[Frontiers in Bioscience 12, 4568-4594, May 1, 2007]

Can graft-versus-leukemia reactivity be dissociated from graft-versus-host disease?

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1. ABSTRACT

Dissociation of graft-versus-leukemia (GvL). effects from graft-versus-host disease (GvHD) is the ultimate goal of allogeneic hematopoietic stem cell transplantation (alloHSCT) in the treatment of hematological malignancies. The pivotal role of donor T cells in both anti-leukemic and anti-host reactivity of allogeneic stem cell grafts has been known since the first transplants for fatal leukemia were performed over 25 years ago. Growing understanding of the T cell-mediated GvL response has revealed the importance of host-type antigen-presenting cells and the capacity of adoptively transferred donor T cells in inducing anti-leukemic responses, and has led to a re-evaluation of the relative roles of the pre-transplant conditioning regimen and the allogeneic stem cell graft. Key advances in clinical practice such as reduced-intensity stem cell transplantation and donor lymphocyte infusions are now routinely applied and allow for the induction of potent antileukemic effects, while GvHD can to some extent be controlled. Other strategies to separate T cell-mediated antileukemic effects from GvHD are antigen-specific adoptive T cell-therapy and recipient lymphocyte infusion (RLI) and these are in an experimental stage. Importantly, a role for alloreactive natural killer cells in mediating GvL without GvHD has emerged in patient studies of MHC haplotype-mismatched alloHSCT. Finally, experimental studies indicate that naturally occurring regulatory T cells may differentially affect GvHD and GvL.

2. HISTORICAL PERSPECTIVE: ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION AND THE GRAFT-VERSUS-LEUKEMIA EFFECT

Allogeneic hematopoietic stem cell transplantation (alloHSCT) currently is a cornerstone in the treatment of hematological malignancies. Depending on the nature of the malignancy and the stage of the disease, alloHSCT can induce durable remission in acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), chronic myeloid leukemia (CML), myelodysplasia (MDS) and high-risk lymphoma. Since the first bone marrow transplantation (BMT) was performed for fatal leukemia over 25 years ago, the practice of alloHSCT has evolved considerably, and the paradigm has shifted from alloHSCT as a means to rescue the patients' immunohematopoietic system following high-dose chemo- and/or radiotherapy, to a potent form of antileukemic immunotherapy.

In 1956 Barnes *et al.* (1), exploring X-ray irradiation plus BMT as a therapeutic regimen for transplantable leukemia in mice, first demonstrated superiority of alloBMT to syngeneic BMT in achieving successful eradication of leukemia. In 1965, Mathé *et al.* (2) described the first case of an irradiated leukemia patient exhibiting successful engraftment of allogeneic bone marrow (alloBM) with prolonged leukemia-free survival, suggesting that the allogeneic transplant exerted an anti-leukemic effect superimposed on that of radiotherapy. At that time it had become clear that immune reactivity of the donor graft against host tissues generates GvHD (3). Another decade later, Odom *et al.* (4) used the term 'graft-versus-leukemia reaction' when they described the case of an alloBMT patient showing remission of relapsed leukemia coincident with a graft-versus-host reaction, and in 1981, Weiden *et al.* (5) published a retrospective study revealing an association between post-transplant GvHD and reduced leukemia relapse rates, supporting Odom's hypothesis that the alloBM graft can confer an immune-mediated anti-tumor effect. Horowitz *et al.* (6) further substantiated the relationship between GvHD, in particular the extent of histo-incompatibility and the role of donor T cells, and anti-leukemic immune reactivity of the alloBM graft. These authors (6) observed that leukemia relapse rates were reduced when alloBMT gave rise to both acute and chronic GvHD, as opposed to when it led to chronic GvHD only. Moreover, relapse rates in recipients of T cell-depleted BM grafts were higher than those in non-T cell-depleted BMT patients, but lower than those in recipients of twin transplants.

Since these early studies, GvHD remains the principal complication of alloHSCT, leading to considerable morbidity and mortality. Effective measures to prevent GvHD, such as HLA matching, T cell depletion or post-transplant immunosuppression have the undesired consequence of mitigating leukemia-free survival and today it is well established that the curative potential of alloHSCT for leukemia critically depends on the GvL effect. This concept has led investigators to test the post-transplant infusion of donor lymphocytes (donor lymphocyte infusions, DLI), in mice and subsequently in humans in the mid 1990s (7), as a means to induce or potentiate antileukemic reactivity in the post-transplant period (8). Although success rates of DLI varied amongst different types of leukemia, its curative potential has supported its introduction into clinical practice (7).

The studies, showing that the benefit of alloHSCT derives not so much from its ability to reconstitute the hematopoietic system, but rather from its specific anti-leukemic potential, led to the idea to tone down the highly toxic conditioning component and to maximally exploit the alloreactivity of the stem cell graft, in *reduced intensity stem cell transplantation* (RIST) strategies (Box 1) (9-11). The first experience with so-called *non-myeloablative conditioning* (Box 1) in alloHSCT procedures was reported on by Slavin and colleagues in 1998 (12). Today, these regimens have proved to be clinically effective (13), and to allow for older and more advanced leukemic patients to undergo alloHSCT (14). Although these key advances have improved the outcome of alloHSCT procedures for leukemias, considerable risks for post-transplant morbidity, including disease relapse continue to exist. Furthermore, the immune mechanisms underlying the GvL effect, in particular those which would allow its dissociation from GvHD, are still incompletely understood. Moreover, as post-transplantation immunosuppressive therapy is often necessary to control GvHD, the GvL effect may rarely be expected to be complete. Since the early days of alloHSCT practice it has been clear that GvL is closely associated with GvHD. Nevertheless, there is now ample experimental and clinical evidence to indicate that, apart from donor T cells, donor Natural Killer (NK) cells can contribute significantly to GvL, and that GvL and GvHD can be separated. Here, we review the immunological mechanisms underlying the GvL reaction after alloHSCT and present the currently available experimental and clinical evidence indicating that GvL can be dissociated from GvHD.

3. IMMUNOBIOLOGY OF GRAFT-VERSUS-HOST DISEASE AND THE GRAFT-VERSUS-LEUKEMIA EFFECT

3.1. Graft-versus-host disease

GvHD is the cause of considerable post-transplant morbidity and mortality, and therefore is the most important obstacle to the overall success of alloHSCT (15,16). According to literature, the incidence of acute GvHD ranges from 16 to 64 %, depending on the type of patient and alloHSCT procedure (17-24). Chronic GvHD occurs in 40 to 60% of long-term survivors of alloHSCT, and this rate is expected to still increase in the future because of the increasing age of the patient population eligible for alloHSCT, the increasing use of peripheral blood as a stem cell source, the application of DLI, and the enlistment of mismatched and unrelated donors (15).

As early as 1966, Billingham stated that acute GvHD develops on the condition that the transplanted graft

Table 1. Antigens involved in the immunobiology of GvL and GvHD

T cells	TAA	Mutated antigen (e.g. bcr/abl[210]). Overexpressed antigen (e.g. WT 1[76], Pr-3[67]). Tissue-specific expressed antigen	
	miHC	Hematopoietic tissue-restricted antigen (e.g. HA-1[60]).	
		Non hematopoietic tissue-restricted antigen	
	MHC	MHC	
		→ Graft versus leukemia	**→ Engraftment, Graft versus host disease**
NK cells	MHC	Lack of self MHC KIR epitope mismatch (e.g. KIR2DL2 and KIR2DL3 & group 1 HLA-C alleles[240])	
		→ Graft versus leukemia	**→ Engraftment**

Box 1: Reduced Intensity Stem Cell Transplantation.Throughout this review, the terms reduced-intensity stem cell transplantation (rist). and nonmyeloablative conditioning are used to refer to any transplant protocol involving a conditioning regimen that was intented not to be myeloablative. In literature, the terms 'reduced intensity conditioning', 'nonmyeloablative conditioning', 'mini-transplant', 'minimal intensity stem cell transplantation' and 'moderate intensity stem cell transplantation' are used. Although they are used often interchangeably, in general these terms refer to the extent to which a particular regimen aims at inducing immunosuppression rather than myeloablation (as reviewed in 11)..

contains (or generates) immunocompetent cells, that the recipient is incapable of rejecting the graft, and that the host expresses tissue antigens that are foreign to the donor cells (3). Today, donor immunocompetent T cells are generally accepted to be the effector cells of GvHD, as is evident from the preventive effects of exhaustive T cell depletion and the well-know correlation of GvHD severity with transplanted donor T cell dose (25,26). GvHD is considered as a three-step process in which innate and adaptive immunity interact. Firstly, the conditioning regimen causes damage to recipient tissues such as the intestinal wall, resulting in translocation of bacterial lipopolysaccharide (LPS) to the blood circulation, and activation of host antigen-presenting-cells (APC). Secondly, donor T cells interact "directly" with host (allo-) antigen expressed on host APC, resulting in activation, massive cytokine production and clonal expansion. Third, the "cytokine storm" and further T cell effector function, including cytotoxicity through perforin- and fas-mediated pathways, result in increasing host tissue damage (27). The immunobiology of chronic GvHD is less well understood. One theory postulates that chronic GvHD is a form of end-stage alloreactivity in which an initial T helper (Th) 1 response evolves into a Th2-mediated process. Another theory holds that chronic GvHD results from a dysfunctional recovery of the immune system, as is thought to occur in what has been called 'autologous GvHD', the auto-immune syndrome that complicates cyclosporine administration after autologous HSCT (15).

Approaches to prevent or minimize the occurrence of GvHD include HLA matching, T cell depletion or T cell-suppressing pharmacotherapy. Although effective in the prevention of GvHD, T cell depletion is well known to be associated with increased risk of graft failure, infection, and malignant relapse (25). The standard pharmacologic GvHD prophylaxis mainly makes use of cyclosporine and methotrexate, either as monotherapy or in combination (28). Other immunosuppressants such as tacrolimus, CAMPATH, anti-thymocyte globulin and mycophenolate mofetil (MMF) have been used to prevent acute GvHD but evidence to their efficacy is limited, and they are therefore not considered standard therapy (29-32). The cornerstone in the treatment of established acute GvHD consists of high-dose corticosteroids (15). For steroid-refractory acute GvHD, which is associated with poor prognosis, various strategies have been used, such as anti-TNF-alpha, anti-IL-2, anti-CD3 or anti-CD147 monoclonal antibodies (moAb)., extracorporeal photo-pheresis and the immunosuppressives pentostatin, rapamycin and MMF, but with limited success (33-38). Standard therapy for chronic GvHD consists of combination therapy with cyclosporine and prednisone (39). In patients with steroid-refractory chronic GvHD, good results were recently reported with anti-CD20 moAb, and various other strategies have been reported on, such as PUVA, thalidomide, MMF and total lymphoid irradiation (40-46).

3.2. Graft-versus-leukemia effect

Since long, donor T lymphocytes are considered the principal GvL effector cells, but the ability of NK cells to also mediate antileukemic effects is increasingly being acknowledged (see section 3.3) (47). The target antigens for allogeneic GvL effector T lymphocytes include major histocompatibility (MHC) antigens, minor histo-compatibility (miHC) antigens and tumor-associated antigens (TAA) (Table 1) (48). The importance of MHC and miHC antigens in GvL is illustrated by the close association between GvL and GvHD in recipients of alloHSC grafts. In 1990 already, Horowitz *et al.* (6) reported their observations that leukemia relapse was less frequent in patients who received alloHSCT and did not develop GvHD, than in patients who received syngeneic or T cell-depleted HSC grafts. The authors suggested the existence of a T cell-mediated GvL effect that selectively targets a subset of miHC antigens preferentially expressed on leukemic cells (and possibly also on normal hematopoietic cells) (6). Today, 9 hematopoietic tissue-restricted miHC have been described: HA-1 (49), HA-2 (50), HA-8 (51), HB-1 (52), ACC-1 and ACC-2 (53), UGT2B17 (54), B8/H-Y (UTY) (55), and LRH-1 (56). MiHC are under consideration as potential targets for anti-tumor therapy because they are highly immunogenic, because miHC-specific T cell clones exhibit high avidity, thus increasing the likelihood of a response against tumor cells that express low levels of miHC antigens, and because miHC-specific T cell responses involve both $CD4^+$ and $CD8^+$ cells (reviewed in (57)). Key studies by Goulmy *et al.*, Mutis *et al.* and Marijt *et al.* in patients receiving miHC-mismatched alloHSCT have demonstrated that miHC antigens play a crucial role in the development of both GvHD and GvL reactions (58-60). Marijt *et al.* and Kircher *et al*. showed that treatment of patients suffering

from relapsed HA-1- and/or HA-2-positive malignancies with DLI from HA-1- and/or HA-2-negative donors, resulted in complete remission, associated with the occurrence of HA-1- and HA-2-specific $CD8^+$ cells (60,61).

