)	79 (2 y)	76 (2 y)
Gupta et al, 2005	24	25 (2 y)	27[4]	52 (2 y)	44 (2 y)
Parker et al, 2002	23	31 (2 y)	31 (2 y)	48 (2 y)	39 (2 y)
Corradini et al, 2005	23	10 (5 y)	–	32 (5 y)	38 (5 y)
Bertz et al, 2003	19	22 (1 y)	21[5]	68 (1 y)	61 (1 y)
Feinstein et al, 2003	18	17 (1 y)	39[6]	54 (1 y)	42 (1 y)
Chan et al, 2003	18	14 (1 y)	13[7]	65 (1 y)	64 (1 y)
Taussig et al, 2003	16	0 (100 d)	–	69 (2 y)	56 (2 y)

TRM = transplant related mortality; OS = overall survival; PFS = progression free survival; y = years; d = days.
FM = fludarabine150 mg/m^2 and melphalan 140-180 mg/m^2; FAI = fludarabine120 mg/m^2, cytarabine 4 g/m^2 and idarubicin 36 mg/m^2.
The median follow-up was: 1) 12 months, 2) 14 months, 3) 31,5 months, 4) 21 months, 5) 825 days, 6) 766 days and 7) 14 months.

Table 8. Chronic myeloid leukemia.

Study	Number of patients	TRM, cumulative incidences, %	Disease progression cumulative incidences, %	OS, %	PFS, %
Crawley et al, 2005b	118[1]	11 (1 y)	35 (3 y)	69 (3y)	45 (3y)
	26[2]	12 (1 y)	45 (3 y)	57 (3 y)	31 (3 y)
	30[3]	29 (1 y)	69 (3 y)	24 (3 y)	11 (3 y)
	12[4]	17 (1 y)	69 (3 y)	8 (3 y)	0 (3 y)
Or et al, 2003	24[5]	13[6]	0[6]	85 (5 y)	85 (5 y)
Kerbauy et al, 2005	14[7]	15 (2 y)	22 (2 y)	70 (2 y)	–
	10[8]	12 (2 y)	64 (2 y)	56 (2 y)	–

TRM = transplant related mortality; OS = overall survival; PFS = progression free survival; y = years.
1) 1st chronic phase, 2) 2nd chronic phase, 3) accelerated phase, 4) blast crisis, 5) 1st chronic phase, 6) median follow-up 37 months, 7) 1st chronic phase and 8) four patients in 2nd chronic phase, six patients in accelerated phase.

patients transplanted with myeloablative or nonmyeloablative conditioning for myelodysplastic syndrome or acute myeloid leukemia.

In patients with chronic myeloid leukemia, Crawley et al (2005b) found that the progression-free survival was higher in patients conditioned with fludarabine, busulfan and ATG (Slavin et al, 1998) when compared to the survival in recipients of other conditioning regimens. Comparable results for overall and progression-free survival between recipients of nonmyeloablative or reduced-intensity HCT and of myeloablative HCT have also been reported in patients with chronic lymphocytic leukemia (Dreger et al, 2005) and in co-

horts of patients with various hematologic malignancies (Alyea et al, 2005; Kojima et al, 2005). In contrast the results of HCT with nonmyeloablative or reduced-intensity conditioning in acute lymphoblastic leukemia have generally been dismal due to high rates of relapse (Kassim et al, 2005).

There are no randomized studies available where nonmyeloablative or reduced-intensity HCT has been compared to conventional chemotherapy. However, Mohty et al (2005) investigated the outcome in patients with acute myeloid leukemia who were potential candidates for HCT with reduced-intensity conditioning. The patients were transplanted if they had an HLA-identical sibling donor but received chemotherapy if no donor was available. The results of this genetic randomization showed a higher 4-year leukemia-free survival in the donor group when compared to the no donor group (Mohty et al, 2005).

In conclusion, more research is needed to establish the role of HCT with nonmyeloablative or reduced-intensity conditioning in the treatment of patients with hematologic malignancies. We now know that responses can be obtained in a variety of diseases, but important issues such as whom to transplant, and when to transplant is currently uncertain. Though prospective comparative studies are needed to address these issues, other kinds of research is also important to improve the results of HCT with reduced-intensity or nonmyeloablative conditioning. Though the TRM in most studies is lower following the nonmyeloablative or reduced-intensity conditioning regimens compared to myeloablative conditioning regimens, it is not negligible as shown in Tables 1-8. Relapse of the malignant disease also continues to be a significant problem in most studies. In HCT with nonmyeloablative conditioning the alloreactive responses of the donor cells represents the only potentially curable principle of the procedure, and at the same time these responses are responsible for a large part of the morbidity and the mortality associated with the treatment. It is thus important to investigate the biological mechanisms involved in GVHD and the GVT effect. The knowledge obtained may then be used to design treatment protocols that reduce the pathology related to alloreactivity without compromising the GVT effect.

4. BIOLOGY OF ALLOREACTIVE RESPONSES

4.1. HEMATOPOIETIC CHIMERISM AND TOLERANCE

In murine and canine models, stable mixed hematopoietic chimerism has been achieved in HCT with nonmyeloablative conditioning regimens (Sharabi & Sachs, 1989; Storb et al, 1997; Sykes et al, 1997). This state of stable mixed hematopoietic chimerism was associated with specific donor-host tolerance exemplified by the lack of GVHD and the ability of donor skin grafts to survive while third party skin grafts were rejected (Sharabi & Sachs, 1989; Storb et al, 1997; Sykes et al, 1997; McSweeney & Storb, 1999). One of the initial goals of HCT with nonmyeloablative conditioning in humans was to obtain stable mixed hematopoietic chimerism and bi-directional donor-host tolerance as in the animal models. If individuals could be tolerized to donor antigens by this procedure it could be beneficial in recipients of solid organ transplantation, obviating the need for life long pharmacologic immunosuppression (Spitzer et al, 1999). Stable mixed hematopoietic chimerism by itself would be curable in a number of non-malignant hematologic diseases as well as creating a platform for immunologic interventions such as DLI to treat malignant diseases (McSweeney & Storb, 1999). The relationship between mixed chimerism and tolerance appears to be more complex in humans than in the animal models. Childs et al (1999) first reported that complete donor T-cell chimerism was necessary before alloresponses could occur. We found, in line with other reports, that acute GVHD could occur in the setting of mixed T-cell chimerism, indicating that mixed T-cell chimerism does not protect against alloreactivity in humans (Mattsson et al, 2001; Dey et al, 2003; Baron et al, 2004; Petersen et al, 2004b). With the onset of acute GVHD, both $CD4^+$ and $CD8^+$ donor T-cell chimerism increased and the $CD8^+$ T cells of recipient origin disappeared faster in patients with acute GVHD grades II-IV than in patients without clinical significant acute GVHD (Petersen et al, 2004b). These findings support the hypothesis that alloreactive donor T cells can contribute to the elimination of recipient hematopoietic tissues through GVH reactions (Kolb et al, 1997; Pelot et al, 1999; Storb et al, 1999). Not only GVHD but also GVT reactions may occur in the context of mixed chimerism in humans (Dey et al, 2003; Baron et al, 2004). In the study by Baron et al (2004) approximately half of the patients were still mixed T-cell chimeras at the time when they achieved complete remission.

4.2. THE GRAFT-VERSUS-TUMOR EFFECT

Intensive research has been performed into the mechanisms involved in the GVT effect. There is growing evidence that mHags are some of the primary target molecules and that alloreactive T cells specific for these mHags are the effector cells (Fontaine et al, 2001; Bleakley & Riddell, 2004). As mHags are peptides derived from polymorphic proteins one would expect a vast number of mHags to be present in any transplant situation. However, only 16 molecules have been identified as mHags in humans so far (Bleakley & Riddell, 2004). One of the intriguing aspects of the GVT effect is that while it is often associated with GVHD, it can occur in the absence of GVHD. One possible explanation for this observation could be related to the tissue distribution of the different mHags. The majority of the identified mHags has an ubiquitous tissue distribution and mismatch for these antigens may therefore be associated with reactivity against both malignant cells and normal host tissues (Bleakley & Riddell, 2004). In contrast, the expression of mHags such as HA-1 and HA-2 are confined to cells of hematopoietic origin (de Bueger et al, 1992). Disparity for these antigens could therefore lead to an alloresponse directed against both normal and malignant hematopoietic cells while sparing the non-hematopoietic tissues of the host, which clinically would be observed as a GVT effect without GVHD (Mutis et al, 1999b; Dickinson et al, 2002; Marijt et al, 2003). This explanation may be overly simplistic because disparity for HA-1 has in some studies been implicated in the development of GVHD (Goulmy et al, 1996; Tseng et al, 1999; Mutis et al, 1999a; Lin et al, 2001). Inflammatory cytokines, released during an alloresponse, can mediate acute GVHD in mice that lack expression of alloantigen on the epithelial cells (Teshima et al, 2002) and inflammation may induce expression of mHags, normally restricted to the hematopoietic tissues, on non-hematopoietic cells (Kloosterboer et al, 2005). This indicates that the separation of GVHD and the GVT effect may be difficult to achieve in the clinical setting. Recently, it has been shown that many of the mHags identified in humans are derived from proteins implicated in tumorigenesis and that solid tumors may aberrantly express mHags such as HA-1 (Klein et al, 2002; Spierings et al, 2004; Tykodi et al, 2004). These observations may encourage further development of allogeneic HCT as immunotherapy not only for hematologic malignancies but also for solid tumors, for example by post-transplant vaccination or infusion of mHag specific T cells (Tykodi et al, 2004; Hambach & Goulmy, 2005). Alloreactive NK cells have also been implicated in GVT responses following HCT, especially in recipients of T-cell depleted grafts. The activity of NK cells is influenced by killer-cell immunoglobulin-like receptors (KIRs) present on the surface of the NK cells. This receptor family includes inhibitory receptors that recognize HLA class I alleles known as KIR epitopes (Parham & McQueen, 2003). According to the missing self recognition model, the NK cells of an individual carries inhibitory KIRs specific for the HLA class I molecules of the individual, and interaction between the inhibitory KIRs and HLA class I on the surface of autologous cells sends a negative signal to the NK cell, which prevents killing of the autologous cells. In contrast, allogeneic cells, which express other HLA class I alleles fail to interact with the inhibitory KIRs on the NK cells of the individual and are killed (Karre, 2002). In haploidentical HCT the donor and

the recipient only share one set of HLA class I alleles and the recipient cells will therefore fail to express KIR epitopes that can interact with the inhibitory KIRs on the donor NK cells. In this transplant setting, where extensive T-cell depletion is used, it has been shown that NK-cell alloreactivity plays an important role in the eradication of acute myeloid leukemia (Ruggeri et al, 1999; Ruggeri et al, 2002). Interestingly the patients with KIR epitope incompatibility did not develop GVHD, and by using murine models the authors showed that this could be due to elimination of the recipient APC by the alloreactive NK cells (Ruggeri et al, 2002). Besides the control of NK-cell activity that is based on the interaction between KIRs and HLA class I, NK-cell activity is also influenced by HLA class I independent mechanisms (Kumar & McNerney, 2005). It is therefore possible for an individual to harbor potentially autoreactive NK cells that carries KIRs for which the individual have no corresponding HLA-alleles and thus KIR epitope incompatibility may also be present in HLA-identical HCT (Shilling et al, 2002; Dupont & Hsu, 2004). That NK-cell alloreactivity may play a role in HLA-identical HCT is suggested by a recent study, where patients who lacked one or more of the HLA class I alleles for the inhibitory KIRs of the donors had a lower relapse incidence than patients with KIR epitope compatibility (Hsu et al, 2005).

4.3. PATHOPHYSIOLOGY OF GRAFT-VERSUS-HOST DISEASE

While the pathophysiology of chronic GVHD remains poorly characterized, acute GVHD has been thoroughly investigated in animal models and a three-step model has been proposed by Ferrara and colleagues (Reddy & Ferrara, 2003; Ferrara et al, 2005). In the first phase of the process, tissue damage by the conditioning regimen results in the translocation of lipopolysaccharide (LPS) from the intestinal lumen to the circulation and release of inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1 (Hill et al, 1997; Hill & Ferrara, 2000). One of the effects of LPS and the inflammatory cytokines is to induce maturation and activation of host APC such as dendritic cells, leading to an increased expression of MHC, costimulatory and adhesion molecules on the APC surface, which in turn enhances donor T-cell activation (Matzinger, 2002; Reddy & Ferrara, 2003; Shlomchik, 2003). Recent studies has highlighted the role of host APC as being essential for donor T-cell activation and initiation of GVHD, but APC of donor origin may intensify and propagate ongoing GVHD and thus contribute to the pathology of GVHD (Shlomchik et al, 1999; Teshima et al, 2002; Matte et al, 2004; Merad et al, 2004; Tivol et al, 2005). When T cells are activated in the second phase of the process, they upregulate the expression of the IL-2 receptor, proliferate and secrete cytokines such as IL-2 and interferon (IFN)-γ (Th1 cytokines) and/or IL-4, IL-5, IL-10 and IL-13 (Th2 cytokines) (Mosmann et al, 1986; Ferrara et al, 2005). The exact role of these and other cytokines in this process named the "cytokine storm" which accompanies acute GVHD is not completely elucidated. However, acute GVHD is primarily a Th1 response where IL-2 is essential for continuous proliferation and amplification of the response. And where IFN-γ lowers the amount of LPS needed to induce production of inflammatory cytokines by macrophages and thereby creates a vicious circle of increasing inflammation (Ferrara et al, 2005). In the third phase of the process, cells in the target tissues of GVHD, which are primarily the skin, liver, gut and lymphohematopoietic tissues, are destroyed by direct killing by donor cytotoxic T cells using the Fas/Fas ligand or the perforin/granzyme pathways (Graubert et al, 1997; van den Brink & Burakoff, 2002; Reddy & Ferrara, 2003) and by the toxicity of inflammatory cytokines such as IL-1 and TNF-α (Teshima et al, 2002).

4.4. CYTOKINES IN ALLOREACTIVE RESPONSES

Though acute GVHD is characterized as a Th1 response, the Th1/Th2 paradigm is not applicable to all aspects of alloreactivity. Interleukin-4 has been shown to be necessary for activating alloreactive $CD4^+$ T cells by an APC dependent mechanism (Bagley et al, 2000) and IL-2 is involved in the development of tolerance (Nelson, 2004). However, T cells that have been experimentally manipulated to express a Th2 phenotype, characterized by the production of IL-4 and IL-10 when stimulated, have been shown to inhibit development of acute GVHD in murine models (Fowler et al, 1994a; Fowler et al, 1994b; Krenger et al, 1995). In addition, human studies have shown that high pre-transplant frequencies of donor IL-2 producing helper T lymphocyte precursors (HTLp) reacting against recipient antigens are associated with an increased risk of acute GVHD (Theobald et al, 1992; Schwarer et al, 1993; Russell, 2002) and with a lower risk of relapse (Russell et al, 2001). Similarly high frequencies of anti-recipient IL-4 producing HTLp have been related to a reduced risk of acute GVHD and an increased risk of relapse (Imami et al, 1998). The combined determination of IL-2 and IL-4 producing HTLp frequencies would potentially give a more accurate estimate of the degree of alloreactivity that could be expected after allogeneic HCT in each donor/recipient combination. With the aim of performing simultaneous determination of IL-2 and IL-4 producing HTLp frequencies by limiting dilution analysis (LDA), we utilized the CTLL-2 bioassay and the CT.h4S bioassay to study the secretion of IL-2 and IL-4 in HLA-mismatched mixed lymphocyte cultures (MLCs) (Petersen et al, 2002). The results showed that though we were able to optimize the CT.h4S bioassay to measure IL-4 concentrations as low as 5 pg/ml, we failed to detect IL-4 in most of the MLCs that were performed with 5×10^4 responder cells and incubated for 3 days (Petersen et al, 2002). High amounts of soluble IL-4 receptor produced by activated T cells could in theory be responsible for this lack of IL-4 detection. To test this hypothesis we used an enzyme linked immunosorbent assay (ELISA) to detect soluble IL-4 receptor, but did not detect significant amounts of this molecule in the MLC supernatants (Petersen et al, 2002). Finally we performed large MLCs with 1×10^6 responder cells and found that whereas 3-6 days of incubation was optimal for IL-2 detection, the optimal incubation period for IL-4 detection was 12 days or more.

In conclusion we found that a combined IL-2/IL-4 HTLp assay was not practical due to low frequencies of IL-4 producing cells in response to HLA-mismatched stimulator cells and due to differences in the optimal incubation period needed to detect the two cytokines (Petersen et al, 2002). The secretion of IL-4 in response to HLA-identical stimulator cells was not investigated in the study mentioned above. However, with the use of real-time quantitative RT-PCR we did not detect significant amounts of IL-4 mRNA in HLA-identical MLCs performed with 5×10^4 responder cells and incubated for 3 days (Petersen et al, 2006).

In vitro methods such as LDA, flowcytometry with intracellular staining and enzyme linked immunospot (ELISPOT) assays have been used after allogeneic HCT to quantify cytokine secreting cells and their relation to the development of GVHD. By using LDA we found that the frequencies of IL-2 producing cells that reacted against recipient antigens were higher in patients with acute GVHD grades II-IV than in patients without clinical significant acute GVHD (Figure 9) (Petersen et al, 2005). This finding confirms that it is possible to quantify the degree of ongoing alloreactivity by use of LDA of IL-2 producing cells following HCT (Nierle et al, 1993; Bunjes et al, 1995). However, due to frequent occurrence of inhibition of the IL-2 production and to the subsequent complexity of the computational analysis, we found that the assay was not suitable as a routine tool in allogeneic HCT (Petersen et al, 2005).

In studies performed with ELISPOT or flowcytometry with intracellular staining, both IFN-γ and IL-4 have been implicated in the development of GVHD (Guo et al, 2004; Hirayama et al, 2005; Takabayashi et al, 2005). Higher frequencies of INF-γ producing cells have been found in patients with acute GVHD when compared to patients without acute GVHD (Guo et al, 2004; Hirayama et al, 2005). The results are more inconsistent for IL-4, one study showing higher frequencies in patients with acute GVHD when compared to

patients without GVHD (Hirayama et al, 2005), one study showing no difference (Guo et al, 2004) and one study showing lower frequencies of IL-4 producing cells in patients with acute GVHD (Takabayashi et al, 2005). Other methods that have been employed in the study of cytokines and development of GVHD have included determination of cytokine gene expression in cells from the peripheral blood, measurement of cytokines and cytokine receptors in serum, plasma or culture supernatants and investigation of polymorphisms in the genes that controls the production of cytokines or the expression of cytokine receptors.

We performed a longitudinal study of the gene expression of IL-2, IL-4, IL-10, IL-18, TNF-α and transforming growth factor β (TGF-β) in peripheral blood mononuclear cells obtained from 21 recipients of HCT with nonmyeloablative conditioning (Petersen et al, 2006). We used real-time quantitative RT-PCR to determine the levels of cytokine mRNA in cells that were unstimulated or stimulated with irradiated recipient-, donor- or third-party mononuclear cells. The results of the study showed that the composition of the responder cells was likely to influence the results (Petersen et al, 2006). This finding may not be surprising but is important in studies of samples obtained from recipients of HCT, as the cell composition of these samples varies widely because of the differences in the reconstitution of monocytes and lymphocytes following HCT. In addition, we observed that on day +14 the levels of IL-10 mRNA in response to stimulation with irradiated recipient- or donor cells were higher in patients without acute GVHD than in patients with acute GVHD grades II-IV (Figure 10) (Petersen et al, 2006). The IL-10 mRNA detected on day +14 was likely to originate from the recipient cells and the patients who experienced progression or relapse of the malignant disease had higher pre-transplant levels of IL-10 mRNA than patients who did not relapse. Based on these findings we concluded that IL-10 might inhibit alloreactivity following allogeneic HCT potentially affecting both the occurrence of GVHD and the GVL effect (Petersen et al, 2006).