Tumor-associated antigens are non-miHC self-antigens that are aberrantly expressed, either qualitatively or quantitatively, by tumor cells, and that can therefore be expected to elicit specific anti-tumor immune responses. TAAs can result either from mutation (e.g. bcr/abl mutation in CML), from overexpression (e.g. Wilms' tumor antigen 1), or from tissue-specific expression (e.g. prostate-specific antigen in prostate cancer, melanoma-associated antigen in testicular cancer) (48). Most of the TAAs identified so far are non-mutated self-antigens that are differently expressed in the tumor and normal host tissue. Wilms' tumor antigen 1, myeloperoxidase (MPO), proteinase 3 (Pr-3), survivin, telomerase reverse transcriptase, CYPB1 and immature laminin receptor are examples of proteins that are expressed by leukemic cells at much higher levels than normal cells do, and that could therefore serve as targets for GvL (48). Pr-3 is a serine protease that is maximally expressed at the promyelocyte stage of myeloid differentiation (62) and that is regulated by the transcription factors PU.1 and C/EBP-alpha, both of which have been implicated in leukemogenesis (63-65). Myeloperoxidase is another myeloid-restricted protein and is expressed during early myeloid differentiation. Both MPO and Pr-3 are over-expressed in a variety of myeloid leukemia cells including approximately 75% of CML patients, 50% of AML patients and 30% of MDS patients. Evidence that Pr-3 and MPO can serve as target antigens for T cells originally stems from the observation that T cells, taken from patients with Wegener's granulomatosis or small vessel vasculitis, respectively, mount a proliferative response when stimulated with the particular antigen (66). In patients with myeloid malignancies it has been demonstrated that CTLs directed against Pr-1 (which is an HLA-A2.1-restricted peptide derived from Pr-3), can lyse leukemic blasts (67-69), and, furthermore, cytogenetic remission of CML following treatment with interferon-alpha has been described to correlate with the occurrence of these Pr-1-specific CTLs (70). Finally, also Wilms' tumor antigen 1 is overexpressed in leukemic cells in AML, CML, ALL, MM and multiple solid tumors (71-75), and evidence exists to indicate that anti-Wilm's tumor 1 CTLs can specifically lyse leukemic cells (76-78).

4. STRATEGIES TO SEPARATE GRAFT-VERSUS-LEUKEMIA FROM GRAFT-VERSUS-HOST DISEASE

Since the first description of the concept of GvL, the emphasis in alloHSCT clinical practice and research lies on the immunotherapeutic capacity of the alloHSC graft rather than on the tumor-reducing effect of high-dose conditioning therapy. Key evolutions include the development of RIST regimens (Box 1), which aim to exploit GvL immune reactivity while reducing treatment-related toxicity and while controlling for GvHD, and the development of post-transplant immunotherapeutic approaches for the induction of GvL, most of which are based on DLIs. In addition, very recently it has become evident from human studies that NK cells can significantly contribute to GvL, and a potential therapeutic role for regulatory T cells (Treg) is emerging.

4.1. The conditioning regimen and the peri-transplant cytokine milieu

Originally, the purpose of the conditioning treatment before alloHSCT was to suppress the recipient's immune system and to eradicate the tumor, and it was therefore designed to be myeloablative. Current myeloablative conditioning regimens most commonly consist of a combination of cyclophosphamide and total body irradiation or busulphan (79). However, it has become clear that even the most intensive regimen rarely eradicates the malignancy and that leukemic relapse relentlessly occurs if a GvL effect is not generated (80-82). By comparing two regimens of high-dose irradiation, Clift *et al*. showed both in patients with AML in first remission (81) and in patients with CML in chronic phase (82) that an increase in the dose of total body irradiation failed to prevent leukemia relapse and to improve survival but resulted in increased mortality from causes other than disease recurrence. In order to minimize conditioning-related toxicity, and thereby render older and frailer patients eligible for alloHSCT procedures, the RIST approach was developed. Through immunosuppression rather than myeloablation, so-called non-myeloablative protocols were designed to create immunological space in the bone marrow and lymphoid organs, so as allow for allogeneic engraftment (14). The resulting, often low-level, mixed donor chimerism can subsequently be used as a platform for the induction or reinforcement of GvL through immunotherapeutic approaches such as DLI. Various regimens have been developed. Most common is the combination of fludarabine with other cytotoxic agents such as busulfan, cyclophopsphamide, melphalan, ATG, or with low-dose total body irradiation and post-transplant cyclosporine and MMF immunosuppressant treatment in the so-called 'mini-transplant' procedure (79). In 1998, Slavin reported on 26 patients given alloHSCT with non-myeloablative conditioning including fludarabine, anti-T-lymphocyte globulin, and low-dose busulfan. A good safety profile and strong antitumor responses were obtained (12). This study was followed by several reports on the efficacy of RIST in inducing donor chimerism and anti-tumor effects in patients with various types of haematological malignancies (9,11). A comparative study by Le Blanc *et al.* showed that the mini-transplant protocol, as compared to a standard non-myeloablative protocol, led to reduced risks for leucopenia and transfusion need, but increased incidences of graft failure, acute GvHD and transplant-related mortality (83).

Similar to what has been observed in myeloablative transplant regimens, anti-leukemic responses in RIST protocols have been shown to occur in association with progression from mixed to full donor chimerism, and often with the development of GvHD (84,85). It is well known that administration of cytotoxic or radiotoxic conditioning regimen results in the release of a range of

inflammatory cytokines, and since long it has been accepted that this so-called 'cytokine storm' in the early post-transplant microenvironment crucially contributes to the pathophysiology of GvHD (86). Chakraverty *et al.* recently showed in a mouse model that inflammation in nonlymphoid tissue is a prerequisite for migration of activated T cells to that site. Despite effective activation and expansion, adoptively transferred donor-type alloreactive T cells entered nonlymphoid tissues only in freshly irradiated mice and not in mixed chimeras (87). Similarly, as discussed below, abatement of the cytokine storm is thought to be one of the factors that account for the reduced risk for DLI-induced GvHD when DLI is administered several weeks or months rather than immediately after conditioning and transplantation; an observation that was made in the clinic and has been experimentally reproduced in mice (14,88-92). Hence, other than to limit direct organ toxicity, RIST may also have the beneficial consequence of reducing the risk for GvHD.

Randomized controlled trials comparing standard myeloablative conditioning with RIST are as yet not available. Uzunel et al. observed similar incidences of molecular remission in patients with CML following alloHSCT using RIST or myeloablative conditioning (93). Also, Sorror et al. found that non-myeloablative conditioning before HLA-matched unrelated alloHSCT led to reduced risks for severe conditioning-related toxicity and severe GvHD (94). In contrast, Mielcarek and coworkers reported that, rather than preventing GvHD, a non-myeloablative regimen consisting of low-dose TBI and post-transplant cyclosporine and MMF altered the kinetics and tissue-specificity of GvHD, resulting in late-onset acute GvHD (after day 100 post transplantation) with skin being more frequently, and gut being more severely affected (95). It is clear that RIST provides treatment opportunities for patients with advanced age, comorbidities or previous transplants. However, randomized controlled trials demonstrating its superior efficacy in inducing GVL without GvHD are as yet not available.

Hypothesizing that the peri-transplant cytokine environment can steer alloreactive T cells towards GvHD, investigators have studied the effect of various cytokines on GvHD and GvL reactivity in experimental alloBMT models, but few have been studied in a clinical context. Administration of anti-interleukin (IL)-1 moAb in a haplotype-mismatched murine alloBMT model resulted in reduced GvHD with preserved GvL effects (96), but clinical studies on the effect of IL-1 inhibition on GvHD have yielded conflicting results. In an open-label, phase I/II trial in 17 patients with steroid-resistant GVHD, treatment with recombinant human IL-1 receptor-antagonist was found to be safe and to improve the severity of GvHD in 10 of 16 patients. However, in a double-blind, placebo-controlled randomized trial involving 186 patients, these results could not be confirmed (97,98). The observation that post alloHSCT GvHD was associated with high tumor necrosis factor alpha (TNF-alpha) serum levels (99) led to clinical studies evaluating the effect of anti-TNF-alpha moAb in the treatment of steroid-refractory GvHD. A multicenter pilot study in 19 such patients showed that infliximab was well tolerated but that it produced a partial response in a limited subset of patients only, and was generally ineffective in preventing GvHD recurrence (100). Similarly, a retrospective study of 134 patients, treated with infliximab for steroid-resistant acute GvHD, showed a partial response, in particular in those with gastrointestinal involvement, but an increase in fungal and viral infections was noted (101). Importantly, the observation in murine models that donor T cell-derived TNF-alpha is involved in the development of both GvHD and GvL suggest that TNF-alpha blockade might abrogate GvL effects (96,102,103), and data on the effect of TNF-alpha blockade on GvL in humans are as yet not available. Various other cytokines have been investigated for their role in GvHD and GvL. Administration of IFN-gamma, IL-2, IL-12 or IL-18 in variable models of experimental alloBMT was found to allow for a dissociation of GvL from GvHD (104-106). Also, administration of IL-11 in a murine model was shown to reduce $CD4^+$ T cell-mediated GvHD without compromising the $CD8^+$ T cell-mediated GVL effect. However, treatment of human transplant patients with IL-11 has been reported to result in serious adverse advents, and this has averted further clinical trials along this line (107). Although these studies clearly demonstrate that interfering with the cytokine network may dissociate the GvHD and GvL effect, available evidence does not support a clear model that explains and unifies these phenomena, and this is probably due to our incomplete understanding of the cytokine network and the various cell populations involved. Furthermore, none of these studies have been translated into successful clinical trials.

Conventional bone marrow alloHSCT is being progressively replaced by peripheral blood alloHSCT. A recent meta-analysis has shown that peripheral blood alloHSCT is associated with enhanced GVL responses, and that - although also associated with an increase in severe acute GvHD and with an increase in chronic GvHD - it results in superior disease-free survival rates (108). In order to mobilize stem cells in the peripheral blood of alloHSC donors, granylocyte-colony stimulating factor (G-CSF) is commonly used, and recent studies have revealed how G-CSF pretreatment of donor cells can contribute to dissociated GvL and GvHD. The group of Morris, Macdonald and Hill et al. was able to show in murine models of mobilized peripheral blood alloHSCT, that G-CSF treatment of donor animals prior to transplantation can attenuate GvHD through effects on donor T cells (bias towards Th2 differerentiation and induction of Tr1 regulatory cells), and through effects on donor APCs (induction of regulatory APCs, immature myeloid precursors and plasmacytoid dendritic cells (DCs)), while these immune effects do not affect cytotoxic T cell function involved in GvL (109) (and as reviewed in (110)). They further showed that donor pretreatment with novel G-CSF analogs additionally results in expansion and activation of donor NK T cells, which upon transplantation critically contribute to superior GvL effects (111) (and as reviewed in (110).).

4.2. Targeting the T cell-mediated GvL effect

T cells are considered the principal effector cells of both GvH and GvL effects. The benefits of non-specific T cell-depletion, as a measure to prevent GvHD (25), is well known to be off-set by increased incidences of graft rejection, disease relapse and delayed immune reconstitution (112,113). Therefore, strategies have been developed that aim at exploiting the GvL reactivity of alloreactive T cells either through post alloHSCT adoptive transfer of donor T cells or subsets thereof, or through manipulation of the antigen-presenting cells with which they interact.

4.2.1. Interaction of GvH and GvL effector T cells with antigen-presenting cells

Both experimental and clinical data indicate that host-type APC play a key role in the generation of a GvL response after DLI (91,92,114). Chimerism analysis in DLI-treated patients shows that responders generally become complete chimeras in conjunction with or before the GvL response, supporting the concept that the graft-versus-hematopoietic-host response functions as a basis for the GvL effect (88,114,115). This is supported by the observations in murine models that DLI yields stronger GvL effects in mixed as opposed to complete donor chimeras and that GvL is associated with T cell-mediated lymphohematopoietic GvH reactions establishing substantial increases in donor chimerism (91,92). Our group has shown in a mouse model of miHC-disparate alloBMT, that DLI yields stronger GvL effects in mixed than in complete donor chimeras and that DLI-induced GvL was associated with T cell-mediated lympho-hematopoietic GvH reactions (Figure 1) (92). Using a model of class I-deficient beta-2-microglobulin knock-out BMT in recipient mice with normal MHC I expression, Mapara *et al.* showed that host APCs were required for DLI to induce GvH and GvL effects (91). Shlomchik *et al.* had previously delivered direct proof that elimination of host APCs after alloBMT led to abrogation of GvHD, but that this also had the undesired consequence of reducing GvL (116). Other elegant mouse studies of alloBMT have subsequently confirmed that host-type APC are both crucial and sufficient for the development of GvHD (116-118), but equally that differential targeting of host APC populations could allow for the dissociation of GvHD and GvL. Merad *et al.* demonstrated that host-type Langherhans cells that persist after allo BMT can trigger GvHD (119), and Teshima *et al.* showed that although GvHD results from the interaction of donor T cells with host APCs, it does not require expression of alloantigen on epithelial target cells (117). Also, Matte *et al* showed that once initiated, GvHD can be intensified by donor-type cells, most probably donor-type APCs, that cross-prime alloreactive $CD8^+$ cells. In contrast, donor-type APCs were not required to obtain a $CD8^+$-mediated GvL effect (118). As mentioned above, rodent studies by Mapara *et al.* revealed the ciritical role of host APC in the generation of GvL (91). This was confirmed by Reddy *et al.* who showed in a rodent model that alloantigen presentation by host APC present within the lymphohematopoietic system, and not by those residing within the GvHD target tissues, crucially determines the occurrence of a GvL response (120). Furthermore, they found donor-derived APC only to contribute to the GvL effect through cross-priming of alloreactive $CD8^+$ T cells in situations where the tumor burden had manifestly decreased (120). These studies indicate that the selective depletion of APCs in GvHD target tissues, e.g. skin, while preserving them at other places such as secondary lymphoid organs where they would be required to initiate GvL, would represent a potential approach to separate GvL from GvHD, and Merad *et al.* recently demonstrated this approach to be effective in preventing murine GvHD (119).

Another potential means to differentially interfere with the T cell – APC interaction involved in GvL and GvHD is to enhance DC presentation of leukemic antigens to effector T cells. Choudhury *et al.* and Harrison *et al.* have demonstrated the feasibility of generating CML- or AML-derived DCs that elicit strong alloreactive and anti-tumor cytotoxic responses by autologous T cells *in vitro*, and thereby deliver evidence to support the rationale of therapeutic vaccines based on leukemic cell-derived DCs (121,122). Similarly, it has been shown in mice that pre-transplant vaccination of donor mice with recipient-type leukemic antigens potentiated post-transplant GvL without increased risk for GvHD (123).

It is well known that, depending on DC subtype and maturation state, and on the type and duration of antigenic stimulus, the interaction between MHC/peptide complexes on the DC and T cells results either in tolerance or immune activation (124). Through *in vitro* cultures, Sato *et al.* obtained a subtype of murine regulatory DCs, characterized by high expression of MHC and low expression of co-stimulatory molecules, which showed potent inhibiting properties both *in vitro* and *in vivo* towards acute GvHD (125). A single injection of these "regulatory" DC in a mouse alloBMT model resulted in the prevention of GvHD while GvL effect was maintained. The authors further established a role for IL-10-producing $CD4^+$ T cells and $CD4^+CD25^+CD152^+$ T cells in this phenomenon (125). Similarly, TRAIL-transduced DC have been shown in a fully MHC-mismatched murine alloBMTmodel to suppress GvHD and preserve GvL through the induction of apoptosis in both alloreactive T cells and leukemia cells (126).

4.2.2. Adoptive transfer of GvL effector T cells

4.2.2.1. Donor lymphocyte infusion

Early alloHSCT studies demonstrated that intensive conditioning regimens rarely eradicate hematological malignancies completely (81,82), and the practice of second alloHSCT (using HSC from another donor) in patients with post-transplant relapse was met with limited success due to high morbidity and mortality (127). These observations led investigators to test whether infusion of lymphocytes, obtained from the original BM donor could be used as a means to induce post-transplant anti-leukemic immunoreactivity. The first reports on DLI in mice and humans were published by Slavin *et al.* in 1988 (7). Today, the success of adoptive immunotherapy with DLI has provided the most compelling evidence that the GvL effect of allografts is crucial to the effectiveness of alloHSCT in the treatment for lymphohematopoietic

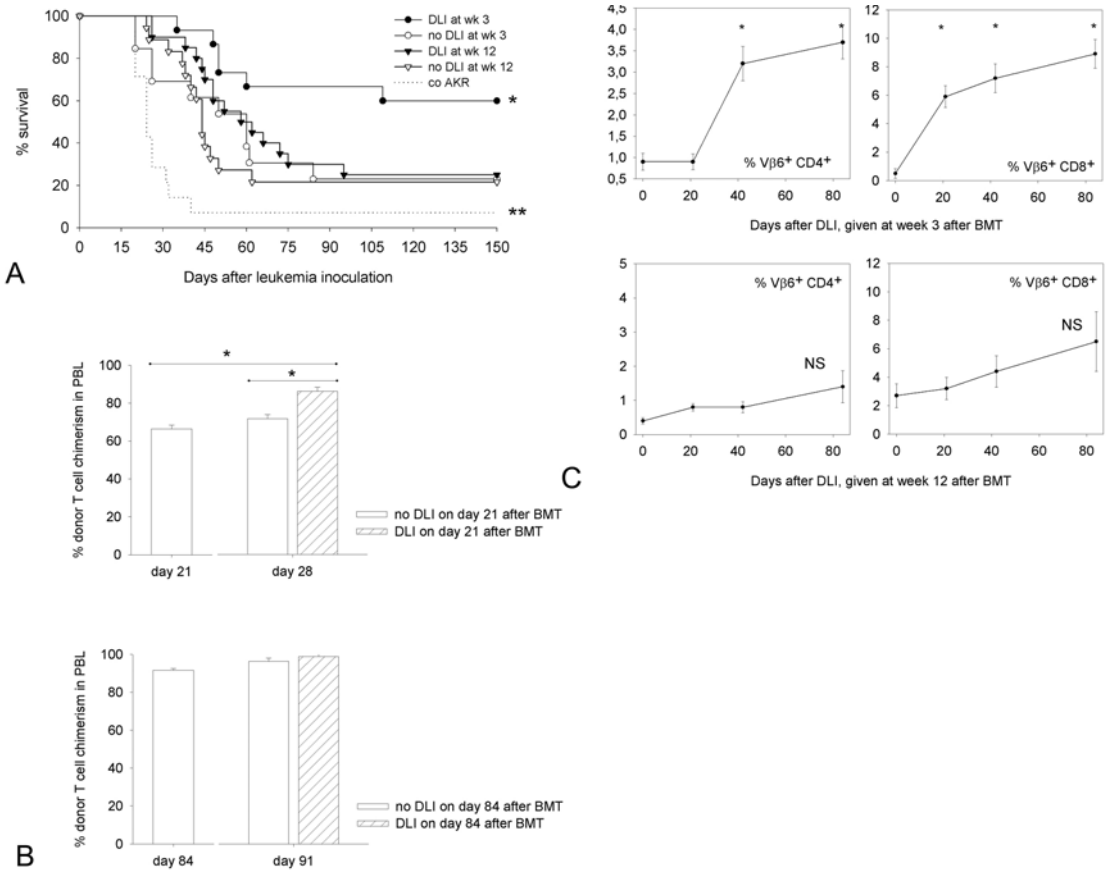

Figure 1. In C3H→AKR miHC disparate bone marrow chimeras, DLI generates lymphohematopoietic GVH reactivity and a GVL effect only in mixed chimeras at 3 weeks after alloBMT, and not in complete donor chimeras at 12 weeks after alloBMT. AKR (H-2k, Thy 1.1^+, Mls1a/2b) recipient mice were lethally irradiated, transplanted with T cell-depleted C3H (H-2k, Thy 1.2^+, Mls1b/2a) bone marrow cells, and given DLI at 3 or 12 weeks. (a) Chimeras given DLI at 3 or 12 weeks, and age-matched chimeras not given DLI were inoculated with BW5147.3 host-type leukemia cells at week 4 and 13, respectively. As a control, untreated host-type AKR mice were given the same leukemia challenge. Kaplan-Meier survival curves are shown for a total of 20 (DLI) and 18 (no DLI) week-12-chimeras, a total of 14 (DLI) and 13 (no DLI) week-3 chimeras and a total of 14 untreated AKR control mice. (b). Spontaneous evolution of donor T cell chimerism after BMT and changes in chimerism induced by DLI at 3 (upper panel) and 12 (lower panel) weeks after BMT. Bars represent mean ± SE of 3-5 identically designed experiments (n = 4 per group and n total = 12-22). (c) Frequency of host-reactive TCR-$V\beta6^+$ T cells in PBL before and after DLI at 3 (upper panels) or 12 weeks (lower panels). Data represent mean ± SE of 2 (3 weeks) and 3 (12 weeks) identically designed experiments (n = 4-5 per group and n total = 9 for 3 weeks, 14 for 12 weeks).. This research was originally published in Blood. A. D. Billiau, S. Fevery, O. Rutgeerts, W. Landuyt and M. Waer: Crucial role of timing of donor lymphocyte infusion in generating dissociated graft-versus-host and graft-versus-leukemia responses in mice receiving allogeneic bone marrow transplants. Reproduced with permission from 92.

malignancies, and has led to its routine application in clinical practice (128-130). Although DLI has been applied in a wide range of hematological malignancies, considerable variability exists in the susceptibility of different types of leukemia to DLI-induced GvL (131). CML is the most susceptible with responses being more frequent and durable for chronic-phase CML than for CML in blast crisis (132). In other types of hematologic malignancies, e.g in AML, MM and MDS, responses are less frequent, and although a clear GvL reactivity for Philadelphia-chromosome-positive ALL has been noted, success in ALL is rare (128,132-140). The differential response of leukemia types to DLI is incompletely understood. Differences in growth kinetics (141,142), variable expression of co-stimulatory molecules (143) and differential ability of leukemic cells to differentiate into DC may play a role (121). Indeed, the process of allogeneic engraftment and development of GvL may be outpaced by rapid proliferating aggressive malignancies such as AML, and GVL effector mechanisms may differ between leukemia types. Whereas it is established that T lymphocytes are extremely important in the generation of graft-versus-CML responses (144), the incidences of AML and ALL relapse have been shown to be less influenced by T cell-depletion (145).