The data in the literature concerning the levels of IL-10 post transplant and the occurrence of GVHD are conflicting. In some studies high post-transplant levels of IL-10 have been related to the development of tolerance (Bacchetta et al, 1994) and a low incidence of acute GVHD (Tanaka et al, 1997; Ju et al, 2005). In other reports increased IL-10 levels have been observed before or during acute GVHD (Carayol et al, 1997; Hempel et al, 1997; Remberger & Ringden, 1997; Liem et al, 1998; Takatsuka et al, 1999; Remberger et al, 2003; Visentainer et al, 2003). The major biological function of IL-10 is to oppose inflammatory responses (Moore et al, 2001) and it is therefore difficult to determine whether IL-10 observed during an inflammatory response such as acute GVHD contribute to the response or is secreted as a regulatory mechanism. High spontaneous IL-10 production by MNC pre-transplant has been associated with a low incidence of acute GVHD (Baker et al, 1999; Holler et al, 2000) and though the genetic control of IL-10 production needs further elucidation (Mullighan & Bardy, 2004; Lin et al, 2005), studies of gene polymorphisms in the IL-10 promoter region have suggested that recipients who are intermediate or high IL-10 producers are protected against severe acute GVHD (Middleton et al, 1998; Cavet et al, 1999; Cavet et al, 2001; Socie et al, 2001; Lin et al, 2003; Dickinson et al, 2004). A recent study by Lin et al (2005) further substantiates the role of IL-10 in regulation of the development of acute GVHD. In that study there was a synergistic relationship between the recipient IL-10 genotype and the IL-10 receptor-β genotype of the donor, suggesting that both cytokine production and the receptor properties are important for regulation of alloreactivity by IL-10 following allogeneic HCT (Lin et al, 2005).

4.5. REGULATORY CELL POPULATIONS AND ALLOREACTIVITY

During the last decade one topic in immunology that has received much interest is the regulation of immuneresponses. The interest

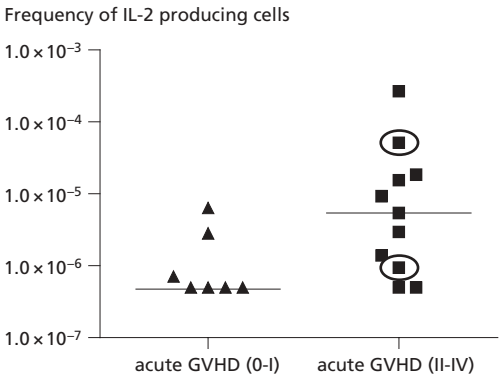


Figure 9. Comparison of the IL-2 producing HTL frequencies in the GVH direction on day 27-33 in the recipients of nonmyeloablative conditioning who developed acute GVHD grades 0-I or grades II-IV. The P-value of the Mann-Whitney test is 0.046 if all the data-points are included. With exclusion of the data-points that represent frequencies from assays performed after the patient had developed GVHD (encircled), the P-value is 0.07. Adapted from *Petersen et al,* 2005.

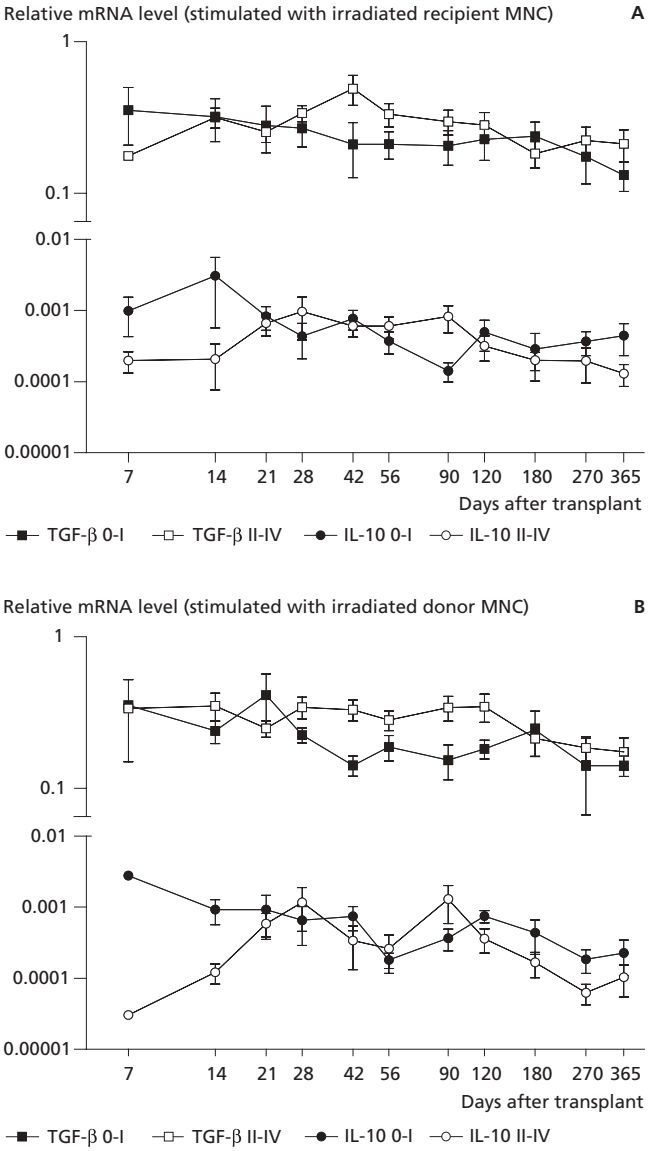


Figure 10. The relative mRNA level of TGF-β and IL-10 during the first year post transplant in cultures from patients with acute GVHD grades 0-I (closed symbols) or grades II-IV (open symbols). The results are shown for cultures stimulated with irradiated recipient mononuclear cells (MNC) (A) or irradiated donor MNC (B). Adapted from *Petersen et al,* 2006.

was prompted by the characterization of a subset of $CD4^+$ T cells that inhibited autoimmunity in mice. These cells were termed regulatory T cells (T_{reg}) and were characterized by constitutive surface expression of CD25 (the α chain of the IL-2 receptor) (Sakaguchi et al, 1995; Sakaguchi, 2004). Resting T cells normally do not express CD25 but the expression of this molecule on the cell surface is up-regulated upon activation. The $CD4^+CD25^+$ T_{reg} originally characterized underwent differentiation in the thymus, had limited potential for proliferation and inhibited the proliferation of $CD4^+CD25^-$ T cells and $CD8^+$ T cells in vitro by a cell-contact dependent mechanism, which involved down regulation of the IL-2 production (Suri-Payer et al, 1998; Thornton & Shevach, 1998; Jordan et al, 2001; Piccirillo & Shevach, 2001). In humans, $CD4^+CD25^+$ T_{reg} with similar characteristics have also been found (Ng et al, 2001). More recently, it has been shown that expression of the forkhead family transcription factor Foxp3 is critical for the generation and function of T_{reg} (Fontenot et al, 2003; Hori et al, 2003; Khattri et al, 2003). In addition it has been shown that $CD4^+CD25^+$ T_{reg} can be generated in the periphery under certain conditions (Karim et al, 2004) and that T_{reg} can be expanded in vitro while retaining their inhibitory capacity (Hoffmann et al, 2004). In addition to the $CD4^+CD25^+$ T_{reg}, several other T-cell subsets with regulatory properties have been identified, including $CD4^+$ T cells that produce high levels of IL-10 (Groux et al, 1997), $CD8^+$ T-cell populations (Jiang & Chess, 2000; Xystrakis et al, 2004) and NK-T cells (Kronenberg, 2005). In the field of HCT, regulatory T-cell populations have been shown to inhibit GVHD in animal models (Zeng et al, 1999; Taylor et al, 2002; Edinger et al, 2003; Trenado et al, 2003; Xystrakis et al, 2004). In humans, Meignin et al (2005) observed no differences in the numbers of $CD4^+CD25^+$ T_{reg} in the blood or in the expression of Foxp3 in sorted $CD4^+CD25^+$ T_{reg} between patients with and without chronic GVHD. Clark et al (2004) found higher absolute numbers of T_{reg} in patients with chronic GVHD than in patients without GVHD and Sanchez et al (2004) observed a similar trend. On the contrary Miura et al (2004) observed lower levels of Foxp3 in peripheral blood mononuclear cells from patients with acute and chronic GVHD than from patients without GVHD, and Zorn et al (2005) confirmed this finding in a larger study of patients with chronic GVHD and also found low frequencies of $CD4^+CD25^+$ T_{reg} in patients with chronic GVHD. Thus more studies are needed before definite conclusions can be made about the relationship between the numbers of $CD4^+CD25^+$ T_{reg} and GVHD development.

Depletion of $CD25^+$ cells by CD25-specific immunotoxin infusions early after transplant has been attempted with the aim of depleting T cells activated by alloantigens and thereby reducing the incidence of GVHD (Martin et al, 2004). The results suggested that this procedure could actually increase the incidence of acute GVHD grades III-IV because the concomitant administration of cyclosporine inhibited the expression of CD25 on the surface of the alloreactive cells and caused a selective depletion of cells with a constitutively high expression of CD25 such as T_{reg} (Martin et al, 2004).

An alternative way to study cell interactions in immune responses is by LDA (Lefkovits & Waldmann H, 1999; Hernandez-Fuentes et al, 2003; Bonnefoix et al, 2005). We performed LDA of IL-2 producing cells using responder cells obtained from patients during the first year following HCT with myeloablative or nonmyeloablative conditioning. In a substantial number of the assays performed, there were deviations from single-hit kinetics and signs of inhibition of the IL-2 production (Petersen et al, 2005). In limiting dilution analysis studies inhibition has been observed previously when mixtures of effector and inhibitory cells are present in the responder cell population, and mathematical models have been developed to calculate the frequencies of the different cell subsets (Fey et al, 1983; Dozmorov et al, 1995; Dozmorov et al, 1996; Dozmorov & Miller, 1996; Lefkovits & Waldmann H, 1999; Dozmorov et al, 2000; Portugal et al, 2001; Bonnefoix et al, 2003; Bonnefoix et al, 2005). Inhibition of alloresponses has previously been observed in LDA experiments with responder cells from recipients of HCT (Rosenkrantz et al, 1990; Kraus et al, 2003) but computational analysis of these cell interactions has not been attempted in a large number of samples. We used a non-linear model to analyze the LDA results and this data analysis suggested the presence of an inhibitory cell population in the responder cells (Petersen et al, 2005). Inhibition occurred throughout the first year post-transplant and was not restricted by factors such as type of graft, conditioning regimen or chimerism status. Inhibition was more often present with high frequencies of IL-2 producing cells suggesting a physiologic role of the supposed inhibitory cell population in down-regulation of immune responses (Figure 11) (Petersen et al, 2005) We do not know the phenotype of the inhibitory cell population proposed by our analysis, but $CD4^+$, $CD25^+$ T_{reg} can inhibit $CD4^+$, $CD25^-$ T-cell responses to alloantigens in a similar manner as we have observed (Ng et al, 2001; Bonnefoix et al, 2005).

In conclusion, both animal models and human studies indicate that regulatory cell populations may affect alloresponses following HCT, but further studies are needed to clarify the exact role of these cells. If this goal is achieved, one possible therapeutic application of regulatory cells could be infusion of in vitro expanded T_{reg} for treatment of GVHD.

5. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Today, almost 10 years after the first reports of allogeneic HCT with nonmyeloablative or reduced-intensity conditioning were published, this treatment modality has expanded worldwide. The procedure is now routinely offered to patients with hematologic malignancies who are ineligible to receive HCT with myeloablative conditioning. The results have shown that disease control can be achieved in many patients and that a survival comparable to the survival following HCT with myeloablative conditioning can be obtained. However, infections, GVHD and relapse of the malignancy are important factors that limit the success of the procedure. One possible way to reduce the occurrence of infections is to increase the rate of reconstitution of the adaptive immune system. Agents such as IL-7, growth hormone and keratinocyte growth factor have been explored in this context (van den Brink et al, 2004). As both GVHD and the GVT effect are manifestations of alloreactivity this phenomenon is central to the success of HCT with nonmyeloablative conditioning. The experimental work, that forms the basis for this thesis, demonstrates the complexity of alloreactive responses and the strengths and limitations of techniques such as LDA and cytokine gene expression analysis in the study of these mechanisms. The use of other

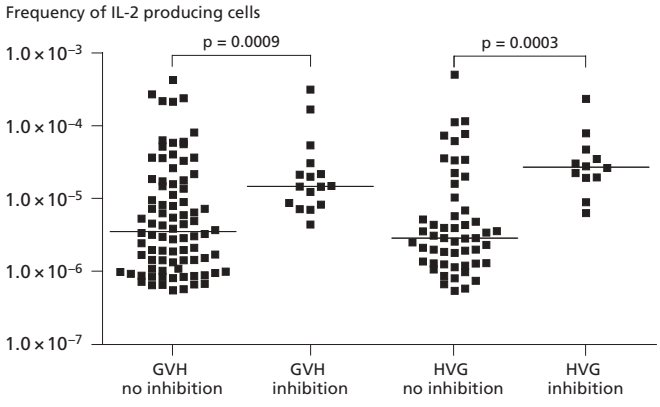


Figure 11. Results of limiting dilution analysis performed with responder cells obtained from 26 patients following hematopoietic stem cell transplantation. Comparison of the frequencies obtained in the assays where the IL-2 producing cells exhibited single-hit kinetics with or without inhibition. The stimulator cells were either of recipient (GVH) or of donor (HVG) origin. The solid line in each group of data-points represents the median. The p-values of the Mann Whitney test are shown. Adapted from *Petersen et al,* 2005.

methods such as T-cell clonotype analysis (Kollgaard et al, 2005), high sensitivity chimerism analysis (Masmas et al, 2005), analysis of gene polymorphisms and global gene expression profiling by gene-chip analysis will hopefully yield a more elaborate knowledge about the cellular and molecular mechanisms that underlie engraftment, GVHD and the GVT effect in the future. One of the challenges that remain in clinical HCT is how to separate GVHD and the GVT effect. The development of treatment schedules that limits the occurrence of the more severe forms of acute GVHD and allows only for chronic GVHD to occur could represent a step in the right direction. Simple and robust tests, such as the determination of the level of donor T-cell chimerism or absolute donor T-cell counts, that are able to estimate the risk of acute GVHD early after transplant could, if verified in a large patient population, be used clinically to devise protocols for individualized immunosuppression. Such protocols could hopefully contribute to a more safe and efficient exploitation of alloreactivity as the therapeutic principle in the treatment of patients with hematologic malignancies. The combination of a safe HCT procedure with adoptive transfer of T cells that are primed against mHags that are tissue specific or tumor specific could ultimately allow for a potent GVT effect without GVHD (Fontaine et al, 2001; Meunier et al, 2005).

An issue that becomes increasingly important as the field of HCT with nonmyeloablative or reduced-intensity conditioning moves from an experimental treatment modality to a routine procedure is the role of HCT with nonmyeloablative conditioning along with other treatment modalities in the care of patients with hematologic malignancies. To achieve this knowledge large prospective studies are needed. Collaboration between centers is therefore necessary and recently the Department of Hematology at Rigshospitalet has been included in the Seattle consortium. The inclusion of patients in the protocols initiated within the Seattle consortium and the continued participation in other multi-center trials such as those launched by the EBMT, The British Medical Research Council or other organizations will hopefully contribute to the development of optimal treatment strategies.

6. SUMMARY

Allogeneic hematopoietic cell transplantation (HCT) represents a potentially curative treatment modality in a range of hematologic malignancies. High-dose myeloablative radio-chemotherapy has conventionally been used as part of the preparative regimen before HCT for two reasons: it has a profound immunosuppressive effect on the host, limiting the ability to reject the graft and it has substantial anti-tumor efficacy. Graft rejection is an example of alloreactivity as alloreactivity denotes the immunologic reactions that occur when tissues are transplanted between two individuals within the same species. If the immune system of the host is suppressed to a degree where rejection does not occur, the possibility arises that immunocompetent donor cells can attack the recipient tissues. This phenomenon is termed Graft-versus-Host disease (GVHD) if healthy tissues of the host are attacked and the Graft-versus-Tumor (GVT) effect if the malignant cells are the targets of the reaction. Clinical studies have shown that patients who develop GVHD have a lower risk of relapse of the malignant disease and that donor lymphocyte infusion can induce durable remissions in patients with relapsed disease following the transplant. These observations indicate that a GVT effect can be present following allogeneic HCT and that this effect, like GVHD, is an alloreactive response. The toxicity of HCT with myeloablative conditioning is considerable and this limits the use of this procedure to patients below 50-60 years of age. A large proportion of the patients with hematologic malignancies are older than 60 years at diagnosis and they are therefore not eligible for this treatment.

During the last decade, conditioning regimens that are nonmyeloablative or have reduced intensity have been developed. The purpose of this development has been to extend the use of allogeneic HCT to older patients and to patients who due to the malignant disease or to comorbidities are unable to tolerate myeloablative conditioning. In allogeneic HCT with nonmyeloablative conditioning the curative potential relies entirely on the ability of the donor cells to elicit a GVT effect. Allogeneic HCT with nonmyeloablative conditioning was introduced at Department of Hematology at Rigshospitalet in March 2000. The results of this treatment modality have been promising and we and others have shown that durable remissions can be obtained in patients who are heavily pretreated. One of the goals of allogeneic HCT with nonmyeloablative conditioning was to perform both the actual transplant procedure and the clinical follow up in the outpatient setting. In the first 30 patients transplanted at Rigshospitalet, we observed that the transplant itself and the first weeks post transplant could be performed as an outpatient procedure in a number of patients. However, all the patients were admitted and the median duration of hospitalization was 44 days during the first year post transplant. Complications such as infections and GVHD were common causes of hospitalization and studies from other centers have shown that infections, GVHD and relapse of the malignancy are the major obstacles to a good result of allogeneic HCT with nonmyeloablative conditioning.

One way to improve the results of this treatment would therefore be to reduce the incidence of GVHD without compromising the GVT effect. In HCT with nonmyeloablative conditioning the relatively well-defined antineoplastic effect of high-dose myeloablative radio-chemotherapy is substituted with the alloreactive effect of the donor cells. Because the level of alloreactivity varies widely between different donor-recipient pairs, the ability to monitor the level of alloreactivity following the transplant would therefore be desirable. To this end we have investigated the ability of different immunologic and molecular methods to quantify the level of ongoing alloreactivity following the transplant. By simultaneous determination of the fraction of T cells of donor origin (donor T-cell chimerism) and the total number of T cells in the peripheral blood, we observed that patients with a high number of donor $CD8^+$ T cells on day +14 had a high risk of acute GVHD. Other studies have shown that the level of donor T-cell chimerism early after transplant predicts the development of acute GVHD. One way to exploit this knowledge could be to individualize the pharmacologic immunosuppression given post transplant. This immunosuppression is given primarily to prevent the development of GVHD but may also inhibit the GVT effect. In patients with a low risk of GVHD early tapering of the immunosuppression could be done, while the period of immunosuppression could be extended in patients with a high risk of GVHD. In this way the GVT effect could theoretically be optimized in each patient and the results of the treatment improved.

In another study we used limiting dilution analysis to monitor the frequencies of interleukin (IL)-2 producing helper T cells responding to recipient or donor antigens following the transplant. The conclusion from this study was that both the technical performance and the data analysis were to complex for this method to be used as a routine clinical tool. However, the study showed that immune responses following HCT are subject to a tight regulation and suggested that this regulation could be due to regulatory cell populations. Such regulatory cell populations have been used successfully in animal models to treat acute GVHD. The secretion of cytokines is an important aspect of immune responses. We analyzed cytokine gene expression in mononuclear cells obtained from patients and donors before and after HCT. Patients with acute GVHD had lower levels of IL-10 mRNA on day +14 than patients who did not develop acute GVHD. Patients who experienced progression or relapse of the malignant disease were characterized by higher levels of IL-10 mRNA before the transplant than patients who remained in remission. The conclusion of this study was that IL-10 might be an inhibitor of alloreactivity following allogeneic HCT with nonmyeloablativ conditioning.

Allogeneic HCT with nonmyeloablative conditioning represents a

major step forward in the treatment of patients with hematologic malignancies. However, many issues such as whom to transplant and when the transplant should be performed remain to be clarified. Large prospective studies, involving collaboration between centers, are needed to define the role of HCT with nonmyeloablative conditioning along with other treatment modalities. In addition, it is important to continue to elucidate the immunologic mechanisms that are responsible for GVHD and the GVT effect.

ABBREVIATIONS

ADAwMTS:	a disintegrinlike and metalloprotease with thrombospondin type I motifs
APC:	antigen presenting cells
ATG:	antithymocyte globulin
BCR-ABL:	breakpoint cluster region-abelson
BEAM:	carmustine-etoposide-cytarabine-melphalan
BM:	bone marrow
CMV:	cytomegalovirus
DLI:	donor lymphocyte infusion
EBMT:	European Group for Blood and Marrow Transplantation
ELISA:	enzyme linked immunosorbent assay
ELISPOT:	enzyme linked immunospot
FAI:	Fludarabine-Cytarabine-Idarubicine
FCy:	Fludarabine-Cyclophosphamide
FHCRC:	Fred Hutchinson Cancer Research Center
FM:	Fludarabine-Melphalan
GVT:	Graft-versus-Tumor
GVHD:	Graft-versus-Host disease
HCT:	hematopoietic cell transplantation
HLA:	human leukocyte antigen
HTLp:	helper T lymphocyte precursor
IFN:	interferon
IL:	interleukin
KIR:	killer-cell immunoglobulin-like receptor
LDA:	limiting dilution analysis
LPS:	lipopolysaccharide
mHag:	minor histocompatibility antigen
MHC:	major histocompatibility complex
MLC:	mixed lymphocyte culture
MMF:	mycophenolate mofetil
OS:	overall survival
PBSC:	peripheral blood stem cells
PFS:	progression free survival
RT-PCR:	reverse transcriptase polymerase chain reaction
STR:	short tandem repeats
TBI:	total body irradiation
TGF:	transforming growth factor
TNF:	tumor necrosis factor
Treg:	regulatory T cells
TRM:	transplant related mortality
TTP:	thrombotic thrombocytopenic purpura
VNTR:	variable number of tandem repeats

8. REFERENCES

Alyea, E., Weller, E., Schlossman, R., Canning, C., Mauch, P., Ng, A., Fisher, D., Gribben, J., Freeman, A., Parikh, B., Richardson, P., Soiffer, R., Ritz, J., & Anderson, K.C. (2003) Outcome after autologous and allogeneic stem cell transplantation for patients with multiple myeloma: impact of graft-versus-myeloma effect. Bone Marrow Transplant., 32, 1145-1151.

Alyea, E.P., Kim, H.T., Ho, V., Cutler, C., Gribben, J., DeAngelo, D.J., Lee, S.J., Windawi, S., Ritz, J., Stone, R.M., Antin, J.H., & Soiffer, R.J. (2005) Comparative outcome of nonmyeloablative and myeloablative allogeneic hematopoietic cell transplantation for patients older than 50 years of age. Blood, 105, 1810-1814.

Anderlini, P., Saliba, R., Acholonu, S., Okoroji, G.J., Donato, M., Giralt, S., Andersson, B., Ueno, N.T., Khouri, I., de Lima, M., Hosing, C., Cohen, A., Ippoliti, C., Romaguera, J., Rodriguez, M.A., Pro, B., Fayad, L., Goy, A., Younes, A., & Champlin, R.E. (2005) Reduced-intensity allogeneic stem cell transplantation in relapsed and refractory Hodgkin's disease: low transplant-related mortality and impact of intensity of conditioning regimen. Bone Marrow Transplant., 35, 943-951.

Antin, J.H., Childs, R., Filipovich, A.H., Giralt, S., Mackinnon, S., Spitzer, T., & Weisdorf, D. (2001) Establishment of complete and mixed donor chimerism after allogeneic lymphohematopoietic transplantation: recommendations from a workshop at the 2001 Tandem Meetings of the International Bone Marrow Transplant Registry and the American Society of Blood and Marrow Transplantation. Biol.Blood Marrow Transplant., 7, 473-485.

Aoudjhane, M., Labopin, M., Gorin, N.C., Shimoni, A., Ruutu, T., Kolb, H.J., Frassoni, F., Boiron, J.M., Yin, J.L., Finke, J., Shouten, H., Blaise, D., Falda, M., Fauser, A.A., Esteve, J., Polge, E., Slavin, S., Niederwieser, D., Nagler, A., & Rocha, V. (2005) Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT). Leukemia, 19, 2304-2312.

Auletta, J.J. & Lazarus, H.M. (2005) Immune restoration following hematopoietic stem cell transplantation: an evolving target. Bone Marrow Transplant., 35, 835-857.

Bacchetta, R., Bigler, M., Touraine, J.L., Parkman, R., Tovo, P.A., Abrams, J., de Waal, M.R., de Vries, J.E., & Roncarolo, M.G. (1994) High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. J Exp.Med., 179, 493-502.

Badros, A., Barlogie, B., Morris, C., Desikan, R., Martin, S.R., Munshi, N., Zangari, M., Mehta, J., Toor, A., Cottler-Fox, M., Fassas, A., Anaissie, E., Schichman, S., & Tricot, G. (2001) High response rate in refractory and poor-risk multiple myeloma after allotransplantation using a nonmyeloablative conditioning regimen and donor lymphocyte infusions. Blood, 97, 2574-2579.

Bagley, J., Sawada, T., Wu, Y., & Iacomini, J. (2000) A critical role for interleukin 4 in activating alloreactive CD4 T cells. Nat.Immunol., 1, 257-261.

Bahceci, E., Epperson, D., Douek, D.C., Melenhorst, J.J., Childs, R.C., & Barrett, A.J. (2003) Early reconstitution of the T-cell repertoire after nonmyeloablative peripheral blood stem cell transplantation is from post-thymic T-cell expansion and is unaffected by graft-versus-host disease or mixed chimaerism. Br.J Haematol., 122, 934-943.

Baker, K.S., Roncarolo, M.G., Peters, C., Bigler, M., DeFor, T., & Blazar, B.R. (1999) High spontaneous IL-10 production in unrelated bone marrow transplant recipients is associated with fewer transplant-related complications and early deaths. Bone Marrow Transplant., 23, 1123-1129.

Banna, G.L., Aversa, S., Sileni, V.C., Favaretto, A., Ghiotto, C., & Monfardini, S. (2004) Nonmyeloablative allogeneic stem cell transplantation (NST) after truly nonmyeloablative and reduced intensity conditioning regimens. Crit Rev.Oncol.Hematol., 51, 171-189.

Barnes, D.W.H., Corp, M.J., Loutit, J.F., & Neal, F.E. (1956) Treatment of Murine Leukaemia with X-Rays and Homologous Bone Marrow – Preliminary Communication. British Medical Journal, 2, 626-627.

Barnes, D.W.H. & Loutit, J.F. (1957) Treatment of Murine Leukaemia with X-Rays and Homologous Bone Marrow. II. British Journal of Haematology, 3, 241-252.

Baron, F., Baker, J.E., Storb, R., Gooley, T.A., Sandmaier, B.M., Maris, M.B., Maloney, D.G., Heimfeld, S., Oparin, D., Zellmer, E., Radich, J.P., Grumet, F.C., Blume, K.G., Chauncey, T.R., & Little, M.T. (2004) Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. Blood, 104, 2254-2262.

Baron, F., Little, M.T., & Storb, R. (2005a) Kinetics of engraftment following allogeneic hematopoietic cell transplantation with reduced-intensity or nonmyeloablative conditioning. Blood Rev., 19, 153-164.

Baron, F., Maris, M.B., Sandmaier, B.M., Storer, B.E., Sorror, M., Diaconescu, R., Woolfrey, A.E., Chauncey, T.R., Flowers, M.E., Mielcarek, M., Maloney, D.G., & Storb, R. (2005b) Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. J Clin.Oncol., 23, 1993-2003.

Baron, F., Maris, M.B., Storer, B.E., Sandmaier, B.M., Panse, J.P., Chauncey, T.R., Sorror, M., Little, M.T., Maloney, D.G., Storb, R., & Heimfeld, S. (2005c) High doses of transplanted $CD34^+$ cells are associated with rapid T-cell engraftment and lessened risk of graft rejection, but not more graft-versus-host disease after nonmyeloablative conditioning and unrelated hematopoietic cell transplantation. Leukemia, 19, 822-828.

Baron, F., Schaaf-Lafontaine, N., Humblet-Baron, S., Meuris, N., Castermans, E., Baudoux, E., Frere, P., Bours, V., Fillet, G., & Beguin, Y. (2003) T-cell reconstitution after unmanipulated, CD8-depleted or CD34-selected nonmyeloablative peripheral blood stem-cell transplantation. Transplantation, 76, 1705-1713.

Baron, F., Storb, R., Gooley, T., Sandmaier, B., Gisburne, S., Shin, S., Stroup, P., Baker, J., Maris, M., Maloney, D., Heimfeld, S., Grumet, F.C., Chauncey, T., Blume, K., & Little, M.T. (2005d) Assessing donor chimerism level

among CD3 T, CD4 T, CD8 T, and NK cells predicts subsequent graft rejection, GVHD, and relapse after allogeneic HCT with nonmyeloablative conditioning. Biology of Blood and Marrow Transplantation, 11, suppl. 1, abstract 11.

Bensinger, W.I., Martin, P.J., Storer, B., Clift, R., Forman, S.J., Negrin, R., Kashyap, A., Flowers, M.E., Lilleby, K., Chauncey, T.R., Storb, R., & Appelbaum, F.R. (2001) Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N.Engl.J Med., 344, 175-181.

Bertz, H., Potthoff, K., & Finke, J. (2003) Allogeneic stem-cell transplantation from related and unrelated donors in older patients with myeloid leukemia. J Clin.Oncol., 21, 1480-1484.

Bethge, W.A., Hegenbart, U., Stuart, M.J., Storer, B.E., Maris, M.B., Flowers, M.E., Maloney, D.G., Chauncey, T., Bruno, B., Agura, E., Forman, S.J., Blume, K.G., Niederwieser, D., Storb, R., & Sandmaier, B.M. (2004) Adoptive immunotherapy with donor lymphocyte infusions after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. Blood, 103, 790-795.

Biedermann, B.C., Sahner, S., Gregor, M., Tsakiris, D.A., Jeanneret, C., Pober, J.S., & Gratwohl, A. (2002) Endothelial injury mediated by cytotoxic T lymphocytes and loss of microvessels in chronic graft versus host disease. Lancet, 359, 2078-2083.

Blaise, D., Kuentz, M., Fortanier, C., Bourhis, J.H., Milpied, N., Sutton, L., Jouet, J.P., Attal, M., Bordigoni, P., Cahn, J.Y., Boiron, J.M., Schuller, M.P., Moatti, J.P., & Michallet, M. (2000) Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Societe Francaise de Greffe de Moelle. J Clin.Oncol., 18, 537-546.

Blaise, D.P., Michel, B.J., Faucher, C., Mohty, M., Bay, J.O., Bardoux, V.J., Perreau, V., Coso, D., Pigneux, A., & Vey, N. (2005) Reduced intensity conditioning prior to allogeneic stem cell transplantation for patients with acute myeloblastic leukemia as a first-line treatment. Cancer, 104, 1931-1938.

Bleakley, M. & Riddell, S.R. (2004) Molecules and mechanisms of the graft-versus-leukaemia effect. Nat.Rev.Cancer, 4, 371-380.

Boeckh, M., Leisenring, W., Riddell, S.R., Bowden, R.A., Huang, M.L., Myerson, D., Stevens-Ayers, T., Flowers, M.E., Cunningham, T., & Corey, L. (2003) Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. Blood, 101, 407-414.

Bonnefoix, T., Bonnefoix, P., Mi, J.Q., Lawrence, J.J., Sotto, J.J., & Leroux, D. (2003) Detection of suppressor T lymphocytes and estimation of their frequency in limiting dilution assays by generalized linear regression modeling. J.Immunol., 170, 2884-2894.

Bonnefoix, T., Bonnefoix, P., Perron, P., Mi, J.Q., Ng, W.F., Lechler, R., Bensa, J.C., Cahn, J.Y., & Leroux, D. (2005) Quantitating effector and regulatory T lymphocytes in immune responses by limiting dilution analysis modeling. J Immunol., 174, 3421-3431.

Bunjes, D., Theobald, M., Nierle, T., Arnold, R., & Heimpel, H. (1995) Presence of host-specific interleukin 2-secreting T helper cell precursors correlates closely with active primary and secondary chronic graft-versus-host disease. Bone Marrow Transplant., 15, 727-732.

Busca, A., Lovisone, E., Aliberti, S., Locatelli, F., Serra, A., Scaravaglio, P., Omede, P., Rossi, G., Cirillo, D., Barbui, A., Ghisetti, V., Dall'Omo, A.M., & Falda, M. (2003) Immune reconstitution and early infectious complications following nonmyeloablative hematopoietic stem cell transplantation. Hematology., 8, 303-311.

Cao, T.M., Shizuru, J.A., Wong, R.M., Sheehan, K., Laport, G.G., Stockerl-Goldstein, K.E., Johnston, L.J., Stuart, M.J., Grumet, F.C., Negrin, R.S., & Lowsky, R. (2005) Engraftment and survival following reduced-intensity allogeneic peripheral blood hematopoietic cell transplantation is affected by $CD8^+$ T-cell dose. Blood, 105, 2300-2306.

Carayol, G., Bourhis, J.H., Guillard, M., Bosq, J., Pailler, C., Castagna, L., Vernant, J.P., Pico, J.L., Hayat, M., Chouaib, S., & Caignard, A. (1997) Quantitative analysis of T helper 1, T helper 2, and inflammatory cytokine expression in patients after allogeneic bone marrow transplantation: relationship with the occurrence of acute graft-versus-host disease. Transplantation, 63, 1307-1313.

Carella, A.M., Giralt, S., & Slavin, S. (2000) Low intensity regimens with allogeneic hematopoietic stem cell transplantation as treatment of hematologic neoplasia. Haematologica, 85, 304-313.

Carella, A.M., Lerma, E., Dejana, A., Corsetti, M.T., Celesti, L., Bruni, R., Benvenuto, F., Figari, O., Parodi, C., Carlier, P., Florio, G., Lercari, G., Valbonesi, M., Casarino, L., De Stefano, F., Geniram, A., Venturino, M., Tedeschi, L., Palmieri, G., Piaggio, G., Podesta, M., Frassoni, F., Van Lint, M.T., Marmont, A.M., & Bacigalupo, A. (1998) Engraftment of HLA-matched sibling hematopoietic stem cells after immunosuppressive conditioning regimen in patients with hematologic neoplasias. Haematologica, 83, 904-909.

Cavet, J., Dickinson, A.M., Norden, J., Taylor, P.R., Jackson, G.H., & Middleton, P.G. (2001) Interferon-gamma and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. Blood, 98, 1594-1600.

Cavet, J., Middleton, P.G., Segall, M., Noreen, H., Davies, S.M., & Dickinson, A.M. (1999) Recipient tumor necrosis factor-alpha and interleukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants. Blood, 94, 3941-3946.

Chakrabarti, S., Mackinnon, S., Chopra, R., Kottaridis, P.D., Peggs, K., O'Gorman, P., Chakraverty, R., Marshall, T., Osman, H., Mahendra, P., Craddock, C., Waldmann, H., Hale, G., Fegan, C.D., Yong, K., Goldstone, A.H., Linch, D.C., & Milligan, D.W. (2002) High incidence of cytomegalovirus infection after nonmyeloablative stem cell transplantation: potential role of Campath-1H in delaying immune reconstitution. Blood, 99, 4357-4363.

Chakraverty, R., Peggs, K., Chopra, R., Milligan, D.W., Kottaridis, P.D., Verfuerth, S., Geary, J., Thuraisundaram, D., Branson, K., Chakrabarti, S., Mahendra, P., Craddock, C., Parker, A., Hunter, A., Hale, G., Waldmann, H., Williams, C.D., Yong, K., Linch, D.C., Goldstone, A.H., & Mackinnon, S. (2002) Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. Blood, 99, 1071-1078.

Champlin, R., Khouri, I., Shimoni, A., Gajewski, J., Kornblau, S., Molldrem, J., Ueno, N., Giralt, S., & Anderlini, P. (2000) Harnessing graft-versus-malignancy: non-myeloablative preparative regimens for allogeneic haematopoietic transplantation, an evolving strategy for adoptive immunotherapy. Br.J Haematol., 111, 18-29.

Chan, G.W., Foss, F.M., Klein, A.K., Sprague, K., & Miller, K.B. (2003) Reduced-intensity transplantation for patients with myelodysplastic syndrome achieves durable remission with less graft-versus-host disease. Biol.Blood Marrow Transplant., 9, 753-759.

Chao, N.J., Liu, C.X., Rooney, B., Chen, B.J., Long, G.D., Vredenburgh, J.J., Morris, A., Gasparetto, C., & Rizzieri, D.A. (2002) Nonmyeloablative regimen preserves "niches" allowing for peripheral expansion of donor T-cells. Biol.Blood Marrow Transplant., 8, 249-256.

Chien, J.W., Maris, M.B., Sandmaier, B.M., Maloney, D.G., Storb, R.F., & Clark, J.G. (2005) Comparison of lung function after myeloablative and 2 Gy of total body irradiation-based regimens for hematopoietic stem cell transplantation. Biol.Blood Marrow Transplant., 11, 288-296.

Childs, R., Chernoff, A., Contentin, N., Bahceci, E., Schrump, D., Leitman, S., Read, E.J., Tisdale, J., Dunbar, C., Linehan, W.M., Young, N.S., & Barrett, A.J. (2000) Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N.Engl.J Med., 343, 750-758.

Childs, R., Clave, E., Contentin, N., Jayasekera, D., Hensel, N., Leitman, S., Read, E.J., Carter, C., Bahceci, E., Young, N.S., & Barrett, A.J. (1999) Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. Blood, 94, 3234-3241.

Childs, R.W. & Barrett, J. (2004) Nonmyeloablative allogeneic immunotherapy for solid tumors. Annu.Rev.Med., 55, 459-475.

Chung, B., Barbara-Burnham, L., Barsky, L., & Weinberg, K. (2001) Radiosensitivity of thymic interleukin-7 production and thymopoiesis after bone marrow transplantation. Blood, 98, 1601-1606.

Clark, F.J., Gregg, R., Piper, K., Dunnion, D., Freeman, L., Griffiths, M., Begum, G., Mahendra, P., Craddock, C., Moss, P., & Chakraverty, R. (2004) Chronic graft-versus-host disease is associated with increased numbers of peripheral blood $CD4^+CD25$high regulatory T cells. Blood, 103, 2410-2416.

Clift, R.A., Buckner, C.D., Appelbaum, F.R., Bearman, S.I., Petersen, F.B., Fisher, L.D., Anasetti, C., Beatty, P., Bensinger, W.I., Doney, K., & . (1990) Allogeneic marrow transplantation in patients with acute myeloid leukemia in first remission: a randomized trial of two irradiation regimens. Blood, 76, 1867-1871.

Clift, R.A., Buckner, C.D., Appelbaum, F.R., Bryant, E., Bearman, S.I., Petersen, F.B., Fisher, L.D., Anasetti, C., Beatty, P., Bensinger, W.I., & . (1991) Allogeneic marrow transplantation in patients with chronic myeloid leukemia in the chronic phase: a randomized trial of two irradiation regimens. Blood, 77, 1660-1665.

Collins, R.H., Jr., Shpilberg, O., Drobyski, W.R., Porter, D.L., Giralt, S., Champlin, R., Goodman, S.A., Wolff, S.N., Hu, W., Verfaillie, C., List, A., Dalton, W., Ognoskie, N., Chetrit, A., Antin, J.H., & Nemunaitis, J. (1997) Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin.Oncol., 15, 433-444.

Cordonnier, C., Maury, S., Esperou, H., Pautas, C., Beaune, J., Rodet, M., Lagrange, J.L., Rouard, H., Beaumont, J.L., Bassompierre, F., Gluckman, E., Kuentz, M., & Durand-Zaleski, I. (2005) Do minitransplants have minicosts? A cost comparison between myeloablative and nonmyeloablative allogeneic stem cell transplant in patients with acute myeloid leukemia. Bone Marrow Transplant., 36, 649-654.