The effector mechanisms underlying the DLI-induced GvL effect are still incompletely understood but,

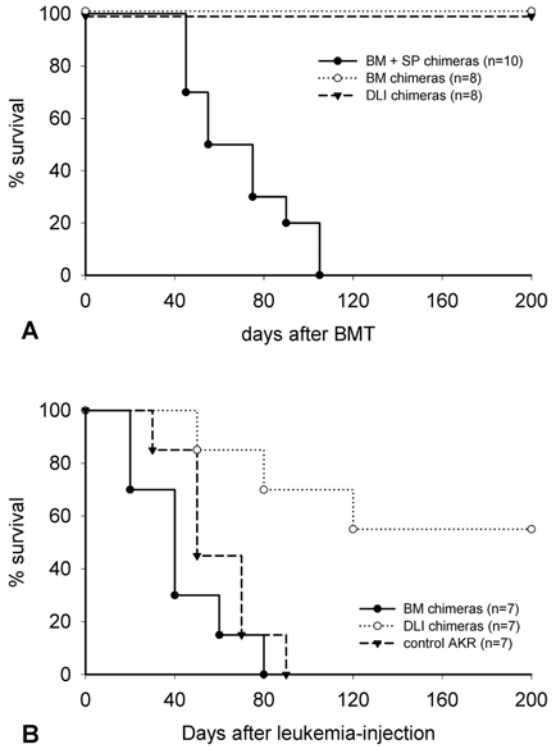

Figure 2. In C3H→AKR miHC-disparate bone marrow chimeras, DLI at the time of BMT induces acute GVHD, but when given 3 weeks after the BMT, DLI induces GVL without GVHD. AKR (H-2k, Thy 1.1^+, Mls1a/2b). recipient mice were lethally irradiated and transplanted with T cell-depleted C3H (H-2k, Thy 1.2^+, Mls1b/2a) bone marrow cells. (a) Survival of mice after transplantation with C3H bone marrow only (BM chimeras), bone marrow together with C3H spleen cells within 24 hr after irradiation (BM+SP chimeras), or bone marrow only followed 3 weeks later by injection with C3H spleen cells (DLI chimeras). (b) Survival of naïve host-type AKR mice and of different groups of bone marrow chimeras (as in (a)) following inoculation of host-type leukemia cells (BW5147.3) cells, 4 weeks after bone marrow transplantation. Reproduced with permission from 267.

as discussed earlier (section 3.1), studies in DLI-treated leukemia patients and in mouse models of alloHSCT have indicated graft-versus-lymphohematopoietic responses as the basis of the GvL effect. Childs *et al*. showed that DLI-treated patients generally exhibit conversion from mixed to complete chimerism prior to, or at the time of the GvL response (114). Moreover, marrow aplasia frequently complicates DLI in recipients who have insufficient donor-derived normal progenitors (146). Our studies in mice showed DLI to induce GvL and lymphohematopoietic GvH reactivity with expansion of host-reactive T cells only in mixed and not in complete donor chimeras (Figure 1) (92). This was in accordance with a simultaneously published study in an MHC-mismatched model by Mapara *et al*., showing that host-type APC are required for the induction of potent DLI-induced GvH and GvL effects (91). These studies therefore corroborate that also DLI-induced antileukemic reactivity is closely associated with broad anti-host reactivity, and therefore carries the risk for the development of GvHD. Also in the clinical setting, DLI does not fully separate GvL from GvHD. Studies by Collins *et al.* and Storb *et al.* showed that disease remission is related to the development of GvHD (131,134), and today, GvHD complicating DLI is still a major problem, occurring in approximately 50% of cases (8,138). Various DLI strategies have been experimentally and clinically explored in an attempt to optimize GvL while controlling for GvHD.

4.2.2.1.1. Timing of donor lymphocyte infusion

From clinical studies it became evident that DLI given early after HSCT led to high incidences of GvHD, whereas when postponed until several weeks or months after the transplant, DLI was associated with reduced GvHD rates. Furthermore, in those cases were GvHD did occur, it was generally mild and controllable (14,90). In accordance with these findings, experiments in murine models of alloBMT showed that DLI given immediately following BMT gave rise to acute GvHD, whereas when DLI was delayed for several weeks, GvHD was mild or even absent, while a potent GvL response could still be observed (92,147-150). This was demonstrated by our group in a miHC-mismatched alloBMT model (Figure 2) (149). As suggested earlier, the higher susceptibility to GvHD in the early post-transplant period is thought to relate to overstimulation of effector donor T cells by the cytokine storm and local inflammation, unleashed by the conditioning regimen (86,87).

These studies of 'delayed' DLI in mice showed that GvHD could be avoided with preservation of GvL reactivity, and thus delivered proof of concept that DLI can induce GvL effects independently from GvHD. In humans also, the practice of giving DLI late after the initial transplant procedure, or with a non-myeloablative alloHSCT regimen designed to give less acute toxicity, has been shown to lead to significant anti-tumor responses with incidences of GvHD that were lower than one would expect when similar doses of donor T lymphocytes were infused at the time of myeloablative alloHSCT (see also section 3.1) (reviewed in (8)). Many studies have reported on efficacy and safety profiles of DLI regimens, the indication for DLI being either mixed chimerism, residual or relapsing disease without GVHD, or high-risk disease (9,84,132,146,151-163). Bader *et al.* have shown that immunotherapy with prophylactic DLI drives the evolution of chimerism towards complete donor chimerism and leads to lower relapse rates (160). This effect of pre-emptive DLI immunotherapy has been confirmed by others (8).

4.2.2.1.2. Dosing of donor lymphocyte infusions: multiple-dose and dose-escalating strategies

Clinical practice has not allowed to reliably define optimal DLI doses to obtain GvL effects. The lowest DLI dose capable of inducing fatal GvHD is on the same order of magnitude as the optimal dose required for the treatment of advanced leukemia (154, 138). Moreover, the minimal therapeutically effective DLI dose varies with the type of malignancy, CML requiring lower doses than MDS or ALL (154).

In a non-randomized clinical trial comparing single-dose with escalating-dose DLI to treat CML relapse, Dazzi *et al.* found that, despite similar cytogenetic remission rates, the 2-year incidence of GvHD was significantly lower in the escalating-dose DLI group than in the single-dose group, although both groups received comparable total lymphocyte doses (164). Similarly, Peggs *et al.* and Mackinnon *et al.* used dose-escalating DLI protocols and found that GvL effects can be induced with DLI doses that are lower than those necessary to provoke GvHD (88,151,155,158). The mechanism underlying the differential effect of repetitive DLI on GvL and GvH reactivity is not known. Repetitive infusion of non-tolerant donor lymphocytes might induce a regulatory circuit that differentially influences GvL and GvHD.

4.2.2.1.3. Composition of the donor lymphocyte inoculum

4.2.2.1.3.1. T cell subset donor lymphocyte infusion

The identification of the effector cells responsible for mediating GvL effects is a key question in alloHSCT research that remains incompletely answered. Studies in mouse models have delivered data to support a direct role for $CD4^+$, $CD8^+$ T cells, or both, in DLI-induced GvL, but their relative contribution seems to vary between animal models and leukemia cell lines used (165-167). Moreover, specific subsets have been identified that mediate both GvL and GvHD, and, hence, depletion of this particular subset resulted both in avoidance of GvHD-associated mortality and abrogation of GvL effect. Also in humans, both leukemia-specific $CD4^+$ and $CD8^+$ T cell lines have been described (168-172), and patient studies on the effect of T cell-subset DLI are emerging (142,173-176). In an early study, Champlin *et al.* found that $CD8^+$ T cell-depleted alloHSCT gave rise to reduced GvHD, while GvL was preserved. However, this was at the expense of an increased incidence of graft failure (174). Similarly, other have reported $CD8^+$ TCD DLIs to exert strong graft-versus-CML and graft-versus-myeloma effects, with reduced rates of severe acute GvHD (142,173,175,176).

Differential involvement of Th1 and Th2 cells in GvL and GvH immune reactivity has been demonstrated in mouse models where adoptively transferred donor Th1 cells mediated a curative GvL effect and moderate GvHD, whereas Th2 cells mediated a variable GvL effect and caused only minimal GvHD (177,178).

4.2.2.1.3.2. Donor lymphocyte inocula depleted of alloreactive T cells

The depletion of alloreactive T cells from the DLI inoculum represents another possible approach to dissociate GvL from GvHD. Various techniques have been reported on, such as identification and elimination of alloreactive T cells based on the expression of activation-induced cell surface markers (such as CD25 and CD69). following *in vitro* stimulation in mixed lymphocyte culture. This approach has been reported to lead to abrogation of *in vitro* alloreactivity while leaving *in vitro* anti-viral or anti-tumor responses intact (179-185). Amrolia and coauthors reported a phase I clinical trial, in which this approach resulted in low incidences of GvHD and infection. However, effects on leukemic relapse remain to be established (186,187). Others have demonstrated the feasibility of establishing non-alloreactive T cell populations with conserved anti-third party and anti-viral specificity using flowcytometric depletion of $CD4^+$ $CD38^+$ T cells, targeting of P-glycoprotein with TH9402 (188-189) and flowcytometric selection of $CFSE^{bright}$ $CD25^-$ cells (190).

Michalek *et al.* have used CD3-size spectratype analysis to demonstrate that human *in vitro* enriched alloreactive and tumor-reactive T cell populations express different T cell receptor V-beta chains (191). Furthermore, the authors established the feasibility of monitoring these clones *in vivo* in relation to GvHD and leukemia relapse. Although these findings provide a basis for a GvL approach that would be theoretically safe, the involvement of specific T cell clones probably varies between experimental and human leukemias. In addition, the identification, isolation and expansion of the relevant T cell clones is likely to be difficult.

In analogy with the approach of depleting alloreactive T cells, Bachar-Lustig *et al.* demonstrated that, in mice, selective infusion of anti-third party T cells could induce a GvL effect without GvHD (192). Similarly, Arditti *et al.*, working with a humanized mouse model of B-CLL, showed that allogeneic, non-hostreactive anti-third-party CTL can establish a strong GvL response, while avoiding GvHD (193).

Of note, it has recently been shown in mouse studies that the memory T cell subset of T cell inocula does not have the capacity to induce GvHD upon transfer in MHC-matched and MHC-mismatched allogeneic hosts (194,195), and that infusion of such T cell subsets allows for the transfer of specific memory (194). Further, Zhang *et al.* showed in a $CD8^+$ T cell-dependent model of GvHD that the depletion of $CD44^{lo}CD8^+$ T cells from an anti-host presensitized $CD8^+$ donor T cell inoculum resulted in prevention of GvHD while GvL effects were not impaired, suggesting that $CD44^{hi}CD8^+$ memory T cells are able to generate GvL effects without GvHD (196).

4.2.2.1.3.3. DLI with suicide-gene transfected effector cells

GvH and GvL responses are thought to follow different kinetics after alloBMT and, consequently, differently timed interventions could result in separation of GvL from GvHD (197). First and foremost, as mentioned above, both in mice and humans, delaying treatments with DLI for some time after the alloHSCT allows for GvL effects with reduced risk for GvHD (92). Second, various methods of suicide gene-transfected T effector cell transfer have been used to demonstrate the feasibility of inducing dissociated GvL and GvH effects. GvL effects without GvHD have been obtained in mouse studies using herpes simplex virus thymidine kinase (HSV-Tk) gene-transfected donor T cells with timed administration of acyclovir (198-200). So far, three patient studies have reported on the use of HSV-TK-transduced DLI, but described variable effects on anti-leukemic responses. Also, clinical application of this approach will need to address problems such as

incomplete elimination of HSV-TK-transgene cells, and excessive elimination due to immunogenicity of the HSV-TK-transgene product (201-203). Other suicide gene transfer-procedures have been developed *in vitro* and involve timed induction of a chimeric FAS protein (204), the death effector domain (DED) of FADD (the human Fas-associated protein with death domain) (205), or of caspase 8 (206).

4.2.2.2. Antigen-specific adoptive T cell therapy

Adoptive transfer of donor T cells specific for viral antigens has been shown to be effective and safe in the treatment of post-transplant CMV and EBV infection (207). Candidate target antigens for antigen-specific adoptive T cell immunotherapy for hematologic malignancies include several miHC antigens and TAA. Marijt *et al.* reported a study in which 3 alloHSCT patients with relapsed HA-1- and/or HA-2-positive malignancies were treated with DLI form HA-1- and/or HA-2-negative donors. They found that all 3 patients achieved complete remission, with detectable levels of HA-1- and HA-2-specific $CD8^+$ cells, and thereby confirmed that these particular miHC-specific CTL can harness GvL in the clinic (61). The observation that in donor-recipient pairs with an HA-1 and/or HA-2 mismatch in the GVH direction, up to 35% of the CTL clones generated after DLI were specific for HA-1 and HA-2 underlines the role of miHC antigens in GvL reactions (208). However, clinically useful miHC antigens for anti-tumortherapy may not be confined to hematopoietic tissue restricted miHC antigens as Fontaine *et al.* have shown that adoptive transfer of $CD8^+$ CTLs, specific for one single recipient miHC antigen (B6dom1) eradicated leukemia without causing GvHD (209). Clinical trials using miHC antigen specific T cells have been initiated (K Van Besien, personal communication).