Corradini, P., Cavo, M., Lokhorst, H., Martinelli, G., Terragna, C., Majolino, I., Valagussa, P., Boccadoro, M., Samson, D., Bacigalupo, A., Russell, N., Montefusco, V., Voena, C., & Gahrton, G. (2003) Molecular remission after myeloablative allogeneic stem cell transplantation predicts a better relapse-free survival in patients with multiple myeloma. Blood, 102, 1927-1929.

Corradini, P., Tarella, C., Olivieri, A., Gianni, A.M., Voena, C., Zallio, F.,

Ladetto, M., Falda, M., Lucesole, M., Dodero, A., Ciceri, F., Benedetti, F., Rambaldi, A., Sajeva, M.R., Tresoldi, M., Pileri, A., Bordignon, C., & Bregni, M. (2002) Reduced-intensity conditioning followed by allografting of hematopoietic cells can produce clinical and molecular remissions in patients with poor-risk hematologic malignancies. Blood, 99, 75-82.

Corradini, P., Zallio, F., Mariotti, J., Farina, L., Bregni, M., Valagussa, P., Ciceri, F., Bacigalupo, A., Dodero, A., Lucesole, M., Patriarca, F., Rambaldi, A., Scime, R., Locasciulli, A., Bandini, G., Gianni, A.M., Tarella, C., & Olivieri, A. (2005) Effect of age and previous autologous transplantation on nonrelapse mortality and survival in patients treated with reduced-intensity conditioning and allografting for advanced hematologic malignancies. J Clin.Oncol., 23, 6690-6698.

Couban, S., Simpson, D.R., Barnett, M.J., Bredeson, C., Hubesch, L., Howson-Jan, K., Shore, T.B., Walker, I.R., Browett, P., Messner, H.A., Panzarella, T., & Lipton, J.H. (2002) A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. Blood, 100, 1525-1531.

Couriel, D.R., Saliba, R.M., Giralt, S., Khouri, I., Andersson, B., de Lima, M., Hosing, C., Anderlini, P., Donato, M., Cleary, K., Gajewski, J., Neumann, J., Ippoliti, C., Rondon, G., Cohen, A., & Champlin, R. (2004) Acute and chronic graft-versus-host disease after ablative and nonmyeloablative conditioning for allogeneic hematopoietic transplantation. Biol.Blood Marrow Transplant., 10, 178-185.

Crawley, C., Lalancette, M., Szydlo, R., Gilleece, M., Peggs, K., Mackinnon, S., Juliusson, G., Ahlberg, L., Nagler, A., Shimoni, A., Sureda, A., Boiron, J.M., Einsele, H., Chopra, R., Carella, A., Cavenagh, J., Gratwohl, A., Garban, F., Zander, A., Bjorkstrand, B., Niederwieser, D., Gahrton, G., & Apperley, J.F. (2005a) Outcomes for reduced-intensity allogeneic transplantation for multiple myeloma: an analysis of prognostic factors from the Chronic Leukaemia Working Party of the EBMT. Blood, 105, 4532-4539.

Crawley, C., Szydlo, R., Lalancette, M., Bacigalupo, A., Lange, A., Brune, M., Juliusson, G., Nagler, A., Gratwohl, A., Passweg, J., Komarnicki, M., Vitek, A., Mayer, J., Zander, A., Sierra, J., Rambaldi, A., Ringden, O., Niederwieser, D., & Apperley, J.F. (2005b) Outcomes of reduced-intensity transplantation for chronic myeloid leukemia: an analysis of prognostic factors from the Chronic Leukemia Working Party of the EBMT. Blood, 106, 2969-2976.

Cull, G.M., Haynes, A.P., Byrne, J.L., Carter, G.I., Miflin, G., Rebello, P., Hale, G., Waldmann, H., & Russell, N.H. (2000) Preliminary experience of allogeneic stem cell transplantation for lymphoproliferative disorders using BEAM-CAMPATH conditioning: an effective regimen with low procedure-related toxicity. Br.J Haematol., 108, 754-760.

Cutler, C., Giri, S., Jeyapalan, S., Paniagua, D., Viswanathan, A., & Antin, J.H. (2001) Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. J Clin.Oncol., 19, 3685-3691.

Daly, A., McAfee, S., Dey, B., Colby, C., Schulte, L., Yeap, B., Sackstein, R., Tarbell, N.J., Sachs, D., Sykes, M., & Spitzer, T.R. (2003) Nonmyeloablative bone marrow transplantation: Infectious complications in 65 recipients of HLA-identical and mismatched transplants. Biol.Blood Marrow Transplant., 9, 373-382.

Daly, A.S., Hasegawa, W.S., Lipton, J.H., Messner, H.A., & Kiss, T.L. (2002) Transplantation-associated thrombotic microangiopathy is associated with transplantation from unrelated donors, acute graft-versus-host disease and venoocclusive disease of the liver. Transfusion and Apheresis Science, 27, 3-12.

Dazzi, F., Szydlo, R.M., Craddock, C., Cross, N.C., Kaeda, J., Chase, A., Olavarria, E., Van Rhee, F., Kanfer, E., Apperley, J.F., & Goldman, J.M. (2000a) Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. Blood, 95, 67-71.

Dazzi, F., Szydlo, R.M., Cross, N.C., Craddock, C., Kaeda, J., Kanfer, E., Cwynarski, K., Olavarria, E., Yong, A., Apperley, J.F., & Goldman, J.M. (2000b) Durability of responses following donor lymphocyte infusions for patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood, 96, 2712-2716.

de Bueger, M., Bakker, A., van Rood, J.J., Van der, W.F., & Goulmy, E. (1992) Tissue distribution of human minor histocompatibility antigens. Ubiquitous versus restricted tissue distribution indicates heterogeneity among human cytotoxic T lymphocyte-defined non-MHC antigens. J Immunol., 149, 1788-1794.

de Lima, M., Anagnostopoulos, A., Munsell, M., Shahjahan, M., Ueno, N., Ippoliti, C., Andersson, B.S., Gajewski, J., Couriel, D., Cortes, J., Donato, M., Neumann, J., Champlin, R., & Giralt, S. (2004) Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. Blood, 104, 865-872.

Delgado, J., Thomson, K., Russell, N., Ewing, J., Stewart, W., Cook, G., Devereux, S., Lovell, R., Chopra, R., Marks, D.I., Mackinnon, S., & Milligan, D.W. (2006) Results of alemtuzumab-based reduced-intensity allogeneic transplantation for chronic lymphocytic leukemia: a british society of blood and marrow transplantation study. Blood, 107, 1724-1730.

Dey, B.R., McAfee, S., Colby, C., Cieply, K., Caron, M., Saidman, S., Preffer, F., Shaffer, J., Tarbell, N., Sackstein, R., Sachs, D., Sykes, M., & Spitzer, T.R. (2005) Anti-tumour response despite loss of donor chimaerism in patients treated with non-myeloablative conditioning and allogeneic stem cell transplantation. Br.J Haematol., 128, 351-359.

Dey, B.R., McAfee, S., Colby, C., Sackstein, R., Saidman, S., Tarbell, N., Sachs, D.H., Sykes, M., & Spitzer, T.R. (2003) Impact of prophylactic donor leukocyte infusions on mixed chimerism, graft-versus-host disease, and antitumor response in patients with advanced hematologic malignancies treated with nonmyeloablative conditioning and allogeneic bone marrow transplantation. Biol.Blood Marrow Transplant., 9, 320-329.

Diaconescu, R., Flowers, C.R., Storer, B., Sorror, M.L., Maris, M.B., Maloney, D.G., Sandmaier, B.M., & Storb, R. (2004) Morbidity and mortality with nonmyeloablative compared with myeloablative conditioning before hematopoietic cell transplantation from HLA-matched related donors. Blood, 104, 1550-1558.

Dickinson, A.M., Middleton, P.G., Rocha, V., Gluckman, E., & Holler, E. (2004) Genetic polymorphisms predicting the outcome of bone marrow transplants. Br.J Haematol., 127, 479-490.

Dickinson, A.M., Wang, X.N., Sviland, L., Vyth-Dreese, F.A., Jackson, G.H., Schumacher, T.N., Haanen, J.B., Mutis, T., & Goulmy, E. (2002) In situ dissection of the graft-versus-host activities of cytotoxic T cells specific for minor histocompatibility antigens. Nat.Med., 8, 410-414.

Dodero, A., Carrabba, M., Milani, R., Rizzo, E., Raganato, A., Montefusco, V., Farina, L., Milanesi, M., Longoni, P., Carlo-Stella, C., & Corradini, P. (2005) Reduced-intensity conditioning containing low-dose alemtuzumab before allogeneic peripheral blood stem cell transplantation: graft-versus-host disease is decreased but T-cell reconstitution is delayed. Exp.Hematol., 33, 920-927.

Dozmorov, I., Eisenbraun, M.D., & Lefkovits, I. (2000) Limiting dilution analysis: from frequencies to cellular interactions. Immunol.Today, 21, 15-18.

Dozmorov, I.M., Kalinichenko, V.V., Sidorov, I.A., & Miller, R.A. (1995) Antagonistic interactions among T cell subsets of old mice revealed by limiting dilution analysis. J.Immunol., 154, 4283-4293.

Dozmorov, I.M., Lutsenko, G.V., Sidorov, L.A., & Miller, R.A. (1996) Analysis of cellular interactions in limiting dilution cultures. J.Immunol. Methods, 189, 183-196.

Dozmorov, I.M. & Miller, R.A. (1996) Regulatory interactions between virgin and memory CD4 T lymphocytes. Cell Immunol., 172, 141-148.

Dreger, P., Brand, R., Hansz, J., Milligan, D., Corradini, P., Finke, J., Deliliers, G.L., Martino, R., Russell, N., van Biezen, A., Michallet, M., & Niederwieser, D. (2003) Treatment-related mortality and graft-versus-leukemia activity after allogeneic stem cell transplantation for chronic lymphocytic leukemia using intensity-reduced conditioning. Leukemia, 17, 841-848.

Dreger, P., Brand, R., Milligan, D., Corradini, P., Finke, J., Lambertenghi, D.G., Martino, R., Russell, N., van Biezen, A., Michallet, M., & Niederwieser, D. (2005) Reduced-intensity conditioning lowers treatment-related mortality of allogeneic stem cell transplantation for chronic lymphocytic leukemia: a population-matched analysis. Leukemia, 19, 1029-1033.

Dupont, B. & Hsu, K.C. (2004) Inhibitory killer Ig-like receptor genes and human leukocyte antigen class I ligands in haematopoietic stem cell transplantation. Curr.Opin.Immunol., 16, 634-643.

Edinger, M., Hoffmann, P., Ermann, J., Drago, K., Fathman, C.G., Strober, S., & Negrin, R.S. (2003) $CD4^{+}CD25^{+}$ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. Nat.Med., 9, 1144-1150.

Einsele, H., Schafer, H.J., Hebart, H., Bader, P., Meisner, C., Plasswilm, L., Liebisch, P., Bamberg, M., Faul, C., & Kanz, L. (2003) Follow-up of patients with progressive multiple myeloma undergoing allografts after reduced-intensity conditioning. Br.J Haematol., 121, 411-418.

Elliott, M.A., Nichols, W.L., Plumhoff, E.A., Ansell, S.M., Dispenzieri, A., Gastineau, D.A., Gertz, M.A., Inwards, D.J., Lacy, M.Q., Micallef, I.N.M., Tefferi, A., & Litzow, M.R. (2003) Posttransplantation thrombotic thrombocytopenic purpura: A single-center experience and a contemporary review. Mayo Clinic Proceedings, 78, 421-430.

Escalon, M.P., Champlin, R.E., Saliba, R.M., Acholonu, S.A., Hosing, C., Fayad, L., Giralt, S., Ueno, N.T., Maadani, F., Pro, B., Donato, M., McLaughlin, P., & Khouri, I.F. (2004) Nonmyeloablative allogeneic hematopoietic transplantation: a promising salvage therapy for patients with non-Hodgkin's lymphoma whose disease has failed a prior autologous transplantation. J Clin.Oncol., 22, 2419-2423.

Fallen, P.R., McGreavey, L., Madrigal, J.A., Potter, M., Ethell, M., Prentice, H.G., Guimaraes, A., & Travers, P.J. (2003) Factors affecting reconstitution of the T cell compartment in allogeneic haematopoietic cell transplant recipients. Bone Marrow Transplant., 32, 1001-1014.

Faulkner, R.D., Craddock, C., Byrne, J.L., Mahendra, P., Haynes, A.P., Prentice, H.G., Potter, M., Pagliuca, A., Ho, A., Devereux, S., McQuaker, G., Mufti, G., Yin, J.L., & Russell, N.H. (2004) BEAM-alemtuzumab reduced-intensity allogeneic stem cell transplantation for lymphoproliferative diseases: GVHD, toxicity, and survival in 65 patients. Blood, 103, 428-434.

Feinstein, L.C., Sandmaier, B.M., Hegenbart, U., McSweeney, P.A., Maloney,

D.G., Gooley, T.A., Maris, M.B., Chauncey, T.R., Bruno, B., Appelbaum, F.R., Niederwieser, D.W., & Storb, R.F. (2003) Non-myeloablative allografting from human leucocyte antigen-identical sibling donors for treatment of acute myeloid leukaemia in first complete remission. Br J Haematol, 120, 281-288.

Ferrara, J.L.M., Cooke, K.R., & Teshima, T. (2005) The Pathophysiology of Graft-vs.-Host Disease. Graft.vs.-Host Disease (ed. by J. L. M. Ferrara, K. R. Cooke, & H. J. Deeg), pp. 1-34. Marcel Dekker, New York, NY, U.S.A.

Fey, K., Melchers, I., & Eichmann, K. (1983) Quantitative studies on T cell diversity. IV. Mathematical analysis of multiple limiting populations of effector and suppressor T cells. J.Exp.Med., 158, 40-52.

Flowers, M.E., Traina, F., Storer, B., Maris, M., Bethge, W.A., Carpenter, P., Appelbaum, F., Storb, R., Sandmaier, B.M., & Martin, P.J. (2005) Serious graft-versus-host disease after hematopoietic cell transplantation following nonmyeloablative conditioning. Bone Marrow Transplant., 35, 277-282.

Fontaine, P., Roy-Proulx, G., Knafo, L., Baron, C., Roy, D.C., & Perreault, C. (2001) Adoptive transfer of minor histocompatibility antigen-specific T lymphocytes eradicates leukemia cells without causing graft-versus-host disease. Nat.Med., 7, 789-794.

Fontenot, J.D., Gavin, M.A., & Rudensky, A.Y. (2003) Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. Nat.Immunol., 4, 330-336.

Fowler, D.H., Kurasawa, K., Husebekk, A., Cohen, P.A., & Gress, R.E. (1994a) Cells of Th2 cytokine phenotype prevent LPS-induced lethality during murine graft-versus-host reaction. Regulation of cytokines and $CD8^+$ lymphoid engraftment. J Immunol., 152, 1004-1013.

Fowler, D.H., Kurasawa, K., Smith, R., Eckhaus, M.A., & Gress, R.E. (1994b) Donor CD4-enriched cells of Th2 cytokine phenotype regulate graft-versus-host disease without impairing allogeneic engraftment in sublethally irradiated mice. Blood, 84, 3540-3549.

Friedman, T.M., Varadi, G., Hopely, D.D., Filicko, J., Wagner, J., Ferber, A., Martinez, J., Brunner, J., Grosso, D., McGuire, L., Korngold, R., & Flomenberg, N. (2001) Nonmyeloablative conditioning allows for more rapid T-cell repertoire reconstitution following allogeneic matched unrelated bone marrow transplantation compared to myeloablative approaches. Biol.Blood Marrow Transplant., 7, 656-664.

Fuge, R., Bird, J.M., Fraser, A., Hart, D., Hunt, L., Cornish, J.M., Goulden, N., Oakhill, A., Pamphilon, D.H., Steward, C.G., & Marks, D.I. (2001) The clinical features, risk factors and outcome of thrombotic thrombocytopenic purpura occurring after bone marrow transplantation. British Journal of Haematology, 113, 58-64.

Fukuda, T., Boeckh, M., Carter, R.A., Sandmaier, B.M., Maris, M.B., Maloney, D.G., Martin, P.J., Storb, R.F., & Marr, K.A. (2003a) Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood, 102, 827-833.

Fukuda, T., Hackman, R.C., Guthrie, K.A., Sandmaier, B.M., Boeckh, M., Maris, M.B., Maloney, D.G., Deeg, H.J., Martin, P.J., Storb, R.F., & Madtes, D.K. (2003b) Risks and outcomes of idiopathic pneumonia syndrome after nonmyeloablative and conventional conditioning regimens for allogeneic hematopoietic stem cell transplantation. Blood, 102, 2777-2785.

Gale, R.P., Horowitz, M.M., Ash, R.C., Champlin, R.E., Goldman, J.M., Rimm, A.A., Ringden, O., Stone, J.A., & Bortin, M.M. (1994) Identical twin bone marrow transplants for leukemia. Ann.Intern.Med., 120, 646-652.

Galimberti, S., Benedetti, E., Morabito, F., Papineschi, F., Callea, V., Fazzi, R., Stelitano, C., Andreazzoli, F., Guerrini, F., Ciabatti, E., Martino, M., Nobile, F., Iacopino, P., & Petrini, M. (2005) Prognostic role of minimal residual disease in multiple myeloma patients after non-myeloablative allogeneic transplantation. Leuk.Res., 29, 961-966.

Ganster, A., Brucker, I., Holler, E., Hahn, J., Bremm, H., Andreesen, R., & Eissner, G. (2004) In vitro monitoring of endothelial complications following hematopoietic allogeneic stem cell transplantation. Bone Marrow Transplant., 33, 355-357.

George, J.N., Li, X., McMinn, J.R., Terrell, D.R., Vesely, S.K., & Selby, G.B. (2004) Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic HPC transplantation: a diagnostic dilemma. Transfusion, 44, 294-304.

Georges, G.E. & Storb, R. (2003) Review of "minitransplantation": nonmyeloablative allogeneic hematopoietic stem cell transplantation. Int.J Hematol., 77, 3-14.

Gerull, S., Goerner, M., Benner, A., Hegenbart, U., Klein, U., Schaefer, H., Goldschmidt, H., & Ho, A.D. (2005) Long-term outcome of nonmyeloablative allogeneic transplantation in patients with high-risk multiple myeloma. Bone Marrow Transplant., 36, 963-969.

Giralt, S., Aleman, A., Anagnostopoulos, A., Weber, D., Khouri, I., Anderlini, P., Molldrem, J., Ueno, N.T., Donato, M., Korbling, M., Gajewski, J., Alexanian, R., & Champlin, R. (2002) Fludarabine/melphalan conditioning for allogeneic transplantation in patients with multiple myeloma. Bone Marrow Transplant., 30, 367-373.

Giralt, S., Estey, E., Albitar, M., van Besien, K., Rondon, G., Anderlini, P., O'Brien, S., Khouri, I., Gajewski, J., Mehra, R., Claxton, D., Andersson, B., Beran, M., Przepiorka, D., Koller, C., Kornblau, S., Korbling, M., Keating, M., Kantarjian, H., & Champlin, R. (1997) Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. Blood, 89, 4531-4536.

Giralt, S., Thall, P.F., Khouri, I., Wang, X., Braunschweig, I., Ippolitti, C., Claxton, D., Donato, M., Bruton, J., Cohen, A., Davis, M., Andersson, B.S., Anderlini, P., Gajewski, J., Kornblau, S., Andreeff, M., Przepiorka, D., Ueno, N.T., Molldrem, J., & Champlin, R. (2001) Melphalan and purine analog-containing preparative regimens: reduced- intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood, 97, 631-637.

Glucksberg, H., Storb, R., Fefer, A., Buckner, C.D., Neiman, P.E., Clift, R.A., Lerner, K.G., & Thomas, E.D. (1974) Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation, 18, 295-304.

Gorak, E., Geller, N., Srinivasan, R., Espinoza-Delgado, I., Donohue, T., Barrett, A.J., Suffredini, A., & Childs, R. (2005) Engraftment syndrome after nonmyeloablative allogeneic hematopoietic stem cell transplantation: incidence and effects on survival. Biol.Blood Marrow Transplant., 11, 542-550.

Goulmy, E., Schipper, R., Pool, J., Blokland, E., Falkenburg, J.H., Vossen, J., Gratwohl, A., Vogelsang, G.B., van Houwelingen, H.C., & van Rood, J.J. (1996) Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. N.Engl.J Med., 334, 281-285.

Gratwohl, A., Baldomero, H., Schmid, O., Horisberger, B., Bargetzi, M., & Urbano-Ispizua, A. (2005) Change in stem cell source for hematopoietic stem cell transplantation (HSCT) in Europe: a report of the EBMT activity survey 2003. Bone Marrow Transplant., 36, 575-590.

Graubert, T.A., DiPersio, J.F., Russell, J.H., & Ley, T.J. (1997) Perforin/granzyme-dependent and independent mechanisms are both important for the development of graft-versus-host disease after murine bone marrow transplantation. J Clin.Invest, 100, 904-911.

Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J.E., & Roncarolo, M.G. (1997) A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature, 389, 737-742.

Guo, H., Qiao, Z., Zhu, L., Wang, H., Su, L., Lu, Y., Cui, Y., Jiang, B., Zhu, Q., & Xu, L. (2004) Th1/Th2 cytokine profiles and their relationship to clinical features in patients following nonmyeloablative allogeneic stem cell transplantation. Am.J Hematol., 75, 78-83.

Gupta, V., Daly, A., Lipton, J.H., Hasegawa, W., Chun, K., Kamel-Reid, S., Tsang, R., Yi, Q.L., Minden, M., Messner, H., & Kiss, T. (2005) Nonmyeloablative stem cell transplantation for myelodysplastic syndrome or acute myeloid leukemia in patients 60 years or older. Biol.Blood Marrow Transplant., 11, 764-772.

Gutman, J.A., Bearman, S.I., Nieto, Y., Sweetenham, J.W., Jones, R.B., Shpall, E.J., Zeng, C., Baron, A., & McSweeney, P.A. (2005) Autologous transplantation followed closely by reduced-intensity allogeneic transplantation as consolidative immunotherapy in advanced lymphoma patients: a feasibility study. Bone Marrow Transplant., 36, 443-451.

Hahn, T., Alam, A.R., Lawrence, D., Ford, L., Baer, M.R., Bambach, B., Bernstein, Z.P., Czuczman, M.S., Silva, J., Slack, J.L., Wetzler, M., Becker, J., & McCarthy, P.L., Jr. (2004) Thrombotic microangiopathy after allogeneic blood and marrow transplantation is associated with dose-intensive myeloablative conditioning regimens, unrelated donor, and methylprednisolone T-cell depletion. Transplantation, 78, 1515-1522.

Hambach, L. & Goulmy, E. (2005) Immunotherapy of cancer through targeting of minor histocompatibility antigens. Curr.Opin.Immunol., 17, 202-210.

Hempel, L., Korholz, D., Nussbaum, P., Bonig, H., Burdach, S., & Zintl, F. (1997) High interleukin-10 serum levels are associated with fatal outcome in patients after bone marrow transplantation. Bone Marrow Transplant., 20, 365-368.

Hernandez-Fuentes, M.P., Warrens, A.N., & Lechler, R.I. (2003) Immunologic monitoring. Immunol.Rev., 196, 247-264.

Hill, G.R., Crawford, J.M., Cooke, K.R., Brinson, Y.S., Pan, L., & Ferrara, J.L. (1997) Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. Blood, 90, 3204-3213.

Hill, G.R. & Ferrara, J.L. (2000) The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. Blood, 95, 2754-2759.

Hirayama, M., Azuma, E., Kumamoto, T., Iwamoto, S., Yamada, H., Nashida, Y., Araki, M., Kageyama, S., Tamaki, S., Kawakami, K., Yamamoto, H., & Komada, Y. (2005) Prediction of acute graft-versus-host disease and detection of distinct end-organ targets by enumeration of peripheral blood cytokine spot-forming cells. Transplantation, 80, 58-65.

Ho, A.Y., Pagliuca, A., Kenyon, M., Parker, J.E., Mijovic, A., Devereux, S., & Mufti, G.J. (2004) Reduced-intensity allogeneic hematopoietic stem cell transplantation for myelodysplastic syndrome and acute myeloid leu-

kemia with multilineage dysplasia using fludarabine, busulphan, and alemtuzumab (FBC) conditioning. Blood, 104, 1616-1623.

Ho, V.T., Cutler, C., Carter, S., Martin, P., Adams, R., Horowitz, M., Ferrara, J., Soiffer, R., & Giralt, S. (2005) Blood and marrow transplant clinical trials network toxicity committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. Biol. Blood Marrow Transplant., 11, 571-575.

Hoffmann, P., Eder, R., Kunz-Schughart, L.A., Andreesen, R., & Edinger, M. (2004) Large-scale in vitro expansion of polyclonal human CD4(+) CD25high regulatory T cells. Blood, 104, 895-903.

Holler, E., Roncarolo, M.G., Hintermeier-Knabe, R., Eissner, G., Ertl, B., Schulz, U., Knabe, H., Kolb, H.J., Andreesen, R., & Wilmanns, W. (2000) Prognostic significance of increased IL-10 production in patients prior to allogeneic bone marrow transplantation. Bone Marrow Transplant., 25, 237-241.

Hori, A., Kami, M., Kim, S.W., Chizuka, A., Kojima, R., Imataki, O., Sakiyama, M., Hamaki, T., Onishi, Y., Usubuchi, N., Kishi, Y., Murashige, N., Tajima, K., Miyakoshi, S., Heike, Y., Masuo, S., Taniguchi, S., & Takaue, Y. (2004) Development of early neutropenic fever, with or without bacterial infection, is still a significant complication after reduced-intensity stem cell transplantation. Biol.Blood Marrow Transplant., 10, 65-72.

Hori, S., Nomura, T., & Sakaguchi, S. (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science, 299, 1057-1061.

Horowitz, M.M., Gale, R.P., Sondel, P.M., Goldman, J.M., Kersey, J., Kolb, H.J., Rimm, A.A., Ringden, O., Rozman, C., Speck, B., & . (1990) Graft-versus-leukemia reactions after bone marrow transplantation. Blood, 75, 555-562.

Hsu, K.C., Keever-Taylor, C.A., Wilton, A., Pinto, C., Heller, G., Arkun, K., O'Reilly, R.J., Horowitz, M.M., & Dupont, B. (2005) Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. Blood, 105, 4878-4884.

Imami, N., Brookes, P.A., Lombardi, G., Hakooz, B., Johns, M., Goldman, J.M., Batchelor, J.R., Lechler, R.I., & Ritter, M.A. (1998) Association between interleukin-4-producing T lymphocyte frequencies and reduced risk of graft-versus-host disease. Transplantation, 65, 979-988.

Jeffreys, A.J., Wilson, V., & Thein, S.L. (1985) Hypervariable "minisatellite" regions in human DNA. Nature, 314, 67-73.

Jiang, H. & Chess, L. (2000) The specific regulation of immune responses by $CD8^+$ T cells restricted by the MHC class Ib molecule, Qa-1. Annu. Rev.Immunol., 18, 185-216.

Jimenez, M., Martinez, C., Ercilla, G., Carreras, E., Urbano-Ispizua, A., Aymerich, M., Villamor, N., Amezaga, N., Rovira, M., Fernandez-Aviles, F., Gaya, A., Martino, R., Sierra, J., & Montserrat, E. (2005) Reduced-intensity conditioning regimen preserves thymic function in the early period after hematopoietic stem cell transplantation. Exp.Hematol., 33, 1240-1248.

Jordan, M.S., Boesteanu, A., Reed, A.J., Petrone, A.L., Holenbeck, A.E., Lerman, M.A., Naji, A., & Caton, A.J. (2001) Thymic selection of $CD4^+$ $CD25^+$ regulatory T cells induced by an agonist self-peptide. Nat.Immunol., 2, 301-306.

Ju, X.P., Xu, B., Xiao, Z.P., Li, J.Y., Chen, L., Lu, S.Q., & Huang, Z.X. (2005) Cytokine expression during acute graft-versus-host disease after allogeneic peripheral stem cell transplantation. Bone Marrow Transplant., 35, 1179-1186.

Junghanss, C., Boeckh, M., Carter, R.A., Sandmaier, B.M., Maris, M.B., Maloney, D.G., Chauncey, T., McSweeney, P.A., Little, M.T., Corey, L., & Storb, R. (2002a) Incidence and outcome of cytomegalovirus infections following nonmyeloablative compared with myeloablative allogeneic stem cell transplantation, a matched control study. Blood, 99, 1978-1985.

Junghanss, C., Marr, K.A., Carter, R.A., Sandmaier, B.M., Maris, M.B., Maloney, D.G., Chauncey, T., McSweeney, P.A., & Storb, R. (2002b) Incidence and outcome of bacterial and fungal infections following non-myeloablative compared with myeloablative allogeneic hematopoietic stem cell transplantation: A matched control study. Biology of Blood and Marrow Transplantation, 8, 512-520.

Karim, M., Kingsley, C.I., Bushell, A.R., Sawitzki, B.S., & Wood, K.J. (2004) Alloantigen-induced $CD25^+CD4^+$ regulatory T cells can develop in vivo from $CD25\text{-}CD4^+$ precursors in a thymus-independent process. J Immunol., 172, 923-928.

Karre, K. (2002) NK cells, MHC class I molecules and the missing self. Scand.J Immunol., 55, 221-228.

Kassim, A.A., Chinratanalab, W., Ferrara, J.L., & Mineishi, S. (2005) Reduced-intensity allogeneic hematopoietic stem cell transplantation for acute leukemias: "what is the best recipe?". Bone Marrow Transplant., 36, 565-574.

Keil, F., Prinz, E., Moser, K., Mannhalter, C., Kalhs, P., Worel, N., Rabitsch, W., Schulenburg, A., Mitterbauer, M., & Greinix, H. (2003) Rapid establishment of long-term culture-initiating cells of donor origin after non-myeloablative allogeneic hematopoietic stem-cell transplantation, and significant prognostic impact of donor T-cell chimerism on stable engraftment and progression-free survival. Transplantation, 76, 230-236.

Kerbauy, F.R., Storb, R., Hegenbart, U., Gooley, T., Shizuru, J., Al Ali, H.K., Radich, J.P., Maloney, D.G., Agura, E., Bruno, B., Epner, E.M., Chauncey, T.R., Blume, K.G., Niederwieser, D., & Sandmaier, B.M. (2005) Hematopoietic cell transplantation from HLA-identical sibling donors after low-dose radiation-based conditioning for treatment of CML. Leukemia, 19, 990-997.

Khattri, R., Cox, T., Yasayko, S.A., & Ramsdell, F. (2003) An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. Nat.Immunol., 4, 337-342.

Khouri, I.F., Keating, M., Korbling, M., Przepiorka, D., Anderlini, P., O'Brien, S., Giralt, S., Ippoliti, C., von Wolff, B., Gajewski, J., Donato, M., Claxton, D., Ueno, N., Andersson, B., Gee, A., & Champlin, R. (1998) Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. J Clin.Oncol., 16, 2817-2824.

Khouri, I.F., Lee, M.S., Romaguera, J., Mirza, N., Kantarjian, H., Korbling, M., Albitar, M., Giralt, S., Samuels, B., Anderlini, P., Rodriguez, J., von Wolff, B., Gajewski, J., Cabanillas, F., & Champlin, R. (1999) Allogeneic hematopoietic transplantation for mantle-cell lymphoma: molecular remissions and evidence of graft-versus-malignancy. Ann.Oncol., 10, 1293-1299.

Khouri, I.F., Lee, M.S., Saliba, R.M., Andersson, B., Anderlini, P., Couriel, D., Hosing, C., Giralt, S., Korbling, M., McMannis, J., Keating, M.J., & Champlin, R.E. (2004) Nonablative allogeneic stem cell transplantation for chronic lymphocytic leukemia: impact of rituximab on immunomodulation and survival. Exp.Hematol., 32, 28-35.

Khouri, I.F., Saliba, R.M., Giralt, S.A., Lee, M.S., Okoroji, G.J., Hagemeister, F.B., Korbling, M., Younes, A., Ippoliti, C., Gajewski, J.L., McLaughlin, P., Anderlini, P., Donato, M.L., Cabanillas, F.F., & Champlin, R.E. (2001) Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. Blood, 98, 3595-3599.

Klein, C.A., Wilke, M., Pool, J., Vermeulen, C., Blokland, E., Burghart, E., Krostina, S., Wendler, N., Passlick, B., Riethmueller, G., & Goulmy, E. (2002) The hematopoietic system-specific minor histocompatibility antigen HA-1 shows aberrant expression in epithelial cancer cells. J Exp. Med., 196, 359-368.

Kloosterboer, F.M., Luxemburg-Heijs, S.A., van Soest, R.A., van Egmond, H.M., Willemze, R., & Falkenburg, J.H. (2005) Up-regulated expression in nonhematopoietic tissues of the BCL2A1-derived minor histocompatibility antigens in response to inflammatory cytokines: relevance for allogeneic immunotherapy of leukemia. Blood, 106, 3955-3957.

Kojima, R., Kami, M., Kanda, Y., Kusumi, E., Kishi, Y., Tanaka, Y., Yoshioka, S., Morishima, S., Fujisawa, S., Mori, S.I., Kasai, M., Hatanaka, K., Tajima, K., Kasai, M., Mitani, K., Ichinohe, T., Hirai, H., Taniguchi, S., Sakamaki, H., Harada, M., & Takaue, Y. (2005) Comparison between reduced intensity and conventional myeloablative allogeneic stem-cell transplantation in patients with hematologic malignancies aged between 50 and 59 years. Bone Marrow Transplant., 36, 667-674.

Kojima, R., Kami, M., Nannya, Y., Kusumi, E., Sakai, M., Tanaka, Y., Kanda, Y., Mori, S., Chiba, S., Miyakoshi, S., Tajima, K., Hirai, H., Taniguchi, S., Sakamaki, H., & Takaue, Y. (2004) Incidence of invasive aspergillosis after allogeneic hematopoietic stem cell transplantation with a reduced-intensity regimen compared with transplantation with a conventional regimen. Biol.Blood Marrow Transplant., 10, 645-652.

Kolb, H.J., Gunther, W., Schumm, M., Holler, E., Wilmanns, W., & Thierfelder, S. (1997) Adoptive immunotherapy in canine chimeras. Transplantation, 63, 430-436.

Kolb, H.J., Mittermuller, J., Clemm, C., Holler, E., Ledderose, G., Brehm, G., Heim, M., & Wilmanns, W. (1990) Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood, 76, 2462-2465.

Kolb, H.J., Schattenberg, A., Goldman, J.M., Hertenstein, B., Jacobsen, N., Arcese, W., Ljungman, P., Ferrant, A., Verdonck, L., Niederwieser, D., Van Rhee, F., Mittermueller, J., de Witte, T., Holler, E., & Ansari, H. (1995) Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood, 86, 2041-2050.

Kolb, H.J., Schmid, C., Barrett, A.J., & Schendel, D.J. (2004) Graft-versus-leukemia reactions in allogeneic chimeras. Blood, 103, 767-776.

Kollgaard, T., Petersen, S.L., Hadrup, S.R., Masmas, T.N., Seremet, T., Andersen, M.H., Madsen, H.O., Vindelov, L., & thor Straten, P. (2005) Evidence for involvement of clonally expanded $CD8^+$ T cells in anticancer immune responses in CLL patients following nonmyeloablative conditioning and hematopoietic cell transplantation. Leukemia, 19, 2273-2280.

Kornacker, M., Luft, T., Ho, A.D., & Schaefer, H.J. (2005) Thrombotic microangiopathy after combined autografting-allografting for multiple myeloma – report of three cases. Eur.J Haematol., 74, 250-253.

Kottaridis, P.D., Milligan, D.W., Chopra, R., Chakraverty, R.K., Chakrabarti, S., Robinson, S., Peggs, K., Verfuerth, S., Pettengell, R., Marsh, J.C., Schey, S., Mahendra, P., Morgan, G.J., Hale, G., Waldmann, H., de Elvira, M.C., Williams, C.D., Devereux, S., Linch, D.C., Goldstone, A.H., & Mackinnon,

S. (2000) In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. Blood, 96, 2419-2425.

Kraus, A.B., Shaffer, J., Toh, H.C., Preffer, F., Dombkowski, D., Saidman, S., Colby, C., George, R., McAfee, S., Sackstein, R., Dey, B., Spitzer, T.R., & Sykes, M. (2003) Early host CD8 T-cell recovery and sensitized anti-donor interleukin-2-producing and cytotoxic T-cell responses associated with marrow graft rejection following nonmyeloablative allogeneic bone marrow transplantation. Exp.Hematol., 31, 609-621.

Krenger, W., Snyder, K.M., Byon, J.C., Falzarano, G., & Ferrara, J.L. (1995) Polarized type 2 alloreactive $CD4^+$ and $CD8^+$ donor T cells fail to induce experimental acute graft-versus-host disease. J Immunol., 155, 585-593.

Kroger, N., Perez-Simon, J.A., Myint, H., Klingemann, H., Shimoni, A., Nagler, A., Martino, R., Alegre, A., Tomas, J.F., Schwerdtfeger, R., Kiehl, M., Fauser, A., Sayer, H.G., Leon, A., Beyer, J., Zabelina, T., Ayuk, F., San Miguel, J.F., Brand, R., & Zander, A.R. (2004a) Relapse to prior autograft and chronic graft-versus-host disease are the strongest prognostic factors for outcome of melphalan/fludarabine-based dose-reduced allogeneic stem cell transplantation in patients with multiple myeloma. Biol.Blood Marrow Transplant., 10, 698-708.

Kroger, N., Sayer, H.G., Schwerdtfeger, R., Kiehl, M., Nagler, A., Renges, H., Zabelina, T., Fehse, B., Ayuk, F., Wittkowsky, G., Schmitz, N., & Zander, A.R. (2002a) Unrelated stem cell transplantation in multiple myeloma after a reduced-intensity conditioning with pretransplantation antithymocyte globulin is highly effective with low transplantation-related mortality. Blood, 100, 3919-3924.

Kroger, N., Schilling, G., Einsele, H., Liebisch, P., Shimoni, A., Nagler, A., Perez-Simon, J.A., San Miguel, J.F., Kiehl, M., Fauser, A., Schwerdtfeger, R., Wandt, H., Sayer, H.G., Myint, H., Klingemann, H., Zabelina, T., Dierlamm, J., Hinke, A., & Zander, A.R. (2004b) Deletion of chromosome band 13q14 as detected by fluorescence in situ hybridization is a prognostic factor in patients with multiple myeloma who are receiving allogeneic dose-reduced stem cell transplantation. Blood, 103, 4056-4061.

Kroger, N., Schwerdtfeger, R., Kiehl, M., Sayer, H.G., Renges, H., Zabelina, T., Fehse, B., Togel, F., Wittkowsky, G., Kuse, R., & Zander, A.R. (2002b) Autologous stem cell transplantation followed by a dose-reduced allograft induces high complete remission rate in multiple myeloma. Blood, 100, 755-760.

Kronenberg, M. (2005) Toward an understanding of NKT cell biology: progress and paradoxes. Annu.Rev.Immunol., 23, 877-900.

Kumar, V. & McNerney, M.E. (2005) A new self: MHC-class-I-independent natural-killer-cell self-tolerance. Nat.Rev.Immunol., 5, 363-374.

Kusumi, E., Kami, M., Kanda, Y., Murashige, N., Kishi, Y., Suzuki, R., Takeuchi, K., Tanimoto, T.E., Mori, T., Muta, K., Tamaki, T., Tanaka, Y., Ogawa, H., Yamane, T., Taniguchi, S., & Takaue, Y. (2005) Reduced-intensity hematopoietic stem-cell transplantation for malignant lymphoma: a retrospective survey of 112 adult patients in Japan. Bone Marrow Transplant., 36, 205-213.

Larosa, F., Marmier, C., Robinet, E., Ferrand, C., Saas, P., Deconinck, E., Bulabois, C.E., Rohrlich, P.S., Ledu, K., Helias, P., Tiberghien, P., & Cahn, J.Y. (2005) Peripheral T-cell expansion and low infection rate after reduced-intensity conditioning and allogeneic blood stem cell transplantation. Bone Marrow Transplant., 35, 859-868.