So far, biologically relevant antigen-specific tumor responses against the known TAA have been difficult to document. It has been shown that CML cells can present peptides derived from the bcr/abl fusion protein (210). Bcr/abl-derived peptide vaccinations have been shown to induce antigen-specific $CD4^+$ T cell proliferative responses, but $CD8^+$ T cell responses were not seen (211). Similarly, in a transplanted CML patient, bcr/abl-specific $CD4^+$ T cells were documented, but these failed to elicit cytotoxicity *ex vivo* (212,213).

Isolation and expansion of sufficient numbers of antigen-specific T cells has proved to be a technically demanding process, and hampers clinical applicability. *Ex vivo* expansion of leukemia-reactive CTLs is most promising in situations where the leukemic cells can be induced *in vitro* to differentiate into APCs, as has been shown for Philadelphia chromosome-positive CML, CLL and follicular lymphoma cells (121,214,215). Alternatively, leukemia-reactive CTLs can be generated through TAA-specific TCR gene transfer, and successful attempts have been reported for both murine and human T cells. Human T cells transfected with the gene for WT1-specific T-cell receptor were shown to retain specificity and to be able to kill human leukemic cells (216). However, in view of reports on malignant transformation of e.g. retroviral transfected human hematopoietic stem cells (217), (pre)-<clinical development of this approach will need to address important safety issues (218). Ongoing intensive research to identify new TAA and to unravel mechanisms of tumor immune recognition or immune escape will likely contribute to a further development of antigen-specific adoptive immunotherapy.

4.2.2.3. Recipient lymphocyte infusion

Although current alloHSCT strategies are designed to make use of the anti-leukemic effect of the graft and its derived cells, and although *in vivo* GvL effects are generally associated with donor-anti-host reactivity, strong anti-leukemic responses with sustained remissions have also been observed in patients that experienced loss of donor chimerism after a non-myeloablative stem cell transplant (219). Moreover, in rodent studies, the group of Sykes *et al.* have documented that alloBMT followed by recipient lymphocyte infusion could also produce significant antileukemic effects, which was mainly mediated by IFN-gamma-producing recipient-type $CD8^+$ and $CD4^+$ T cells, and not by donor T cells (220,221). These observations suggest that not only donor-anti-host but also a host-anti-donor response can initiate and contribute to an anti-tumor response.

4.3. Targeting the NK cell-mediated GvL effect

The normal function of NK cells consists in recognizing and attacking virus-infected and malignantly transformed cells by non-antigen-specific mechanisms. However, clinical as well as experimental evidence indicates that NK cells can fulfil effector functions in GvH and GvL reactions associated with alloHSCT procedures. Activation of the NK cell depends on the balance between positive and negative signals transmitted through sets of stimulatory and inhibitory receptors for ligands on potential target cells (222,223). These receptors belong to different molecular classes: the killer cell immunoglobulin-like receptors (KIRs), the C-type lectins (NKG2 family) and the natural cytotoxicity receptors (NCRs). Triggering receptors are those that recognize ligands expressed by cells under stress (virus-infected and cancer cells). Inhibitory receptors recognize self MHC Class I molecules enabling NK cells to spare normal host cells while mounting their attack against prospective targets that miss these self MHC molecules (224). Of note, this 'missing self' theory does not explain all aspects of NK cell biology, since MHC class I expression is not in all instances sufficient to prevent NK cytotoxicity nor is it always a necessary condition (225).

Each single inhibitory KIR recognizes antigenic determinants defining a group of 'self' MHC I alleles. For instance, in humans, the inhibitory KIR2DL2 and KIR2DL3 recognize group 1 HLA-C alleles (Cw1, Cw3, Cw7 and Cw8) having Ser77 and Asn80. In general, the inhibitory KIRs tend to have a greater affinity for their corresponding ligands than do the activating KIRs (226). Thus diversity in KIR repertoire between individuals that are mismatched for subgroups HLA-C alleles establishes the basis for NK-cell mediated alloreactivity (Table 1) (47).

The inhibitory C-type lectin receptors NKG2A, NKG2C, NKG2E, NKG2F are heterodimers containing a shared subunit (CD94) (227). The activating NKG2D is a homodimer and may play a role in anti-tumor responses since its ligands are induced by neoplastic cell transformation. In humans, these ligands include two families, the MHC class I chain-related antigens (MIC) and the UL16-binding proteins (ULBPs) (228-230). Besides NKG2D, other activating receptors without specificity for MHC class I molecules have been identified. Most of these seem to act as co-activators of NK cytotoxicity. In humans, these receptors are collectively called natural cytotoxicity receptors (NCRs) (231-235). They are thought to be complementary to NKG2D in the MHC-independent recognition of tumor cells.

Evidence that NK cells can contribute to the GvL effect of BMT procedures stems from studies in patients undergoing MHC-mismatched haploidentical alloHSCT (47). In this setting, the MHC Class I molecules of host cells may not correspond to the inhibitory receptors of the donor NK cell clones. In addition, leukemic cells may express ligands that trigger stimulatory NK cell receptors. Together, this may result in killing of host leukemic cells. NK cell alloreactivity is mainly limited to lymphohaematopoietic lineage cells, as epithelial cells are generally insensitive to NK cell-mediated killing, and this may result in engraftment while leaving clinical GvHD target organs (e.g. the gut) intact (223,225). MHC-mismatched haploidentical alloHSCT is applied when an MHC-matched HSC donor is not available, and often involves multiple mismatches at MHC class I and II loci, a situation that is expected to result in severe GvHD. In order to prevent graft rejection and GvHD, such patients are given very intensive conditioning treatment and HSC grafts that are extensively depleted of T cells (236). In several studies, the group of Martelli *et al.* have reported this approach to result in high rates of engraftment with low rate of severe GvHD (236-239). In addition to being the effect of preventive T cell depletion, the low rate of GvHD is thought to result from NK cell-mediated killing of host-type APCs in GvHD target tissues. As mentioned earlier, these play a key role in eliciting GvHD (116) and Ruggeri *et al.* could show in mice that alloreactive NK cells can very rapidly eliminate host lymphohematopoietic cells, including DCs (240).

Exhaustive T cell depletion in this setting precludes significant T cell mediated GvL responses, and on the basis of studies in AML patients, Ruggeri *et al.* have reported data to indicate that in this setting, alloreactive NK cells contribute to GvL (240). This has subsequently been confirmed by others for other types of haematological malignancies (241,242). Ruggeri *et al* (240) studied 57 high-risk AML patients given MHC-mismatched haploidentical HSCT. Twenty donor/recipient pairs showed a KIR epitope mismatch, with presence of donor-type alloreactive NK cells, whereas 37 pairs were KIR epitope compatible. In the patient group that received alloHSC with alloreactive NK cells, the five-year disease-free survival was 60%, as opposed to only 5% in the KIR ligand-matched group. Within the same study, such an effect of KIR epitope incompatibility could not be documented in ALL patients, and this is supported by the recent demonstration that NK cells fail to kill ALL cells *in vitro* (243). Unfortunately, the results of KIR ligand incompatibility in recipients of alloHSCT are not unequivocal. This discrepancy remains unexplained but may be related to differences in patient populations, underlying diseases, conditioning regimens, graft composition, and post-transplant immunosuppressive regimens (244,245).

4.4. The role of regulatory cells in the dissociation of Graft-versus-host disease and Graft-versus-leukemia

Naturally occurring Treg were originally described as key regulators of autoreactive T cells (246,247). In addition to their role in maintaining tolerance to self, the role of Treg in transplantation tolerance is increasingly being acknowledged (248). In MHC class II-mismatched or fully MHC-mismatched BMT models, $CD4^+CD25^+$ Treg were shown to inhibit GvHD (249,250). Taylor *et al.* demonstrated that both the depletion of $CD25^+$ T cells in the recipient, and the depletion of these cells from the donor inoculum led to increased rates of GvHD. In contrast, Hoffmann *et al.* found that only donor-derived and not recipient-type Tregs reduced GvHD (251). In these settings, Treg did not influence engraftment, but instead facilitated multilineage hematopoietic reconstitution and the induction of donor chimerism. Importantly, subsequent studies showed that although Treg suppressed GvHD, they did not inhibit GvL effects (252,253). The potential of adoptively transferred Treg to obtain dissociated GvL and GvHD has led to the development of techniques to expand Treg and this has been successfully achieved for Treg in mice (254). Human Treg have been successfully expanded but their *in vivo* regulatory role needs to be established (255). The mode of action underlying the differential effect of Treg on GvHD versus GvL is as yet not understood. Possibly, Treg suppress the process of GvHD, which depends on indirect presentation of host antigens, at a time point when GVL reactivity has already taken place through a process of direct presentation (256).

Total lymphoid irradiation (TLI) has been shown in mice to result in temporal non-specific immunosuppression and, when followed by reconstitution with MHC-mismatched BM, to lead to stable mixed chimerism without GvHD (257-260). Strober *et al.* found this protection from GvHD to depend on IL-4-producing host-type $NK1.1^+$ T cells (259), and recently, this group of investigators used NK T cell-deficient $CD1^{-/-}$ mice to definitively show the critical involvement of NK T cells in attenuating GvHD (260). Consistent with these findings, several murine studies have shown that administration of activating NK T cell ligands (e.g. glucocerebroside or alpha-galactosylceramide)., or treatment of donor lymphocytes with alpha-galactosylceramide (and IL-2) before adoptive transfer, results in an attenuation of GvHD (261-263). Finally, as mentioned above, Morris *et al.* have documented the critical involvement of NK T cells in dissociated GVL and GvHD in models of G-CSF-analog-mobilized peripheral blood alloHSCT (111). With regard to the effect of TLI on GvL, Salam *et al.* found that TLI-

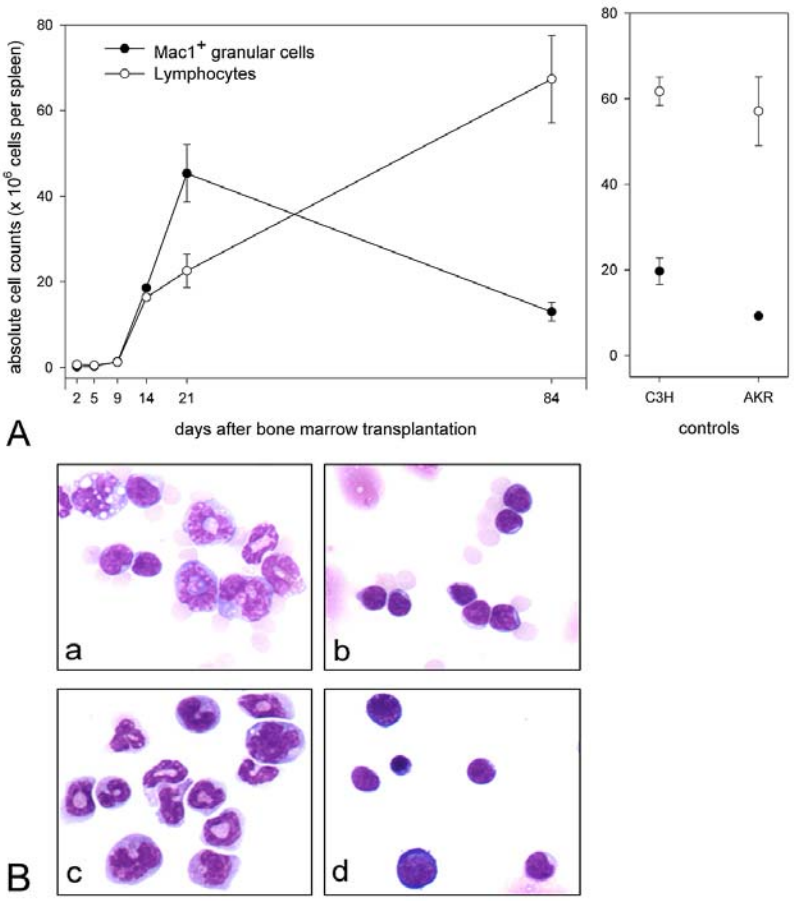

Figure 3. Mac1$^+$ early myeloid cells with suppressor activity transiently expand after C3H→AKR miHC-disparate alloBMT. AKR (H-2k, Thy 1.1$^+$, Mls1a/2b) recipient mice were lethally irradiated and transplanted with T cell-depleted C3H (H-2k, Thy 1.2$^+$, Mls1b/2a) bone marrow cells. Flowcytometric (a) and morphological (b) analysis of spleen cells revealed a transient expansion of early Mac1$^+$ myeloid cells. (a) Lymphoid and myeloid cell repopulation in spleen after BMT: cell numbers in spleens of chimeras (left panel) and untreated control AKR and C3H mice (right panel) (mean values +/- SD of 4-6 chimeric mice). (b) Morphology of Mac1$^+$ granular cells on HE-stained cytospin preparations of spleen cells from week-3 chimeras and control host-type mice. An example is shown for unseparated spleen cells from a week-3 chimera (A) and a control host-type mouse (B), and for Mac1$^+$ (C) and Mac1$^-$ (D) populations following MACS-separation (on the basis of Mac1-expression) of spleen cells from a week-3 chimera. This research was originally published in Blood. A. D. Billiau, S. Fevery, O. Rutgeerts, W. Landuyt and M. Waer: Transient expansion of Mac1$^+$Ly6-G$^+$Ly6-C$^+$ early myeloid cells with suppressor activity in spleens of murine radiation marrow chimeras: possible implications for the graft-versus-host and graft-versus-leukemia reactivity of donor lymphocyte infusions. Reproduced with permission from 273.

treated mice exhibited superior GvL effects as compared to total body irradiation-conditioned mixed chimeras (258). In support of this, it was recently shown that pre-transplant TLI and antithymocyte globulin-treatment in patients with malignant lymphoid diseases or acute leukemia lowered incidences of acute GvHD while GvL was preserved (264).