Lee, C.K., Badros, A., Barlogie, B., Morris, C., Zangari, M., Fassas, A., Van Rhee, F., Cottler-Fox, M., Jacobson, J., Thertulien, R., Muwalla, F., Mazher, S., Anaissie, E., & Tricot, G. (2003a) Prognostic factors in allogeneic transplantation for patients with high-risk multiple myeloma after reduced intensity conditioning. Exp.Hematol., 31, 73-80.

Lee, S.J., Vogelsang, G., & Flowers, M.E. (2003b) Chronic graft-versus-host disease. Biol.Blood Marrow Transplant., 9, 215-233.

Lefkovits, I. & Waldmann H (1999) Limiting dilution analysis of cells in the immune system, 2 edn, Oxford University Press, Oxford, Great Britain.

Liem, L.M., van Houwelingen, H.C., & Goulmy, E. (1998) Serum cytokine levels after HLA-identical bone marrow transplantation. Transplantation, 66, 863-871.

Lin, M.T., Gooley, T., Hansen, J.A., Tseng, L.H., Martin, E.G., Singleton, K., Smith, A.G., Mickelson, E., Petersdorf, E.W., & Martin, P.J. (2001) Absence of statistically significant correlation between disparity for the minor histocompatibility antigen-HA-1 and outcome after allogeneic hematopoietic cell transplantation. Blood, 98, 3172-3173.

Lin, M.T., Storer, B., Martin, P.J., Tseng, L.H., Gooley, T., Chen, P.J., & Hansen, J.A. (2003) Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. N.Engl.J Med., 349, 2201-2210.

Lin, M.T., Storer, B., Martin, P.J., Tseng, L.H., Grogan, B., Chen, P.J., Zhao, L.P., & Hansen, J.A. (2005) Genetic variation in the IL-10 pathway modulates severity of acute graft-versus-host disease following hematopoietic cell transplantation: synergism between IL-10 genotype of patient and IL-10 receptor β genotype of donor. Blood, 106, 3995-4001.

Little, M.T. & Storb, R. (2002) History of haematopoietic stem-cell transplantation. Nat.Rev.Cancer, 2, 231-238.

Mackinnon, S., Papadopoulos, E.B., Carabasi, M.H., Reich, L., Collins, N.H., Boulad, F., Castro-Malaspina, H., Childs, B.H., Gillio, A.P., Kernan, N.A., & . (1995) Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. Blood, 86, 1261-1268.

Maloney, D.G., Molina, A.J., Sahebi, F., Stockerl-Goldstein, K.E., Sandmaier, B.M., Bensinger, W., Storer, B., Hegenbart, U., Somlo, G., Chauncey, T., Bruno, B., Appelbaum, F.R., Blume, K.G., Forman, S.J., McSweeney, P., & Storb, R. (2003) Allografting with nonmyeloablative conditioning following cytoreductive autografts for the treatment of patients with multiple myeloma. Blood, 102, 3447-3454.

Margulies, D.H. & McCluskey, J. (2003) The Major Histocompatibility Complex and Its Encoded Proteins. Fundamental Immunology (ed. by W. E. Paul), pp. 571-612. Lippincott Williams & Wilkins, Philadelphia, PA, U.S.A.

Marijt, W.A., Heemskerk, M.H., Kloosterboer, F.M., Goulmy, E., Kester, M.G., van der Hoorn, M.A., Luxemburg-Heys, S.A., Hoogeboom, M., Mutis, T., Drijfhout, J.W., van Rood, J.J., Willemze, R., & Falkenburg, J.H. (2003) Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. Proc.Natl.Acad.Sci.U.S.A, 100, 2742-2747.

Maris, M., Boeckh, M., Storer, B., Dawson, M., White, K., Keng, M., Sandmaier, B., Maloney, D., Storb, R., & Storek, J. (2003a) Immunologic recovery after hematopoietic cell transplantation with nonmyeloablative conditioning. Exp.Hematol., 31, 941-952.

Maris, M.B., Niederwieser, D., Sandmaier, B.M., Storer, B., Stuart, M., Maloney, D., Petersdorf, E., McSweeney, P., Pulsipher, M., Woolfrey, A., Chauncey, T., Agura, E., Heimfeld, S., Slattery, J., Hegenbart, U., Anasetti, C., Blume, K., & Storb, R. (2003b) HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood, 102, 2021-2030.

Maris, M.B., Sandmaier, B.M., Storer, B., Shizuru, J., Maloney, D., Agura, E., Niederwieser, D., Pulsipher, M., Maziarz, R.T., McSweeney, P., Wade, J., Langston, A., Chauncey, T., Bruno, B., Woolfrey, A., Little, M.T., Blume, K., & Storb, R. (2004a) Unrelated donor peripheral blood stem cell (PBSC) transplantation using nonmyeloablative conditioning and mycophenolate mofetil (MMF) TID results in high engraftment rates. Blood, 104, abstract 503A.

Maris, M.B., Sandmaier, B.M., Storer, B.E., Chauncey, T., Stuart, M.J., Maziarz, R.T., Agura, E., Langston, A.A., Pulsipher, M., Storb, R., & Maloney, D.G. (2004b) Allogeneic hematopoietic cell transplantation after fludarabine and 2 Gy total body irradiation for relapsed and refractory mantle cell lymphoma. Blood, 104, 3535-3542.

Marks, D.I., Lush, R., Cavenagh, J., Milligan, D.W., Schey, S., Parker, A., Clark, F.J., Hunt, L., Yin, J., Fuller, S., Vandenberghe, E., Marsh, J., Littlewood, T., Smith, G.M., Culligan, D., Hunter, A., Chopra, R., Davies, A., Towlson, K., & Williams, C.D. (2002) The toxicity and efficacy of donor lymphocyte infusions given after reduced-intensity conditioning allogeneic stem cell transplantation. Blood, 100, 3108-3114.

Marsh, S.G., Albert, E.D., Bodmer, W.F., Bontrop, R.E., Dupont, B., Erlich, H.A., Geraghty, D.E., Hansen, J.A., Hurley, C.K., Mach, B., Mayr, W.R., Parham, P., Petersdorf, E.W., Sasazuki, T., Schreuder, G.M., Strominger, J.L., Svejgaard, A., Terasaki, P.I., & Trowsdale, J. (2005) Nomenclature for factors of the HLA system, 2004. Tissue Antigens, 65, 301-369.

Martin, P.J., Pei, J., Gooley, T., Anasetti, C., Appelbaum, F.R., Deeg, J., Hansen, J.A., Nash, R.A., Petersdorf, E.W., Storb, R., Ghetie, V., Schindler, J., & Vitetta, E.S. (2004) Evaluation of a CD25-specific immunotoxin for prevention of graft-versus-host disease after unrelated marrow transplantation. Biol.Blood Marrow Transplant., 10, 552-560.

Martinez, M.T., Bucher, C., Stussi, G., Heim, D., Buser, A., Tsakiris, D.A., Tichelli, A., Gratwohl, A., & Passweg, J.R. (2005) Transplant-associated microangiopathy (TAM) in recipients of allogeneic hematopoetic stem cell transplants. Bone Marrow Transplant., 36, 993-1000.

Martino, R., Caballero, M.D., Canals, C., San Miguel, J., Sierra, J., Rovira, M., Solano, C., Bargay, J., Perez-Simon, J., Leon, A., Sarra, J., Brunet, S., & de La, C.R. (2001a) Reduced-intensity conditioning reduces the risk of severe infections after allogeneic peripheral blood stem cell transplantation. Bone Marrow Transplant., 28, 341-347.

Martino, R., Caballero, M.D., Canals, C., Simon, J.A., Solano, C., Urbano-Ispizua, A., Bargay, J., Rayon, C., Leon, A., Sarra, J., Odriozola, J., Conde, J.G., Sierra, J., & San Miguel, J. (2001b) Allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning: results of a prospective multicentre study. Br.J Haematol., 115, 653-659.

Martino, R., Caballero, M.D., Simon, J.A., Canals, C., Solano, C., Urbano-Ispizua, A., Bargay, J., Leon, A., Sarra, J., Sanz, G.F., Moraleda, J.M., Brunet, S., San Miguel, J., & Sierra, J. (2002) Evidence for a graft-versus-leukemia effect after allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning in acute myelogenous leukemia and myelodysplastic syndromes. Blood, 100, 2243-2245.

Masmas, T.N., Madsen, H.O., Petersen, S.L., Ryder, L.P., Svejgaard, A., Alizadeh, M., & Vindelov, L.L. (2005) Evaluation and automation of hematopoietic chimerism analysis based on real-time quantitative polymerase chain reaction. Biol.Blood Marrow Transplant., 11, 558-566.

Massenkeil, G., Nagy, M., Neuburger, S., Tamm, I., Lutz, C., Le Coutre, P., Rosen, O., Wernecke, K.D., Dorken, B., & Arnold, R. (2005) Survival after reduced-intensity conditioning is not inferior to standard high-dose conditioning before allogeneic haematopoietic cell transplantation in acute leukaemias. Bone Marrow Transplant., 36, 683-689.

Matte, C.C., Liu, J., Cormier, J., Anderson, B.E., Athanasiadis, I., Jain, D., McNiff, J., & Shlomchik, W.D. (2004) Donor APCs are required for maximal GVHD but not for GVL. Nat.Med., 10, 987-992.

Mattsson, J., Uzunel, M., Brune, M., Hentschke, P., Barkholt, L., Stierner, U., Aschan, J., & Ringden, O. (2001) Mixed chimaerism is common at the time of acute graft-versus-host disease and disease response in patients receiving non-myeloablative conditioning and allogeneic stem cell transplantation. Br.J Haematol., 115, 935-944.

Matzinger, P. (2002) The danger model: a renewed sense of self. Science, 296, 301-305.

McSweeney, P.A., Niederwieser, D., Shizuru, J.A., Sandmaier, B.M., Molina, A.J., Maloney, D.G., Chauncey, T.R., Gooley, T.A., Hegenbart, U., Nash, R.A., Radich, J., Wagner, J.L., Minor, S., Appelbaum, F.R., Bensinger, W.I., Bryant, E., Flowers, M.E., Georges, G.E., Grumet, F.C., Kiem, H.P., Torok-Storb, B., Yu, C., Blume, K.G., & Storb, R.F. (2001) Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus- tumor effects. Blood, 97, 3390-3400.

McSweeney, P.A. & Storb, R. (1999) Mixed chimerism: preclinical studies and clinical applications. Biol.Blood Marrow Transplant., 5, 192-203.

Meignin, V., de Latour, R.P., Zuber, J., Regnault, A., Mounier, N., Lemaitre, F., Dastot, H., Itzykson, R., Devergie, A., Cumano, A., Gluckman, E., Janin, A., Bandeira, A., & Socie, G. (2005) Numbers of Foxp3-expressing $CD4^{+}CD25$high T cells do not correlate with the establishment of long-term tolerance after allogeneic stem cell transplantation. Exp.Hematol., 33, 894-900.

Merad, M., Hoffmann, P., Ranheim, E., Slaymaker, S., Manz, M.G., Lira, S.A., Charo, I., Cook, D.N., Weissman, I.L., Strober, S., & Engleman, E.G. (2004) Depletion of host Langerhans cells before transplantation of donor alloreactive T cells prevents skin graft-versus-host disease. Nat.Med., 10, 510-517.

Meunier, M.C., Delisle, J.S., Bergeron, J., Rineau, V., Baron, C., & Perreault, C. (2005) T cells targeted against a single minor histocompatibility antigen can cure solid tumors. Nat.Med., 11, 1222-1229.

Michallet, M., Archimbaud, E., Bandini, G., Rowlings, P.A., Deeg, H.J., Gahrton, G., Montserrat, E., Rozman, C., Gratwohl, A., & Gale, R.P. (1996) HLA-identical sibling bone marrow transplantation in younger patients with chronic lymphocytic leukemia. European Group for Blood and Marrow Transplantation and the International Bone Marrow Transplant Registry. Ann.Intern.Med., 124, 311-315.

Mickelson, E. & Petersdorf, E.W. (2004) Histocompatibility. Thomas' Hematopoietic Cell Transplantation (ed. by K. G. Blume, S. J. Forman, & F. R. Appelbaum), pp. 31-42. Blackwell Publishing, Malden, MA, U.S.A.

Middleton, P.G., Taylor, P.R., Jackson, G., Proctor, S.J., & Dickinson, A.M. (1998) Cytokine gene polymorphisms associating with severe acute graft-versus-host disease in HLA-identical sibling transplants. Blood, 92, 3943-3948.

Mielcarek, M., Burroughs, L., Leisenring, W., Diaconescu, R., Martin, P.J., Sandmaier, B.M., Maloney, D.G., Maris, M.B., Chauncey, T.R., Shizuru, J.A., Blume, K.G., Hegenbart, U., Niederwieser, D., Forman, S., Bruno, B., Woolfrey, A., & Storb, R. (2005) Prognostic relevance of "early-onset" graft-versus-host disease following non-myeloablative haematopoietic cell transplantation. Br.J Haematol., 129, 381-391.

Mielcarek, M., Martin, P.J., Leisenring, W., Flowers, M.E., Maloney, D.G., Sandmaier, B.M., Maris, M.B., & Storb, R. (2003) Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. Blood, 102, 756-762.

Miller, K.B., Roberts, T.F., Chan, G., Schenkein, D.P., Lawrence, D., Sprague, K., Gorgun, G., Relias, V., Grodman, H., Mahajan, A., & Foss, F.M. (2004) A novel reduced intensity regimen for allogeneic hematopoietic stem cell transplantation associated with a reduced incidence of graft-versus-host disease. Bone Marrow Transplant., 33, 881-889.

Mineishi, S., Kanda, Y., Saito, T., Nakai, K., Makimoto, A., Kami, M., Tanosaki, R., Wakasugi, H., Tobinai, K., & Takaue, Y. (2003) Impact of graft-versus-host disease in reduced-intensity stem cell transplantation (RIST) for patients with haematological malignancies. Br.J Haematol., 121, 296-303.

Miura, Y., Thoburn, C.J., Bright, E.C., Phelps, M.L., Shin, T., Matsui, E.C., Matsui, W.H., Arai, S., Fuchs, E.J., Vogelsang, G.B., Jones, R.J., & Hess, A.D. (2004) Association of Foxp3 regulatory gene expression with graft-versus-host disease. Blood, 104, 2187-2193.

Mohty, M., Bilger, K., Jourdan, E., Kuentz, M., Michallet, M., Bourhis, J.H., Milpied, N., Sutton, L., Jouet, J.P., Attal, M., Bordigoni, P., Cahn, J.Y., Sadoun, A., Ifrah, N., Guyotat, D., Faucher, C., Fegueux, N., Reiffers, J., Maraninchi, D., & Blaise, D. (2003) Higher doses of $CD34^{+}$ peripheral blood stem cells are associated with increased mortality from chronic graft-versus-host disease after allogeneic HLA-identical sibling transplantation. Leukemia, 17, 869-875.

Mohty, M., Boiron, J.M., Damaj, G., Michallet, A.S., Bay, J.O., Faucher, C., Perreau, V., Bilger, K., Coso, D., Stoppa, A.M., Tabrizi, R., Gastaut, J.A., Michallet, M., Maraninchi, D., & Blaise, D. (2004) Graft-versus-myeloma effect following antithymocyte globulin-based reduced intensity conditioning allogeneic stem cell transplantation. Bone Marrow Transplant., 34, 77-84.

Mohty, M., de Lavallade, H., Ladaique, P., Faucher, C., Vey, N., Coso, D., Stoppa, A.M., Gastaut, J.A., & Blaise, D. (2005) The role of reduced intensity conditioning allogeneic stem cell transplantation in patients with acute myeloid leukemia: a donor vs no donor comparison. Leukemia, 19, 916-920.

Mohty, M., Faucher, C., Vey, N., Stoppa, A.M., Viret, F., Chabbert, I., Chabannon, C., Bouabdallah, R., Ladaique, P., Collet, L., Zandotti, C., Maraninchi, D., & Blaise, D. (2000) High rate of secondary viral and bacterial infections in patients undergoing allogeneic bone marrow mini-transplantation. Bone Marrow Transplant., 26, 251-255.

Mohty, M., Kuentz, M., Michallet, M., Bourhis, J.H., Milpied, N., Sutton, L., Jouet, J.P., Attal, M., Bordigoni, P., Cahn, J.Y., Boiron, J.M., & Blaise, D. (2002) Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study. Blood, 100, 3128-3134.

Moore, K.W., de Waal, M.R., Coffman, R.L., & O'Garra, A. (2001) Interleukin-10 and the interleukin-10 receptor. Annu.Rev.Immunol., 19, 683-765.

Morris, E., Thomson, K., Craddock, C., Mahendra, P., Milligan, D., Cook, G., Smith, G.M., Parker, A., Schey, S., Chopra, R., Hatton, C., Tighe, J., Hunter, A., Peggs, K., Linch, D., Goldstone, A., & Mackinnon, S. (2004) Outcomes after alemtuzumab-containing reduced-intensity allogeneic transplantation regimen for relapsed and refractory non-Hodgkin lymphoma. Blood, 104, 3865-3871.

Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., & Coffman, R.L. (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J.Immunol., 136, 2348-2357.

Mullighan, C.G. & Bardy, P.G. (2004) Advances in the genomics of allogeneic haemopoietic stem cell transplantation. Drug Dev.Res., 62, 273-292.

Mutis, T., Gillespie, G., Schrama, E., Falkenburg, J.H., Moss, P., & Goulmy, E. (1999a) Tetrameric HLA class I-minor histocompatibility antigen peptide complexes demonstrate minor histocompatibility antigen-specific cytotoxic T lymphocytes in patients with graft-versus-host disease. Nat.Med., 5, 839-842.

Mutis, T., Verdijk, R., Schrama, E., Esendam, B., Brand, A., & Goulmy, E. (1999b) Feasibility of immunotherapy of relapsed leukemia with ex vivo-generated cytotoxic T lymphocytes specific for hematopoietic system-restricted minor histocompatibility antigens. Blood, 93, 2336-2341.

Nash, R.A., Pepe, M.S., Storb, R., Longton, G., Pettinger, M., Anasetti, C., Appelbaum, F.R., Bowden, R.A., Deeg, H.J., Doney, K., & . (1992) Acute graft-versus-host disease: analysis of risk factors after allogeneic marrow transplantation and prophylaxis with cyclosporine and methotrexate. Blood, 80, 1838-1845.

Nelson, B.H. (2004) IL-2, regulatory T cells, and tolerance. J Immunol., 172, 3983-3988.

Ng, W.F., Duggan, P.J., Ponchel, F., Matarese, G., Lombardi, G., Edwards, A.D., Isaacs, J.D., & Lechler, R.I. (2001) Human CD4(+)CD25(+) cells: a naturally occurring population of regulatory T cells. Blood, 98, 2736-2744.

Niederwieser, D., Maris, M., Shizuru, J.A., Petersdorf, E., Hegenbart, U., Sandmaier, B.M., Maloney, D.G., Storer, B., Lange, T., Chauncey, T., Deininger, M., Ponisch, W., Anasetti, C., Woolfrey, A., Little, M.T., Blume, K.G., McSweeney, P.A., & Storb, R.F. (2003) Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. Blood, 101, 1620-1629.

Nierle, T., Bunjes, D., Arnold, R., Heimpel, H., & Theobald, M. (1993) Quantitative assessment of posttransplant host-specific interleukin-2-secreting T-helper cell precursors in patients with and without acute graft-versus-host disease after allogeneic HLA-identical sibling bone marrow transplantation. Blood, 81, 841-848.

Oehler, V.G., Radich, J.P., Storer, B., Blume, K.G., Chauncey, T., Clift, R., Snyder, D.S., Forman, S.J., Flowers, M.E., Martin, P., Guthrie, K.A., Negrin, R.S., Appelbaum, F.R., & Bensinger, W. (2005) Randomized trial of allogeneic related bone marrow transplantation versus peripheral blood stem cell transplantation for chronic myeloid leukemia. Biol.Blood Marrow Transplant., 11, 85-92.

Olavarria, E., Kanfer, E., Szydlo, R., Kaeda, J., Rezvani, K., Cwynarski, K., Pocock, C., Dazzi, F., Craddock, C., Apperley, J.F., Cross, N.C., & Goldman, J.M. (2001) Early detection of BCR-ABL transcripts by quantitative reverse transcriptase-polymerase chain reaction predicts outcome after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood, 97, 1560-1565.

Omazic, B., Hentschke, P., Nasman-Bjork, I., Mattsson, J., Oxelius, V.A., Ringden, O., Barkholt, L., Permert, J., & Lundkvist, I. (2005) Reconstitution of the Ig heavy chain CDR3 repertoire after allogeneic haematopoietic stem cell transplantation with myeloablative or reduced-intensity conditioning regimens. Scand.J Immunol., 61, 72-81.

Or, R., Shapira, M.Y., Resnick, I., Amar, A., Ackerstein, A., Samuel, S., Aker, M., Naparstek, E., Nagler, A., & Slavin, S. (2003) Nonmyeloablative allogeneic stem cell transplantation for the treatment of chronic myeloid leukemia in first chronic phase. Blood, 101, 441-445.

Ottinger, H.D., Beelen, D.W., Scheulen, B., Schaefer, U.W., & Grosse-Wilde, H. (1996) Improved immune reconstitution after allotransplantation of peripheral blood stem cells instead of bone marrow. Blood, 88, 2775-2779.

Panse, J.P., Heimfeld, S., Guthrie, K.A., Maris, M.B., Maloney, D.G., Baril, B.B., Little, M.T., Chauncey, T.R., Storer, B.E., Storb, R., & Sandmaier, B.M. (2005) Allogeneic peripheral blood stem cell graft composition affects early T-cell chimaerism and later clinical outcomes after nonmyeloablative conditioning. Br.J Haematol., 128, 659-667.

Parham, P. & McQueen, K.L. (2003) Alloreactive killer cells: hindrance and help for haematopoietic transplants. Nat.Rev.Immunol., 3, 108-122.

Parikh, C.R., Schrier, R.W., Storer, B., Diaconescu, R., Sorror, M.L., Maris, M.B., Maloney, D.G., McSweeney, P., Storb, R., & Sandmaier, B.M. (2005) Comparison of ARF after myeloablative and nonmyeloablative hematopoietic cell transplantation. Am.J Kidney Dis., 45, 502-509.

Parker, J.E., Shafi, T., Pagliuca, A., Mijovic, A., Devereux, S., Potter, M., Prentice, H.G., Garg, M., Yin, J.A., Byrne, J., Russell, N.H., & Mufti, G.J. (2002) Allogeneic stem cell transplantation in the myelodysplastic syndromes: interim results of outcome following reduced-intensity conditioning compared with standard preparative regimens. Br.J Haematol., 119, 144-154.

Peggs, K.S., Hunter, A., Chopra, R., Parker, A., Mahendra, P., Milligan, D., Craddock, C., Pettengell, R., Dogan, A., Thomson, K.J., Morris, E.C., Hale, G., Waldmann, H., Goldstone, A.H., Linch, D.C., & Mackinnon, S. (2005) Clinical evidence of a graft-versus-Hodgkin's-lymphoma effect after reduced-intensity allogeneic transplantation. Lancet, 365, 1934-1941.

Peggs, K.S., Thomson, K., Hart, D.P., Geary, J., Morris, E.C., Yong, K., Goldstone, A.H., Linch, D.C., & Mackinnon, S. (2004) Dose-escalated donor lymphocyte infusions following reduced intensity transplantation: toxicity, chimerism, and disease responses. Blood, 103, 1548-1556.

Pelot, M.R., Pearson, D.A., Swenson, K., Zhao, G., Sachs, J., Yang, Y.G., & Sykes, M. (1999) Lymphohematopoietic graft-vs.-host reactions can be induced without graft-vs.-host disease in murine mixed chimeras established with a cyclophosphamide-based nonmyeloablative conditioning regimen. Biol.Blood Marrow Transplant., 5, 133-143.

Peniket, A.J., Ruiz de Elvira, M.C., Taghipour, G., Cordonnier, C., Gluckman, E., de Witte, T., Santini, G., Blaise, D., Greinix, H., Ferrant, A., Cornelissen, J., Schmitz, N., & Goldstone, A.H. (2003) An EBMT registry matched study of allogeneic stem cell transplants for lymphoma: allogeneic transplantation is associated with a lower relapse rate but a higher procedure-related mortality rate than autologous transplantation. Bone Marrow Transplant., 31, 667-678.

Perez-Simon, J.A., Caballero, D., Diez-Campelo, M., Lopez-Perez, R., Mateos, G., Canizo, C., Vazquez, L., Vidriales, B., Mateos, M.V., Gonzalez, M., & San Miguel, J.F. (2002a) Chimerism and minimal residual disease monitoring after reduced intensity conditioning (RIC) allogeneic transplantation. Leukemia, 16, 1423-1431.

Perez-Simon, J.A., Diez-Campelo, M., Martino, R., Brunet, S., Urbano, A., Caballero, M.D., de Leon, A., Valcarcel, D., Carreras, E., del Canizo, M.C., Lopez-Fidalgo, J., Sierra, J., & San Miguel, J.F. (2005) Influence of the intensity of the conditioning regimen on the characteristics of acute and chronic graft-versus-host disease after allogeneic transplantation. Br.J Haematol., 130, 394-403.

Perez-Simon, J.A., Kottaridis, P.D., Martino, R., Craddock, C., Caballero, D., Chopra, R., Garcia-Conde, J., Milligan, D.W., Schey, S., Urbano-Ispizua, A., Parker, A., Leon, A., Yong, K., Sureda, A., Hunter, A., Sierra, J., Goldstone, A.H., Linch, D.C., San Miguel, J.F., & Mackinnon, S. (2002b) Nonmyeloablative transplantation with or without alemtuzumab: comparison between 2 prospective studies in patients with lymphoproliferative disorders. Blood, 100, 3121-3127.

Perez-Simon, J.A., Martino, R., Alegre, A., Tomas, J.F., de Leon, A., Caballero, D., Sureda, A., Sierra, J., & San Miguel, J.F. (2003) Chronic but not acute graft-versus-host disease improves outcome in multiple myeloma patients after non-myeloablative allogeneic transplantation. Br.J Haematol., 121, 104-108.

Petersen, S.L., Madsen, H.O., Ryder, L.P., Svejgaard, A., Dickmeiss, E., & Vindelov, L.L. (2006) Cytokine gene expression in peripheral blood mononuclear cells and alloreactivity in hematopoietic cell transplantation with nonmyeloablative conditioning. Biol.Blood Marrow Transplant., 12, 48-60.

Petersen, S.L., Madsen, H.O., Ryder, L.P., Svejgaard, A., Jakobsen, B.K., Sengelov, H., Heilmann, C., Dickmeiss, E., & Vindelov, L.L. (2004a) Haematopoietic stem cell transplantation with non-myeloablative conditioning in the outpatient setting: results, complications and admission requirements in a single institution. Br.J.Haematol., 125, 225-231.

Petersen, S.L., Madsen, H.O., Ryder, L.P., Svejgaard, A., Masmas, T.N., Dickmeiss, E., Heilmann, C., & Vindelov, L.L. (2004b) Chimerism studies in HLA-identical nonmyeloablative hematopoietic stem cell transplantation point to the donor CD8 T-cell count on day +14 as a predictor of acute graft-versus-host disease. Biol.Blood Marrow Transplant., 10, 337-346.

Petersen, S.L., Russell, C.A., Bendtzen, K., & Vindelov, L.L. (2002) Optimisation of the CT h4S bioassay for detection of human interleukin-4 secreted by mononuclear cells stimulated by phytohaemaglutinin or by human leukocyte antigen mismatched mixed lymphocyte culture. Immunol.Lett., 84, 29-39.

Petersen, S.L., Ryder, L.P., Bjork, P., Madsen, H.O., Heilmann, C., Jacobsen, N., Sengelov, H., & Vindelov, L.L. (2003) A comparison of T-, B- and NK-cell reconstitution following conventional or nonmyeloablative conditioning and transplantation with bone marrow or peripheral blood stem cells from human leucocyte antigen identical sibling donors. Bone Marrow Transplant., 32, 65-72.

Petersen, S.L., Sidorov, I.A., Russell, C.A., Dickmeiss, E., & Vindelov, L.L. (2005) Limiting Dilution Analysis of Interleukin-2 Producing Helper T-cell Frequencies as a Tool in Allogeneic Hematopoietic Cell Transplantation. Transplantation, 80, 573-581.

Piccirillo, C.A. & Shevach, E.M. (2001) Cutting edge: control of $CD8^+$ T cell activation by $CD4^+CD25^+$ immunoregulatory cells. J Immunol., 167, 1137-1140.

Portugal, K., Dozmorov, I., Sidorov, I., Marrero, I., Fonseca, J.A., Spadafora-Ferreira, M., Kalil, J., & Coelho, V. (2001) Renal transplant patients show variations in their self-reactive repertoires: a serial study. Int.Immunol., 13, 747-755.

Powles, R., Mehta, J., Kulkarni, S., Treleaven, J., Millar, B., Marsden, J., Shepherd, V., Rowland, A., Sirohi, B., Tait, D., Horton, C., Long, S., & Singhal, S. (2000) Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: a randomised trial. Lancet, 355, 1231-1237.

Reddy, P. & Ferrara, J.L. (2003) Immunobiology of acute graft-versus-host disease. Blood Rev., 17, 187-194.

Remberger, M., Jaksch, M., Uzunel, M., & Mattsson, J. (2003) Serum levels of cytokines correlate to donor chimerism and acute graft-vs.-host disease after haematopoietic stem cell transplantation. Eur.J Haematol., 70, 384-391.

Remberger, M. & Ringden, O. (1997) Serum levels of cytokines after bone marrow transplantation: increased IL-8 levels during severe veno-occlusive disease of the liver. Eur.J Haematol., 59, 254-262.

Ringden, O., Labopin, M., Bacigalupo, A., Arcese, W., Schaefer, U.W., Willemze, R., Koc, H., Bunjes, D., Gluckman, E., Rocha, V., Schattenberg, A., & Frassoni, F. (2002) Transplantation of peripheral blood stem cells as compared with bone marrow from HLA-identical siblings in adult patients with acute myeloid leukemia and acute lymphoblastic leukemia. J Clin. Oncol., 20, 4655-4664.

Ritgen, M., Stilgenbauer, S., von Neuhoff, N., Humpe, A., Bruggemann, M., Pott, C., Raff, T., Krober, A., Bunjes, D., Schlenk, R., Schmitz, N., Dohner, H., Kneba, M., & Dreger, P. (2004) Graft-versus-leukemia activity may overcome therapeutic resistance of chronic lymphocytic leukemia with unmutated immunoglobulin variable heavy-chain gene status: implications of minimal residual disease measurement with quantitative PCR. Blood, 104, 2600-2602.

Robinson, S.P., Goldstone, A.H., Mackinnon, S., Carella, A., Russell, N., de Elvira, C.R., Taghipour, G., & Schmitz, N. (2002) Chemoresistant or aggressive lymphoma predicts for a poor outcome following reduced-intensity allogeneic progenitor cell transplantation: an analysis from the Lymphoma Working Party of the European Group for Blood and Bone Marrow Transplantation. Blood, 100, 4310-4316.

Robinson, S.P., Schmitz, N., Taghipour, G., & Sureda, A. (2004) Reduced intensity allogeneic stem cell transplantation for mantle cell lymphoma is associated with substantial late transplant related mortality and a poor outcome in patients with chemoresistant disease. Blood, 104, 622A.

Rosenkrantz, K., Keever, C., Bhimani, K., Horvath, A., Brochstein, J., O'Reilly, R., Dupont, B., & Flomenberg, N. (1990) Both ongoing suppression and clonal elimination contribute to graft-host tolerance after transplantation of HLA mismatched T cell-depleted marrow for severe combined immunodeficiency. J.Immunol., 144, 1721-1728.

Ruggeri, L., Capanni, M., Casucci, M., Volpi, I., Tosti, A., Perruccio, K., Urbani, E., Negrin, R.S., Martelli, M.F., & Velardi, A. (1999) Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood, 94, 333-339.

Ruggeri, L., Capanni, M., Urbani, E., Perruccio, K., Shlomchik, W.D., Tosti, A., Posati, S., Rogaia, D., Frassoni, F., Aversa, F., Martelli, M.F., & Velardi, A. (2002) Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science, 295, 2097-2100.

Russell, C.A. (2002) Alloreactivity and the predictive value of anti-recipient specific interleukin 2 producing helper T lymphocyte precursor frequencies for alloreactivity after bone marrow transplantation. Dan.Med. Bull., 49, 89-108.

Russell, C.A., Heilmann, C., Madsen, H.O., & Vindelov, L.L. (2001) The pre-

dictive value of helper T lymphocyte precursor frequencies for graft-versus-host disease and graft-versus-leukaemia effects in allogeneic bone marrow transplantation. Bone Marrow Transplant., 27, 949-958.

Ruutu, T., Hermans, J., Niederwieser, D., Gratwohl, A., Kiehl, M., Volin, L., Bertz, H., Ljungman, P., Spence, D., Verdonck, L.F., Prentice, H.G., Bosi, A., du Toit, C.E., Brinch, L., & Apperley, J.F. (2002) Thrombotic thrombocytopenic purpura after allogeneic stem cell transplantation: a survey of the European Group for Blood and Marrow Transplantation (EBMT). British Journal of Haematology, 118, 1112-1119.

Sadler, J.E., Moake, J.L., Miyata, T., & George, J.N. (2004) Recent advances in thrombotic thrombocytopenic purpura. Hematology (Am.Soc.Hematol.Educ.Program.), 407-423.

Saito, T., Kanda, Y., Nakai, K., Kim, S.W., Arima, F., Kami, M., Tanosaki, R., Tobinai, K., Wakasugi, H., Heike, Y., Mineishi, S., & Takaue, Y. (2003) Immune reconstitution following reduced-intensity transplantation with cladribine, busulfan, and antithymocyte globulin: serial comparison with conventional myeloablative transplantation. Bone Marrow Transplant., 32, 601-608.

Sakaguchi, S. (2004) Naturally arising $CD4^+$ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu.Rev. Immunol., 22, 531-562.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., & Toda, M. (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol., 155, 1151-1164.

Sanchez, J., Casano, J., Alvarez, M.A., Roman-Gomez, J., Martin, C., Martinez, F., Gomez, P., Serrano, J., Herrera, C., & Torres, A. (2004) Kinetic of regulatory CD25high and activated $CD134^+$ (OX40) T lymphocytes during acute and chronic graft-versus-host disease after allogeneic bone marrow transplantation. Br.J Haematol., 126, 697-703.

Sandmaier, B.M., Maris, M., Maloney, D.G., Gooley, T.A., Stuart, M.J., Hegenbart, U., Sahebi, F., Chauncey, T., Agura, E., Bruno, B., McSweeney, P.A., Maziarz, R., Pulsipher, M., Epner, E., Little, M.T., Forman, S.J., Niederwieser, D.W., Blume, K.G., & Storb, R. (2003) Low-Dose total body irradiation conditioning for hematopoietic cell transplants from HLA-matched related and unrelated donors for patients with hematologic malignancies: A five-year experience. Abstract Blood, 102, 78a-79a.

Santos, G.W., Tutschka, P.J., Brookmeyer, R., Saral, R., Beschorner, W.E., Bias, W.B., Braine, H.G., Burns, W.H., Elfenbein, G.J., Kaizer, H. (1983) Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. N.Engl.J Med., 309, 1347-1353.

Sayer, H.G., Kroger, M., Beyer, J., Kiehl, M., Klein, S.A., Schaefer-Eckart, K., Schwerdtfeger, R., Siegert, W., Runde, V., Theuser, C., Martin, H., Schetelig, J., Beelen, D.W., Fauser, A., Kienast, J., Hoffken, K., Ehninger, G., & Bornhauser, M. (2003) Reduced intensity conditioning for allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia: disease status by marrow blasts is the strongest prognostic factor. Bone Marrow Transplant., 31, 1089-1095.

Schetelig, J., Kroger, N., Held, T.K., Thiede, C., Krusch, A., Zabelina, T., Dubiel, M., Rick, O., Bornhauser, M., Ehninger, G., Zander, A.R., & Siegert, W. (2002) Allogeneic transplantation after reduced conditioning in high risk patients is complicated by a high incidence of acute and chronic graft-versus-host disease. Haematologica, 87, 299-305.

Schetelig, J., Oswald, O., Steuer, N., Radonic, A., Thulke, S., Held, T.K., Oertel, J., Nitsche, A., & Siegert, W. (2003a) Cytomegalovirus infections in allogeneic stem cell recipients after reduced-intensity or myeloablative conditioning assessed by quantitative PCR and pp65-antigenemia. Bone Marrow Transplant., 32, 695-701.

Schetelig, J., Thiede, C., Bornhauser, M., Schwerdtfeger, R., Kiehl, M., Beyer, J., Sayer, H.G., Kroger, N., Hensel, M., Scheffold, C., Held, T.K., Hoffken, K., Ho, A.D., Kienast, J., Neubauer, A., Zander, A.R., Fauser, A.A., Ehninger, G., & Siegert, W. (2003b) Evidence of a graft-versus-leukemia effect in chronic lymphocytic leukemia after reduced-intensity conditioning and allogeneic stem-cell transplantation: the Cooperative German Transplant Study Group. J Clin.Oncol., 21, 2747-2753.

Schmid, C., Schleuning, M., Ledderose, G., Tischer, J., & Kolb, H.J. (2005) Sequential regimen of chemotherapy, reduced-intensity conditioning for allogeneic stem-cell transplantation, and prophylactic donor lymphocyte transfusion in high-risk acute myeloid leukemia and myelodysplastic syndrome. J Clin.Oncol., 23, 5675-5687.

Schmitz, N., Beksac, M., Hasenclever, D., Bacigalupo, A., Ruutu, T., Nagler, A., Gluckman, E., Russell, N., Apperley, J.F., Gorin, N.C., Szer, J., Bradstock, K., Buzyn, A., Clark, P., Borkett, K., & Gratwohl, A. (2002) Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. Blood, 100, 761-767.

Schwarer, A.P., Jiang, Y.Z., Brookes, P.A., Barrett, A.J., Batchelor, J.R., Goldman, J.M., & Lechler, R.I. (1993) Frequency of anti-recipient alloreactive helper T-cell precursors in donor blood and graft-versus-host disease after HLA-identical sibling bone-marrow transplantation. Lancet, 341, 203-205.

Scott, B.L., Sandmaier, B.M., Storer, B., Maris, M.B., Sorror, M.L., Maloney, D.G., Chauncey, T.R., Storb, R., & Deeg, H.J. (2006) Myeloablative vs nonmyeloablative allogeneic transplantation for patients with myelodysplastic syndrome or acute myelogenous leukemia with multilineage dysplasia: a retrospective analysis. Leukemia, 20, 128-135.

Sharabi, Y. & Sachs, D.H. (1989) Mixed chimerism and permanent specific transplantation tolerance induced by a nonlethal preparative regimen. J.Exp.Med., 169, 493-502.

Shilling, H.G., Young, N., Guethlein, L.A., Cheng, N.W., Gardiner, C.M., Tyan, D., & Parham, P. (2002) Genetic control of human NK cell repertoire. J Immunol., 169, 239-247.

Shimazaki, C., Fujii, H., Yoshida, T., Chou, T., Nishimura, M., Asaoku, H., Miyawaki, S., Ishii, A., Ishida, T., Taniwaki, M., Iida, S., Takagi, T., & Takatsuki, K. (2005) Reduced-intensity conditioning allogeneic stem cell transplantation for multiple myeloma: results from the Japan Myeloma Study Group. Int.J Hematol., 81, 342-348.

Shimoni, A., Yeshurun, M., Hardan, I., Avigdor, A., Ben Bassat, I., & Nagler, A. (2004) Thrombotic microangiopathy after allogeneic stem cell transplantation in the era of reduced-intensity conditioning: The incidence is not reduced. Biol.Blood Marrow Transplant., 10, 484-493.

Shlomchik, W.D. (2003) Antigen presentation in graft-vs-host disease. Exp.Hematol., 31, 1187-1197.