Double-negative T cells were originally identified as a distinct immunoregulatory cell type, involved in tolerance induction after allo blood transfusion (265). Recently, these cells were documented to be involved in regulating GvL without GvHD following single MHC I locus-mismatched lymphocyte infusion (266). A role for double negative T cells in regulating alloreactivity has also been proposed in a murine model of miHC alloBMT and recipient lymphocyte infusion (267).

CD11b$^+$ myeloid suppressor cells have been implicated to play a role in tumor-specific and tumor-non-specific T cell dysfunction in carcinoma patients and in murine models of solid tumors (268-272). Our group has shown that such cells transiently expand following myeloablation and murine miHC-disparate alloBMT (Figure 3). As these CD11b$^+$ early myeloid cells were capable of IFN-gamma-induced nitric oxide-mediated suppression of alloreactive T cell responses *ex vivo* (not shown) we proposed a role for their involvement in dissociated GvL and GvH reactivity following DLI (273).

Mesenchymal stem cells have anti-inflammatory and immunomodulatory effects. In particular, they have been shown to suppress alloreactive T cell responses while the cells themselves are poorly immunogenic (274,275)

(and reviewed in (276)). A recent pilot study by Ringden *et al* (277) has demonstrated the potential of mesenchymal stem cells, obtained from a third-party individual, in attenuating GvHD, but the effect of this approach on GvL responses remains to be evaluated. In this study, third-party mesenchymal stem cell treatment was given to 8 patients with steroid-refractory grade III-IV acute GvHD: acute GvHD resolved completely in all 6 evaluable patients, and survival in the treated group was significantly better than that of similar patients not treated with mesenchymal stem cells (277).

4.5. Biological, immunosuppressive and immunomodulatory agents

Agents of various kinds have been explored for a potential effect on GvHD and/or GvL. In most of these studies, the mechanism underlying differential immune effects was not explored in detail. It is well known that LPS and inflammatory cytokines play a key role in the initial events of GvHD (278). Antagonisation of LPS, by the synthetic lipid-A analogue B975 resulted in attenuation of murine GvHD while GvL was preserved (278). Furthermore, in mouse models of alloBMT, peri-transplant administration of the histone deacetylase inhibitor Suberoylanilide hydroxamic acid (SAHA) and the cytoprotective agent keratinocyte growth factor was found – through a repressive effect on the production of proinflammatory cytokines – to inhibit the inflammatory events leading to GvHD, while both CTL responses to host antigens and GvL reactivity were maintained (279,280). Similarly, administration of the metalloproteinase inhibitor KB-R7785, which inhibits release of TNF-alpha and FAS-L release, reduced GvHD while strong antitumor effects persisted (281).

The proteasome inhibitor bortezomib, which is used in the treatment of myeloma, has been shown to inhibit alloreactive T cell responses *in vitro*, and, when timely administered in mouse alloBMT models, to segregate GvL from GvHD (282,283). Also, pre-treatment of DLI inocula with the lysosomotropic agent L-leucyl methyl ester, known to inhibit murine and canine GvHD through depletion of dipeptidyl peptidase I-expressing cytotoxic cellular subsets, has been demonstrated to lead to attenuated murine GvHD with preserved GvL (284).

Most immunosuppressive agents, such as FK506, rapamycin and deoxyspergualin, have been shown to inhibit both GvHD and GvL (285-287), and although for some other agents such as MMF, FTY720 and the Janus kinase 3 inhibitor WHI-P131/JANEX-1 (288-291), differential effects on GvHD and GvL have been demonstrated, currently they are not clinically used to modulate GvHD and GvL.

The T cell co-stimulatory pathways CD28/CTLA-4/CD80-CD86 and CD40/CD40L represent potential targets for modulating graft rejection and GVHD. Ohata *et al.* showed in a murine alloBMT model that inhibition of CD28 co-stimulation (through CD80 and CD86 blockade) allowed for the separation of GvL from GvHD (292), but in a model of post-transplant DLI, Blazar *et al.* showed that this strategy resulted in the abrogation of both GvHD and GvL (293). Importantly however, Guinan *et al.* recently reported a clinical trial in which transplantation of BM allografts, rendered anergic through *in vitro* culture with host-type cells in the presence of CTLA4-Ig, gave rise to diminished GvHD with a preserved GvL effect (294). CTLA-4-blocking therapy has been reported to elicit potent anti-cancer effects in solid tumors (295-298). Blockade of CTLA-4 in a murine model of MHC-mismatched BMT and DLI was shown by Blazar *et al.* to elicit a significant anti-tumor effect, but severe GVHD precluded a survival advantage (299). Finally, Blazar *et al.* also demonstrated a regulatory role for 4-1BB in murine GvHD and GvL (300).

Using murine alloBMT models Schmaltz *et al.* have shown that donor T cells from TNF-related apoptosis-inducing ligand (TRAIL)-deficient mice exhibited a weaker GvL effect than did those of wild-type mice, but possessed unaltered potential to induce GvHD (301), suggesting that strategies to augment TRAIL-dependent antitumor reactivity might allow for dissociation of GvL and GvHD (302,303).

5. PERSPECTIVE

More than 2 decades of intensive clinical and experimental research on the therapeutic effects of alloHSCT for leukemias has allowed for major advances in the understanding of the immunobiology of GvL. Clinical and experimental strategies that induce GvL while controlling for GvHD have been developed, but the dissociation is rarely complete, and GvHD as well as leukemia relapse remain major causes of post-transplant morbidity and mortality. Strategies designed to exploit the well-known anti-leukemic properties of alloreactive donor T cells are DLI and RIST protocols. Although these have been shown clinically effective in eliciting GvL effects with reduced risks for GvHD, they will need to be further refined in order to achieve optimal GvL and, ultimately, eliminate the risk for GvHD. Antigen-specific adoptive T cell therapy theoretically would allow for a complete separation of anti-leukemic effects and broad anti-host reactivity. Although haematopoietic tissue-restricted miHC antigens are interesting candidate antigens, the required specific mismatches are not always present in selected donor-recipient combinations. The major challenge in this area is the identification of additional tumor-specific antigens, and the development of procedures that are not only technically adequate to isolate and expand antigen-specific T cells, but that are also clinically safe. The potential of alloreactive NK cells in mediating anti-leukemic effects on the basis of KIR-ligand mismatches is emerging. Although these NK cell-dependent GvL effects would require specific HLA allele mismatches, ongoing investigations of the immunobiology of NK cells and NK cell receptors may allow for the discovery of additional pathways of host- or leukemia-specific NK cell reactivity. Finally, potential future approaches to influence the balance between GvL and GvH reactivity of alloHSC grafts may rely upon the recently discovered naturally occurring Treg, which have been shown to differentially influence allo- and anti-leukemic reactivity in experimental models.

6. ACKNOWLEDGEMENTS

BS, BVW and LD are doctoral fellows, and ADB a postdoctoral fellow of the Flanders Fund for Scientific Research (FWO). Belgium, SF holds a grant of the Flanders FWO Belgium.

7. REFERENCES

1. D. W. Barnes, m. J. Corp, j. F. Loutit and F. E. Neal: Treatment of murine leukaemia with X rays and homologous bone marrow; preliminary communication. *Br Med J* 32, 626-627 (1956)
2. G. MATHE, J. L. AMIEL, L. SCHWARZENBERG, A. CATTAN, M. SCHNEIDER, M. J. DEVRIES, M. TUBIANA, C. LALANNE, J. L. BINET, M. PAPIERNIK, G. Seman, M. Atsukura, A. M. MERY, V. SCHWARZMANN and A. FLAISLER: Successful allogeneic bone marrow transplantation in man: chimerism, induced specific tolerance and possible anti-leukemic effects. *Blood* 25, 179-196 (1965)
3. R. E. Billingham: The biology of graft-versus-host reactions. *Harvey Lect* 62, 21-78 (1966)
4. L. F. Odom, C. S. August, J. H. Githens, J. R. Humbert, H. Morse, D. Peakman, B. Sharma, S. L. Rusnak and F. B. Johnson: Remission of relapsed leukaemia during a graft-versus-host reaction. A "graft-versus-leukaemia reaction" in man? *Lancet* 2, 537-540 (1978)
5. P. L. Weiden, N. Flournoy, E. D. Thomas, R. Prentice, A. Fefer, C. D. Buckner and R. Storb: Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 300, 1068-1073 (1979)
6. M. M. Horowitz, R. P. Gale, P. M. Sondel, J. M. Goldman, J. Kersey, H. J. Kolb, A. A. Rimm, O. Ringden, C. Rozman and B. Speck: Graft-versus-leukemia reactions after bone marrow transplantation. *Blood* 75, 555-562 (1990)
7. S. Slavin, R. Or, E. Naparstek, A. Ackerstein and L. Weiss: Cellular-mediated immunotherapy of leukemia in conjunction with autologous and allogeneic bone marrow transplantation in experimental animals and man. *Blood* 72 (suppl 1), 407a (1988)
8. D. Porter and J. E. Levine: Graft-versus-host disease and graft-versus-leukemia after donor leukocyte infusion. *Semin Hematol* 43, 53-61 (2006)
9. S. Slavin, S. Morecki, L. Weiss, M. Y. Shapira, I. Resnick and R. Or: Nonmyeloablative stem cell transplantation: reduced-intensity conditioning for cancer immunotherapy--from bench to patient bedside. *Semin Oncol* 31, 4-21 (2004)
10. L. Feinstein and R. Storb: Nonmyeloablative hematopoietic cell transplantation. *Curr Opin Oncol* 13, 95-100 (2001)
11. A. A. Kassim, W. Chinratanalab, J. L. Ferrara and S. Mineishi: Reduced-intensity allogeneic hematopoietic stem cell transplantation for acute leukemias: 'what is the best recipe?'. *Bone Marrow Transplant* 36, 565-574 (2005)
12. S. Slavin, A. Nagler, E. Naparstek, Y. Kapelushnik, M. Aker, G. Cividalli, G. Varadi, M. Kirschbaum, A. Ackerstein, S. Samuel, A. Amar, C. Brautbar, O. Ben Tal, A. Eldor and R. Or: 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 (1998)
13. A. Shimoni, N. Kroger, T. Zabelina, F. Ayuk, I. Hardan, M. Yeshurun, N. Shem-Tov, A. Avigdor, I. Ben Bassat, A. R. Zander and A. Nagler: Hematopoietic stem-cell transplantation from unrelated donors in elderly patients (age >55 years) with hematologic malignancies: older age is no longer a contraindication when using reduced intensity conditioning. *Leukemia* 19, 7-12 (2005)
14. P. A. McSweeney, D. Niederwieser, J. A. Shizuru, B. M. Sandmaier, A. J. Molina, D. G. Maloney, T. R. Chauncey, T. A. Gooley, U. Hegenbart, R. A. Nash, J. Radich, J. L. Wagner, S. Minor, F. R. Appelbaum, W. I. Bensinger, E. Bryant, M. E. Flowers, G. E. Georges, F. C. Grumet, H. P. Kiem, B. Torok-Storb, C. Yu, K. G. Blume and R. F. Storb: Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 97, 3390-3400 (2001)
15. G. B. Vogelsang, L. Lee and D. M. Bensen-Kennedy: Pathogenesis and treatment of graft-versus-host disease after bone marrow transplant. *Annu Rev Med* 54, 29-52 (2003)
16. G. Socie, J. V. Stone, J. R. Wingard, D. Weisdorf, P. J. Henslee-Downey, C. Bredeson, J. Y. Cahn, J. R. Passweg, P. A. Rowlings, H. C. Schouten, H. J. Kolb and J. P. Klein: 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 (1999)
17. C. Cutler, S. Giri, S. Jeyapalan, D. Paniagua, A. Viswanathan and J. H. Antin: 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 (2001)
18. W. I. Bensinger, R. Clift, P. Martin, F. R. Appelbaum, T. Demirer, T. Gooley, K. Lilleby, S. Rowley, J. Sanders, R. Storb and C. D. Buckner: Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: a retrospective comparison with marrow transplantation. *Blood* 88, 2794-2800 (1996)
19. W. I. Bensinger, P. J. Martin, B. Storer, R. Clift, S. J. Forman, R. Negrin, A. Kashyap, M. E. Flowers, K. Lilleby, T. R. Chauncey, R. Storb and F. R. Appelbaum: 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 (2001)
20. D. Blaise, M. Kuentz, C. Fortanier, J. H. Bourhis, N. Milpied, L. Sutton, J. P. Jouet, M. Attal, P. Bordigoni, J. Y. Cahn, J. M. Boiron, M. P. Schuller, J. P. Moatti and M. Michallet: 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 (2000)
21. R. Powles, J. Mehta, S. Kulkarni, J. Treleaven, B. Millar, J. Marsden, V. Shepherd, A. Rowland, B. Sirohi, D. Tait, C. Horton, S. Long and S. Singhal: Allogeneic blood