Shlomchik, W.D., Couzens, M.S., Tang, C.B., McNiff, J., Robert, M.E., Liu, J., Shlomchik, M.J., & Emerson, S.G. (1999) Prevention of graft versus host disease by inactivation of host antigen-presenting cells. Science, 285, 412-415.

Shulman, H.M., Sullivan, K.M., Weiden, P.L., McDonald, G.B., Striker, G.E., Sale, G.E., Hackman, R., Tsoi, M.S., Storb, R., & Thomas, E.D. (1980) Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am.J Med., 69, 204-217.

Simpson, E., Scott, D., James, E., Lombardi, G., Cwynarski, K., Dazzi, F., Millrain, M., & Dyson, P.J. (2002) Minor H antigens: genes and peptides. Transpl.Immunol., 10, 115-123.

Slavin, S. (2004) Reduced intensity versus truly nonmyeloablative conditioning for stem-cell transplant recipients. Transplantation, 78, 964-965.

Slavin, S., Nagler, A., Naparstek, E., Kapelushnik, Y., Aker, M., Cividalli, G., Varadi, G., Kirschbaum, M., Ackerstein, A., Samuel, S., Amar, A., Brautbar, C., Ben Tal, O., Eldor, A., & Or, R. (1998) Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood, 91, 756-763.

Slavin, S., Naparstek, E., Nagler, A., Ackerstein, A., Kapelushnik, J., & Or, R. (1995) Allogeneic cell therapy for relapsed leukemia after bone marrow transplantation with donor peripheral blood lymphocytes. Exp.Hematol., 23, 1553-1562.

Socie, G., Loiseau, P., Tamouza, R., Janin, A., Busson, M., Gluckman, E., & Charron, D. (2001) Both genetic and clinical factors predict the development of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Transplantation, 72, 699-706.

Socie, G., Stone, J.V., Wingard, J.R., Weisdorf, D., Henslee-Downey, P.J., Bredeson, C., Cahn, J.Y., Passweg, J.R., Rowlings, P.A., Schouten, H.C., Kolb, H.J., & Klein, J.P. (1999) Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry. N.Engl.J Med., 341, 14-21.

Sorror, M.L., Maris, M.B., Sandmaier, B.M., Storer, B.E., Stuart, M.J., Hegenbart, U., Agura, E., Chauncey, T.R., Leis, J., Pulsipher, M., McSweeney, P., Radich, J.P., Bredeson, C., Bruno, B., Langston, A., Loken, M.R., Al Ali, H., Blume, K.G., Storb, R., & Maloney, D.G. (2005) Hematopoietic cell transplantation after nonmyeloablative conditioning for advanced chronic lymphocytic leukemia. J Clin.Oncol., 23, 3819-3829.

Sorror, M.L., Maris, M.B., Storer, B., Sandmaier, B.M., Diaconescu, R., Flowers, C., Maloney, D.G., & Storb, R. (2004) Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplantation comorbidities. Blood, 104, 961-968.

Spierings, E., Wieles, B., & Goulmy, E. (2004) Minor histocompatibility antigens – big in tumour therapy. Trends Immunol., 25, 56-60.

Spitzer, T.R. (2001) Engraftment syndrome following hematopoietic stem cell transplantation. Bone Marrow Transplantation, 27, 893-898.

Spitzer, T.R., Delmonico, F., Tolkoff-Rubin, N., McAfee, S., Sackstein, R., Saidman, S., Colby, C., Sykes, M., Sachs, D.H., & Cosimi, A.B. (1999) Combined histocompatibility leukocyte antigen-matched donor bone marrow and renal transplantation for multiple myeloma with end stage renal disease: the induction of allograft tolerance through mixed lymphohematopoietic chimerism. Transplantation, 68, 480-484.

Spitzer, T.R., McAfee, S., Sackstein, R., Colby, C., Multani, P., Saidman, S., Weymouth, D., Preffer, F., Poliquin, C., Foley, A., Cox, B., Andrews, D., Sachs, D.H., & Sykes, M. (2000) Intentional induction of mixed chimerism and achievement of antitumor responses after nonmyeloablative conditioning therapy and HLA-matched donor bone marrow transplantation for refractory hematologic malignancies. Biology of Blood and Marrow Transplantation, 6, 309-320.

Stem Cell Trialists' Collaborative Group (2005) Allogeneic peripheral blood

stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. J Clin.Oncol., 23, 5074-5087.
Storb, R. (2003) Allogeneic hematopoietic stem cell transplantation – yesterday, today, and tomorrow. Exp.Hematol., 31, 1-10.
Storb, R., Doney, K.C., Thomas, E.D., Appelbaum, F., Buckner, C.D., Clift, R.A., Deeg, H.J., Goodell, B.W., Hackman, R., Hansen, J.A., Sanders, J., Sullivan, K., Weiden, P.L., & Witherspoon, R.P. (1982) Marrow transplantation with or without donor buffy coat cells for 65 transfused aplastic anemia patients. Blood, 59, 236-246.
Storb, R., Raff, R.F., Appelbaum, F.R., Deeg, H.J., Graham, T.C., Schuening, F.G., Shulman, H., Yu, C., Bryant, E., Burnett, R., & . (1994) DLA-identical bone marrow grafts after low-dose total body irradiation: the effect of canine recombinant hematopoietic growth factors. Blood, 84, 3558-3566.
Storb, R., Raff, R.F., Appelbaum, F.R., Schuening, F.W., Sandmaier, B.M., Graham, T.C., & Thomas, E.D. (1988) What radiation dose for DLA-identical canine marrow grafts? Blood, 72, 1300-1304.
Storb, R., Raff, R.F., Graham, T., Appelbaum, F.R., Deeg, H.J., Schuening, F.G., Shulman, H., & Pepe, M. (1993) Marrow toxicity of fractionated vs. single dose total body irradiation is identical in a canine model. Int.J Radiat.Oncol.Biol.Phys., 26, 275-283.
Storb, R., Yu, C., Barnett, T., Wagner, J.L., Deeg, H.J., Nash, R.A., Kiem, H.P., McSweeney, P., Seidel, K., Georges, G., & Zaucha, J.M. (1999) Stable mixed hematopoietic chimerism in dog leukocyte antigen-identical littermate dogs given lymph node irradiation before and pharmacologic immunosuppression after marrow transplantation. Blood, 94, 1131-1136.
Storb, R., Yu, C., Wagner, J.L., Deeg, H.J., Nash, R.A., Kiem, H.P., Leisenring, W., & Shulman, H. (1997) Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. Blood, 89, 3048-3054.
Storb, R.F., Champlin, R., Riddell, S.R., Murata, M., Bryant, S., & Warren, E.H. (2001) Non-myeloablative transplants for malignant disease. Hematology (Am.Soc.Hematol.Educ.Program.), 375-391.
Storek, J., Dawson, M.A., Storer, B., Stevens-Ayers, T., Maloney, D.G., Marr, K.A., Witherspoon, R.P., Bensinger, W., Flowers, M.E., Martin, P., Storb, R., Appelbaum, F.R., & Boeckh, M. (2001a) Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. Blood, 97, 3380-3389.
Storek, J., Espino, G., Dawson, M.A., Storer, B., Flowers, M.E., & Maloney, D.G. (2000) Low B-cell and monocyte counts on day 80 are associated with high infection rates between days 100 and 365 after allogeneic marrow transplantation. Blood, 96, 3290-3293.
Storek, J., Gooley, T., Witherspoon, R.P., Sullivan, K.M., & Storb, R. (1997) Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. Am.J Hematol., 54, 131-138.
Storek, J., Joseph, A., Dawson, M.A., Douek, D.C., Storer, B., & Maloney, D.G. (2002) Factors influencing T-lymphopoiesis after allogeneic hematopoietic cell transplantation. Transplantation, 73, 1154-1158.
Storek, J., Joseph, A., Espino, G., Dawson, M.A., Douek, D.C., Sullivan, K.M., Flowers, M.E., Martin, P., Mathioudakis, G., Nash, R.A., Storb, R., Appelbaum, F.R., & Maloney, D.G. (2001b) Immunity of patients surviving 20 to 30 years after allogeneic or syngeneic bone marrow transplantation. Blood, 98, 3505-3512.
Storek, J., Wells, D., Dawson, M.A., Storer, B., & Maloney, D.G. (2001c) Factors influencing B lymphopoiesis after allogeneic hematopoietic cell transplantation. Blood, 98, 489-491.
Sullivan, K.M. (1999) Graft-vs-Host Disease. Hematopoietic Cell Transplantation (ed. by E. D. Thomas, K. G. Blume, & S. J. Forman), pp. 518-525. Blackwell Science, Malden, MA.
Sullivan, K.M., Weiden, P.L., Storb, R., Witherspoon, R.P., Fefer, A., Fisher, L., Buckner, C.D., Anasetti, C., Appelbaum, F.R., Badger, C., & . (1989) Influence of acute and chronic graft-versus-host disease on relapse and survival after bone marrow transplantation from HLA-identical siblings as treatment of acute and chronic leukemia. Blood, 73, 1720-1728.
Suri-Payer, E., Amar, A.Z., Thornton, A.M., & Shevach, E.M. (1998) $CD4^+$ $CD25^+$ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. J Immunol., 160, 1212-1218.
Sykes, M., Preffer, F., McAfee, S., Saidman, S.L., Weymouth, D., Andrews, D.M., Colby, C., Sackstein, R., Sachs, D.H., & Spitzer, T.R. (1999) Mixed lymphohaemopoietic chimerism and graft-versus-lymphoma effects after non-myeloablative therapy and HLA-mismatched bone-marrow transplantation. Lancet, 353, 1755-1759.
Sykes, M., Szot, G.L., Swenson, K.A., & Pearson, D.A. (1997) Induction of high levels of allogeneic hematopoietic reconstitution and donor-specific tolerance without myelosuppressive conditioning. Nat.Med., 3, 783-787.
Takabayashi, M., Kanamori, H., Takasaki, H., Yamaji, S., Koharazawa, H., Taguchi, J., Tomita, N., Fujimaki, K., Fujisawa, S., Maruta, A., & Ishigatsubo, Y. (2005) A possible association between the presence of interleukin-4-secreting cells and a reduction in the risk of acute graft-versus-host disease. Exp.Hematol., 33, 251-257.
Takatsuka, H., Takemoto, Y., Okamoto, T., Fujimori, Y., Tamura, S., Wada, H., Okada, M., Yamada, S., Kanamaru, A., & Kakishita, E. (1999) Predicting the severity of graft-versus-host disease from interleukin-10 levels after bone marrow transplantation. Bone Marrow Transplant., 24, 1005-1007.
Tanaka, J., Imamura, M., Kasai, M., Hashino, S., Kobayashi, S., Noto, S., Higa, T., Sakurada, K., & Asaka, M. (1997) The important balance between cytokines derived from type 1 and type 2 helper T cells in the control of graft-versus-host disease. Bone Marrow Transplant., 19, 571-576.
Tanimoto, T.E., Yamaguchi, T., Tanaka, Y., Saito, A., Tajima, K., Karasuno, T., Kasai, M., Kishi, K., Mori, T., Maseki, N., Morishima, S., Miyakoshi, S., Kasai, M., Ohno, Y., Kim, S.W., Numata, A., Kami, M., Takaue, Y., Mori, S., & Harada, M. (2004) Comparative analysis of clinical outcomes after allogeneic bone marrow transplantation versus peripheral blood stem cell transplantation from a related donor in Japanese patients. Br.J Haematol., 125, 480-493.
Taussig, D.C., Davies, A.J., Cavenagh, J.D., Oakervee, H., Syndercombe-Court, Kelsey, S., Amess, J.A., Rohatiner, A.Z., Lister, T.A., & Barnett, M.J. (2003) Durable remissions of myelodysplastic syndrome and acute myeloid leukemia after reduced-intensity allografting. J Clin.Oncol., 21, 3060-3065.
Taylor, P.A., Lees, C.J., & Blazar, B.R. (2002) The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. Blood, 99, 3493-3499.
Teshima, T., Ordemann, R., Reddy, P., Gagin, S., Liu, C., Cooke, K.R., & Ferrara, J.L. (2002) Acute graft-versus-host disease does not require alloantigen expression on host epithelium. Nat.Med., 8, 575-581.
Theobald, M., Nierle, T., Bunjes, D., Arnold, R., & Heimpel, H. (1992) Host-specific interleukin-2-secreting donor T-cell precursors as predictors of acute graft-versus-host disease in bone marrow transplantation between HLA-identical siblings. N.Engl.J.Med., 327, 1613-1617.
Thomas, E., Storb, R., Clift, R.A., Fefer, A., Johnson, F.L., Neiman, P.E., Lerner, K.G., Glucksberg, H., & Buckner, C.D. (1975) Bone-marrow transplantation. N.Engl.J Med., 292, 832-843-895-902.
Thornton, A.M. & Shevach, E.M. (1998) $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J Exp.Med., 188, 287-296.
Tivol, E., Komorowski, R., & Drobyski, W.R. (2005) Emergent autoimmunity in graft-versus-host disease. Blood, 105, 4885-4891.
Trenado, A., Charlotte, F., Fisson, S., Yagello, M., Klatzmann, D., Salomon, B.L., & Cohen, J.L. (2003) Recipient-type specific $CD4^+CD25^+$ regulatory T cells favor immune reconstitution and control graft-versus-host disease while maintaining graft-versus-leukemia. J.Clin.Invest, 112, 1688-1696.
Tseng, L.H., Lin, M.T., Hansen, J.A., Gooley, T., Pei, J., Smith, A.G., Martin, E.G., Petersdorf, E.W., & Martin, P.J. (1999) Correlation between disparity for the minor histocompatibility antigen HA-1 and the development of acute graft-versus-host disease after allogeneic marrow transplantation. Blood, 94, 2911-2914.
Tykodi, S.S., Warren, E.H., Thompson, J.A., Riddell, S.R., Childs, R.W., Otterud, B.E., Leppert, M.F., Storb, R., & Sandmaier, B.M. (2004) Allogeneic hematopoietic cell transplantation for metastatic renal cell carcinoma after nonmyeloablative conditioning: toxicity, clinical response, and immunological response to minor histocompatibility antigens. Clin.Cancer Res., 10, 7799-7811.
van Besien, K., Artz, A., Smith, S., Cao, D., Rich, S., Godley, L., Jones, D., Del Cerro, P., Bennett, D., Casey, B., Odenike, O., Thirman, M., Daugherty, C., Wickrema, A., Zimmerman, T., Larson, R.A., & Stock, W. (2005) Fludarabine, melphalan, and alemtuzumab conditioning in adults with standard-risk advanced acute myeloid leukemia and myelodysplastic syndrome. J Clin.Oncol., 23, 5728-5738.
van den Brink, M.R., Alpdogan, O., & Boyd, R.L. (2004) Strategies to enhance T-cell reconstitution in immunocompromised patients. Nat.Rev. Immunol., 4, 856-867.
van den Brink, M.R. & Burakoff, S.J. (2002) Cytolytic pathways in haematopoietic stem-cell transplantation. Nat.Rev.Immunol., 2, 273-281.
van der Plas, R.M., Schiphorst, M.E., Huizinga, E.G., Hene, R.J., Verdonck, L.F., Sixma, J.J., & Fijnheer, R. (1999) von Willebrand factor proteolysis is deficient in classic, but not in bone marrow transplantation-associated, thrombotic thrombocytopenic purpura. Blood, 93, 3798-3802.
Vesely, S.K., George, J.N., Lammle, B., Studt, J.D., Alberio, L., El Harake, M.A., & Raskob, G.E. (2003) ADAMTS13 activity in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: relation to presenting features and clinical outcomes in a prospective cohort of 142 patients. Blood, 102, 60-68.
Visentainer, J.E., Lieber, S.R., Persoli, L.B., Vigorito, A.C., Aranha, F.J., Brito Eid, K.A., Oliveira, G.B., Miranda, E.C., & de Souza, C.A. (2003) Serum cytokine levels and acute graft-versus-host disease after HLA-identical hematopoietic stem cell transplantation. Exp.Hematol., 31, 1044-1050.
Weiden, P.L., Flournoy, N., Thomas, E.D., Prentice, R., Fefer, A., Buckner, C.D., & Storb, R. (1979) Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N.Engl.J Med., 300, 1068-1073.

Weiden, P.L., Sullivan, K.M., Flournoy, N., Storb, R., & Thomas, E.D. (1981) Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N.Engl.J Med., 304, 1529-1533.

Weinberg, K., Blazar, B.R., Wagner, J.E., Agura, E., Hill, B.J., Smogorzewska, M., Koup, R.A., Betts, M.R., Collins, R.H., & Douek, D.C. (2001) Factors affecting thymic function after allogeneic hematopoietic stem cell transplantation. Blood, 97, 1458-1466.

Weissinger, F., Sandmaier, B.M., Maloney, D.G., Bensinger, W.I., Gooley, T., & Storb, R. (2001) Decreased transfusion requirements for patients receiving nonmyeloablative compared with conventional peripheral blood stem cell transplants from HLA-identical siblings. Blood, 98, 3584-3588.

Wong, R., Giralt, S.A., Martin, T., Couriel, D.R., Anagnostopoulos, A., Hosing, C., Andersson, B.S., Cano, P., Shahjahan, M., Ippoliti, C., Estey, E.H., McMannis, J., Gajewski, J.L., Champlin, R.E., & de Lima, M. (2003) Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years. Blood, 102, 3052-3059.

Xystrakis, E., Dejean, A.S., Bernard, I., Druet, P., Liblau, R., Gonzalez-Dunia, D., & Saoudi, A. (2004) Identification of a novel natural regulatory CD8 T-cell subset and analysis of its mechanism of regulation. Blood, 104, 3294-3301.

Yoshihara, S., Tateishi, U., Ando, T., Kunitoh, H., Suyama, H., Onishi, Y., Tanosaki, R., & Mineishi, S. (2005) Lower incidence of Bronchiolitis obliterans in allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning compared with myeloablative conditioning. Bone Marrow Transplant., 35, 1195-1200.

Yu, C., Seidel, K., Nash, R.A., Deeg, H.J., Sandmaier, B.M., Barsoukov, A., Santos, E., & Storb, R. (1998) Synergism between mycophenolate mofetil and cyclosporine in preventing graft-versus-host disease among lethally irradiated dogs given DLA-nonidentical unrelated marrow grafts. Blood, 91, 2581-2587.

Yu, C., Storb, R., Mathey, B., Deeg, H.J., Schuening, F.G., Graham, T.C., Seidel, K., Burnett, R., Wagner, J.L., Shulman, H., & Sandmaier, B.M. (1995) DLA-identical bone marrow grafts after low-dose total body irradiation: effects of high-dose corticosteroids and cyclosporine on engraftment. Blood, 86, 4376-4381.

Zaucha, J.M., Gooley, T., Bensinger, W.I., Heimfeld, S., Chauncey, T.R., Zaucha, R., Martin, P.J., Flowers, M.E., Storek, J., Georges, G., Storb, R., & Torok-Storb, B. (2001) CD34 cell dose in granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell grafts affects engraftment kinetics and development of extensive chronic graft-versus-host disease after human leukocyte antigen-identical sibling transplantation. Blood, 98, 3221-3227.

Zeng, D., Lewis, D., Dejbakhsh-Jones, S., Lan, F., Garcia-Ojeda, M., Sibley, R., & Strober, S. (1999) Bone marrow NK1.1(–) and NK1.1(+) T cells reciprocally regulate acute graft versus host disease. J Exp.Med., 189, 1073-1081.

Zimmerli, W., Zarth, A., Gratwohl, A., & Speck, B. (1991) Neutrophil function and pyogenic infections in bone marrow transplant recipients. Blood, 77, 393-399.

Zinkernagel, R.M. & Doherty, P.C. (1997) The discovery of MHC restriction. Immunol.Today, 18, 14-17.

Zorn, E., Kim, H.T., Lee, S.J., Floyd, B.H., Litsa, D., Arumugarajah, S., Bellucci, R., Alyea, E.P., Antin, J.H., Soiffer, R.J., & Ritz, J. (2005) Reduced frequency of FOXP3+ $CD4^{+}CD25^{+}$ regulatory T cells in patients with chronic graft-versus-host disease. Blood, 106, 2903-2911.