and bone-marrow stem-cell transplantation in haematological malignant diseases: a randomised trial. *Lancet* 355, 1231-1237 (2000)
22. N. Schmitz, A. Bacigalupo, D. Hasenclever, A. Nagler, E. Gluckman, P. Clark, P. Bourquelot, H. Greinix, N. Frickhofen, O. Ringden, A. Zander, J. F. Apperley, C. Gorin, K. Borkett, G. Schwab, M. Goebel, N. H. Russell and A. Gratwohl: Allogeneic bone marrow transplantation vs filgrastim-mobilised peripheral blood progenitor cell transplantation in patients with early leukaemia: first results of a randomised multicentre trial of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 21, 995-1003 (1998)
23. N. Schmitz, M. Beksac, D. Hasenclever, A. Bacigalupo, T. Ruutu, A. Nagler, E. Gluckman, N. Russell, J. F. Apperley, N. C. Gorin, J. Szer, K. Bradstock, A. Buzyn, P. Clark, K. Borkett and A. Gratwohl: Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. *Blood* 100, 761-767 (2002)
24. A. C. Vigorito, W. M. Azevedo, J. F. Marques, A. M. Azevedo, K. A. Eid, F. J. Aranha, I. Lorand-Metze, G. B. Oliveira, M. E. Correa, A. R. Reis, E. C. Miranda and C. A. de Souza: A randomised, prospective comparison of allogeneic bone marrow and peripheral blood progenitor cell transplantation in the treatment of haematological malignancies. *Bone Marrow Transplant* 22, 1145-1151 (1998)
25. V. T. Ho and R. J. Soiffer: The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. *Blood* 98, 3192-3204 (2001)
26. N. A. Kernan, N. H. Collins, L. Juliano, T. Cartagena, B. Dupont and R. J. O'Reilly: Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-v-host disease. *Blood* 68, 770-773 (1986)
27. J. L. Ferrara and P. Reddy: Pathophysiology of graft-versus-host disease. *Semin Hematol* 43, 3-10 (2006)
28. R. Storb, H. J. Deeg, J. Whitehead, F. Appelbaum, P. Beatty, W. Bensinger, C. D. Buckner, R. Clift, K. Doney and V. Farewell: Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med* 314, 729-735 (1986)
29. R. A. Nash, J. H. Antin, C. Karanes, J. W. Fay, B. R. Avalos, A. M. Yeager, D. Przepiorka, S. Davies, F. B. Petersen, P. Bartels, D. Buell, W. Fitzsimmons, C. Anasetti, R. Storb and V. Ratanatharathorn: Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood* 96, 2062-2068 (2000)
30. G. Hale, M. J. Zhang, D. Bunjes, H. G. Prentice, D. Spence, M. M. Horowitz, A. J. Barrett and H. Waldmann: Improving the outcome of bone marrow transplantation by using CD52 monoclonal antibodies to prevent graft-versus-host disease and graft rejection. *Blood* 92, 4581-4590 (1998)
31. A. Bacigalupo, T. Lamparelli, P. Bruzzi, S. Guidi, P. E. Alessandrino, P. di Bartolomeo, R. Oneto, B. Bruno, M. Barbanti, N. Sacchi, M. T. Van Lint and A. Bosi: Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood* 98, 2942-2947 (2001)
32. N. Basara, S. Gunzelmann, W. Willenbacher, A. A. Fauser and M. G. Kiehl: New immunosuppressants in BMT/GVHD. *Transplant Proc* 33, 2220-2222 (2001)
33. T. Alcindor, G. Gorgun, K. B. Miller, T. F. Roberts, K. Sprague, D. P. Schenkein and F. M. Foss: Immunomodulatory effects of extracorporeal photochemotherapy in patients with extensive chronic graft-versus-host disease. *Blood* 98, 1622-1625 (2001)
34. P. A. Carpenter, F. R. Appelbaum, L. Corey, H. J. Deeg, K. Doney, T. Gooley, J. Krueger, P. Martin, S. Pavlovic, J. Sanders, J. Slattery, D. Levitt, R. Storb, A. Woolfrey and C. Anasetti: A humanized non-FcR-binding anti-CD3 antibody, visilizumab, for treatment of steroid-refractory acute graft-versus-host disease. *Blood* 99, 2712-2719 (2002)
35. H. J. Deeg, B. R. Blazar, B. J. Bolwell, G. D. Long, F. Schuening, J. Cunningham, R. M. Rifkin, S. Abhyankar, A. D. Briggs, R. Burt, J. Lipani, L. K. Roskos, J. M. White, N. Havrilla, G. Schwab and H. E. Heslop: Treatment of steroid-refractory acute graft-versus-host disease with anti-CD147 monoclonal antibody ABX-CBL. *Blood* 98, 2052-2058 (2001)
36. G. Kobbe, P. Schneider, U. Rohr, R. Fenk, F. Neumann, M. Aivado, L. Dietze, R. Kronenwett, A. Hunerliturkoglu and R. Haas: Treatment of severe steroid refractory acute graft-versus-host disease with infliximab, a chimeric human/mouse antiTNFalpha antibody. *Bone Marrow Transplant* 28, 47-49 (2001)
37. J. Margolis and G. Vogelsang: An old drug for a new disease: pentostatin (Nipent) in acute graft-versus-host disease. *Semin Oncol* 27, 72-77 (2000)
38. D. Przepiorka, N. A. Kernan, C. Ippoliti, E. B. Papadopoulos, S. Giralt, I. Khouri, J. G. Lu, J. Gajewski, A. Durett, K. Cleary, R. Champlin, B. S. Andersson and S. Light: Daclizumab, a humanized anti-interleukin-2 receptor alpha chain antibody, for treatment of acute graft-versus-host disease. *Blood* 95, 83-89 (2000)
39. K. M. Sullivan, R. P. Witherspoon, R. Storb, H. J. Deeg, S. Dahlberg, J. E. Sanders, F. R. Appelbaum, K. C. Doney, P. Weiden and C. Anasetti: Alternating-day cyclosporine and prednisone for treatment of high-risk chronic graft-v-host disease. *Blood* 72, 555-561 (1988)
40. G. B. Vogelsang: How I treat chronic graft-versus-host disease. *Blood* 97, 1196-1201 (2001)
41. G. B. Vogelsang, D. Wolff, V. Altomonte, E. Farmer, W. L. Morison, R. Corio and T. Horn: Treatment of chronic graft-versus-host disease with ultraviolet irradiation and psoralen (PUVA). *Bone Marrow Transplant* 17, 1061-1067 (1996)
42. G. B. Vogelsang, E. R. Farmer, A. D. Hess, V. Altamonte, W. E. Beschorner, D. A. Jabs, R. L. Corio, L. S. Levin, O. M. Colvin and J. R. Wingard: Thalidomide for the treatment of chronic graft-versus-host disease. *N Engl J Med* 326, 1055-1058 (1992)
43. A. L. Gilman, K. W. Chan, A. Mogul, C. Morris, F. D. Goldman, M. Boyer, E. Cirenza, A. Mazumder, E. Gehan, R. Cahill, S. Frankel and K. Schultz: Hydroxychloroquine for the treatment of chronic graft-versus-host disease. *Biol Blood Marrow Transplant* 6, 327-334 (2000)

44. D. C. Marcellus, V. L. Altomonte, E. R. Farmer, T. D. Horn, C. S. Freemer, J. Grant and G. B. Vogelsang: Etretinate therapy for refractory sclerodermatous chronic graft-versus-host disease. *Blood* 93, 66-70 (1999)
45. B. Mookerjee, V. Altomonte and G. Vogelsang: Salvage therapy for refractory chronic graft-versus-host disease with mycophenolate mofetil and tacrolimus. *Bone Marrow Transplant* 24, 517-520 (1999)
46. C. Cutler, D. Miklos, H. T. Kim, N. Treister, S. B. Woo, D. Bienfang, L. B. Klickstein, J. Levin, K. Miller, C. Reynolds, R. Macdonell, M. Pasek, S. J. Lee, V. Ho, R. Soiffer, J. H. Antin, J. Ritz and E. Alyea: Rituximab for steroid-refractory chronic graft-versus-host disease. *Blood* 108, 756-762 (2006)
47. L. Ruggeri, A. Mancusi, M. Capanni, M. F. Martelli and A. Velardi: Exploitation of alloreactive NK cells in adoptive immunotherapy of cancer. *Curr Opin Immunol* 17, 211-217 (2005)
48. M. Bleakley and S. R. Riddell: Molecules and mechanisms of the graft-versus-leukaemia effect. *Nat Rev Cancer* 4, 371-380 (2004)
49. J. M. den Haan, L. M. Meadows, W. Wang, J. Pool, E. Blokland, T. L. Bishop, C. Reinhardus, J. Shabanowitz, R. Offringa, D. F. Hunt, V. H. Engelhard and E. Goulmy: The minor histocompatibility antigen HA-1: a diallelic gene with a single amino acid polymorphism. *Science* 279, 1054-1057 (1998)
50. J. M. den Haan, N. E. Sherman, E. Blokland, E. Huczko, F. Koning, J. W. Drijfhout, J. Skipper, J. Shabanowitz, D. F. Hunt and V. H. Engelhard: Identification of a graft versus host disease-associated human minor histocompatibility antigen. *Science* 268, 1476-1480 (1995)
51. A. G. Brickner, E. H. Warren, J. A. Caldwell, Y. Akatsuka, T. N. Golovina, A. L. Zarling, J. Shabanowitz, L. C. Eisenlohr, D. F. Hunt, V. H. Engelhard and S. R. Riddell: The immunogenicity of a new human minor histocompatibility antigen results from differential antigen processing. *J Exp Med* 193, 195-206 (2001)
52. H. Dolstra, H. Fredrix, F. Maas, P. G. Coulie, F. Brasseur, E. Mensink, G. J. Adema, T. M. de Witte, C. G. Figdor and van de Wiel-van Kemenade: A human minor histocompatibility antigen specific for B cell acute lymphoblastic leukemia. *J Exp Med* 189, 301-308 (1999)
53. Y. Akatsuka, T. Nishida, E. Kondo, M. Miyazaki, H. Taji, H. Iida, K. Tsujimura, M. Yazaki, T. Naoe, Y. Morishima, Y. Kodera, K. Kuzushima and T. Takahashi: Identification of a polymorphic gene, BCL2A1, encoding two novel hematopoietic lineage-specific minor histocompatibility antigens. *J Exp Med* 197, 1489-1500 (2003)
54. M. Murata, E. H. Warren and S. R. Riddell: A human minor histocompatibility antigen resulting from differential expression due to a gene deletion. *J Exp Med* 197, 1279-1289 (2003)
55. E. H. Warren, M. A. Gavin, E. Simpson, P. Chandler, D. C. Page, C. Disteche, K. A. Stankey, P. D. Greenberg and S. R. Riddell: The human UTY gene encodes a novel HLA-B8-restricted H-Y antigen. *J Immunol* 164, 2807-2814 (2000)
56. B. D. de Rijke, A. van Horssen-Zoetbrood, J. M. Beekman, B. Otterud, F. Maas, R. Woestenenk, M. Kester, M. Leppert, A. V. Schattenberg, T. de Witte, van de Wiel-van Kemenade and H. Dolstra: A frameshift polymorphism in P2X5 elicits an allogeneic cytotoxic T lymphocyte response associated with remission of chronic myeloid leukemia. *J Clin Invest* 115, 3506-3516 (2005)
57. S. R. Riddell, M. Bleakley, T. Nishida, C. Berger and E. H. Warren: Adoptive transfer of allogeneic antigen-specific T cells. *Biol Blood Marrow Transplant* 12, 9-12 (2006)
58. E. Goulmy, R. Schipper, J. Pool, E. Blokland, J. H. Falkenburg, J. Vossen, A. Gratwohl, G. B. Vogelsang, H. C. van Houwelingen and J. J. van Rood: 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 (1996)
59. T. Mutis, G. Gillespie, E. Schrama, J. H. Falkenburg, P. Moss and E. Goulmy: 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 (1999)
60. W. A. Marijt, M. H. Heemskerk, F. M. Kloosterboer, E. Goulmy, M. G. Kester, M. A. van der Hoorn, S. A. Luxemburg-Heys, M. Hoogeboom, T. Mutis, J. W. Drijfhout, J. J. van Rood, R. Willemze and J. H. Falkenburg: 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 (2003)
61. B. Kircher, S. Stevanovic, M. Urbanek, A. Mitterschiffthaler, H. G. Rammensee, K. Grunewald, G. Gastl and D. Nachbaur: Induction of HA-1-specific cytotoxic T-cell clones parallels the therapeutic effect of donor lymphocyte infusion. *Br J Haematol* 117, 935-939 (2002)
62. A. B. Sturrock, K. F. Franklin, G. Rao, B. C. Marshall, M. B. Rebentisch, R. S. Lemons and J. R. Hoidal: Structure, chromosomal assignment, and expression of the gene for proteinase-3. The Wegener's granulomatosis autoantigen. *J Biol Chem* 267, 21193-21199 (1992)
63. N. Muller-Berat, J. Minowada, K. Tsuji-Takayama, H. Drexler, M. Lanotte, J. Wieslander and A. Wiik: The phylogeny of proteinase 3/myeloblastin, the autoantigen in Wegener's granulomatosis, and myeloperoxidase as shown by immunohistochemical studies on human leukemic cell lines. *Clin Immunol Immunopathol* 70, 51-59 (1994)
64. G. Behre, P. Zhang, D. E. Zhang and D. G. Tenen: Analysis of the modulation of transcriptional activity in myelopoiesis and leukemogenesis. *Methods* 17, 231-237 (1999)
65. P. Zhang, E. Nelson, H. S. Radomska, J. Iwasaki-Arai, K. Akashi, A. D. Friedman and D. G. Tenen: Induction of granulocytic differentiation by 2 pathways. *Blood* 99, 4406-4412 (2002)
66. C. F. Franssen, C. A. Stegeman, C. G. Kallenberg, R. O. Gans, P. E. De Jong, S. J. Hoorntje and J. W. Tervaert: Antiproteinase 3- and antimyeloperoxidase-associated vasculitis. *Kidney Int* 57, 2195-2206 (2000)
67. J. Molldrem, S. Dermime, K. Parker, Y. Z. Jiang, D. Mavroudis, N. Hensel, P. Fukushima and A. J. Barrett: Targeted T-cell therapy for human leukemia: cytotoxic T

lymphocytes specific for a peptide derived from proteinase 3 preferentially lyse human myeloid leukemia cells. *Blood* 88, 2450-2457 (1996)
68. J. J. Molldrem, E. Clave, Y. Z. Jiang, D. Mavroudis, A. Raptis, N. Hensel, V. Agarwala and A. J. Barrett: Cytotoxic T lymphocytes specific for a nonpolymorphic proteinase 3 peptide preferentially inhibit chronic myeloid leukemia colony-forming units. *Blood* 90, 2529-2534 (1997)
69. J. J. Molldrem, P. P. Lee, S. Kant, E. Wieder, W. Jiang, S. Lu, C. Wang and M. M. Davis: Chronic myelogenous leukemia shapes host immunity by selective deletion of high-avidity leukemia-specific T cells. *J Clin Invest* 111, 639-647 (2003)
70. J. J. Molldrem, P. P. Lee, C. Wang, K. Felio, H. M. Kantarjian, R. E. Champlin and M. M. Davis: Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. *Nat Med* 6, 1018-1023 (2000)
71. L. Gao, I. Bellantuono, A. Elsasser, S. B. Marley, M. Y. Gordon, J. M. Goldman and H. J. Stauss: Selective elimination of leukemic CD34(+) progenitor cells by cytotoxic T lymphocytes specific for WT1. *Blood* 95, 2198-2203 (2000)
72. A. Gaiger, V. Reese, M. L. Disis and M. A. Cheever: Immunity to WT1 in the animal model and in patients with acute myeloid leukemia. *Blood* 96, 1480-1489 (2000)
73. T. Azuma, T. Otsuki, K. Kuzushima, C. J. Froelich, S. Fujita and M. Yasukawa: Myeloma cells are highly sensitive to the granule exocytosis pathway mediated by WT1-specific cytotoxic T lymphocytes. *Clin Cancer Res* 10, 7402-7412 (2004)
74. Y. Nakahara, H. Okamoto, T. Mineta and K. Tabuchi: Expression of the Wilms' tumor gene product WT1 in glioblastomas and medulloblastomas. *Brain Tumor Pathol* 21, 113-116 (2004)
75. M. Makita, A. Hiraki, T. Azuma, A. Tsuboi, Y. Oka, H. Sugiyama, S. Fujita, M. Tanimoto, M. Harada and M. Yasukawa: Antilung cancer effect of WT1-specific cytotoxic T lymphocytes. *Clin Cancer Res* 8, 2626-2631 (2002)
76. Y. Oka, A. Tsuboi, T. Taguchi, T. Osaki, T. Kyo, H. Nakajima, O. A. Elisseeva, Y. Oji, M. Kawakami, K. Ikegame, N. Hosen, S. Yoshihara, F. Wu, F. Fujiki, M. Murakami, T. Masuda, S. Nishida, T. Shirakata, S. Nakatsuka, A. Sasaki, K. Udaka, H. Dohy, K. Aozasa, S. Noguchi, I. Kawase and H. Sugiyama: Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proc Natl Acad Sci U S A* 101, 13885-13890 (2004)
77. I. Bellantuono, L. Gao, S. Parry, S. Marley, F. Dazzi, J. Apperley, J. M. Goldman and H. J. Stauss: Two distinct HLA-A0201-presented epitopes of the Wilms tumor antigen 1 can function as targets for leukemia-reactive CTL. *Blood* 100, 3835-3837 (2002)
78. H. Ohminami, M. Yasukawa and S. Fujita: HLA class I-restricted lysis of leukemia cells by a CD8(+) cytotoxic T-lymphocyte clone specific for WT1 peptide. *Blood* 95, 286-293 (2000)
79. O. Ringden and K. Le Blanc: Allogeneic hematopoietic stem cell transplantation: state of the art and new perspectives. *APMIS* 113, 813-830 (2005)
80. S. A. Giralt and R. E. Champlin: Leukemia relapse after allogeneic bone marrow transplantation: a review. *Blood* 84, 3603-3612 (1994)
81. R. A. Clift, C. D. Buckner, F. R. Appelbaum, S. I. Bearman, F. B. Petersen, L. D. Fisher, C. Anasetti, P. Beatty, W. I. Bensinger and K. Doney: Allogeneic marrow transplantation in patients with acute myeloid leukemia in first remission: a randomized trial of two irradiation regimens. *Blood* 76, 1867-1871 (1990)
82. R. A. Clift, C. D. Buckner, F. R. Appelbaum, E. Bryant, S. I. Bearman, F. B. Petersen, L. D. Fisher, C. Anasetti, P. Beatty and W. I. Bensinger: Allogeneic marrow transplantation in patients with chronic myeloid leukemia in the chronic phase: a randomized trial of two irradiation regimens. *Blood* 77, 1660-1665 (1991)
83. K. Le Blanc, M. Remberger, M. Uzunel, J. Mattsson, L. Barkholt and O. Ringden: A comparison of nonmyeloablative and reduced-intensity conditioning for allogeneic stem-cell transplantation. *Transplantation* 78, 1014-1020 (2004)
84. G. Massenkeil, M. Nagy, M. Lawang, O. Rosen, I. Genvresse, G. Geserick, B. Dorken and R. Arnold: Reduced intensity conditioning and prophylactic DLI can cure patients with high-risk acute leukaemias if complete donor chimerism can be achieved. *Bone Marrow Transplant* 31, 339-345 (2003)
85. F. Baron, M. B. Maris, B. M. Sandmaier, B. E. Storer, M. Sorror, R. Diaconescu, A. E. Woolfrey, T. R. Chauncey, M. E. Flowers, M. Mielcarek, D. G. Maloney and R. Storb: Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol* 23, 1993-2003 (2005)
86. J. H. Antin and J. L. Ferrara: Cytokine dysregulation and acute graft-versus-host disease. *Blood* 80, 2964-2968 (1992)
87. R. Chakraverty, D. Cote, J. Buchli, P. Cotter, R. Hsu, G. Zhao, T. Sachs, C. M. Pitsillides, R. Bronson, T. Means, C. Lin and M. Sykes: An inflammatory checkpoint regulates recruitment of graft-versus-host reactive T cells to peripheral tissues. *J Exp Med* 203, 2021-2031 (2006)
88. S. Mackinnon, E. B. Papadopoulos, M. H. Carabasi, L. Reich, N. H. Collins, F. Boulad, H. Castro-Malaspina, B. H. Childs, A. P. Gillio and N. A. Kernan: 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 (1995)
89. M. de Lima, M. Bonamino, Z. Vasconcelos, M. Colares, H. Diamond, I. Zalcberg, R. Tavares, D. Lerner, R. Byington, L. Bouzas, J. da Matta, C. Andrade, L. Carvalho, V. Pires, B. Barone, C. Maciel and D. Tabak: Prophylactic donor lymphocyte infusions after moderately ablative chemotherapy and stem cell transplantation for hematological malignancies: high remission rate among poor prognosis patients at the expense of graft-versus-host disease. *Bone Marrow Transplant* 27, 73-78 (2001)
90. A. Badros, B. Barlogie, C. Morris, R. Desikan, S. R. Martin, N. Munshi, M. Zangari, J. Mehta, A. Toor, M. Cottler-Fox, A. Fassas, E. Anaissie, S. Schichman and G. Tricot: 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 (2001)
91. M. Y. Mapara, Y. M. Kim, S. P. Wang, R. Bronson, D. H. Sachs and M. Sykes: Donor lymphocyte infusions

mediate superior graft-versus-leukemia effects in mixed compared to fully allogeneic chimeras: a critical role for host antigen-presenting cells. *Blood* 100, 1903-1909 (2002)
92. A. D. Billiau, S. Fevery, O. Rutgeerts, W. Landuyt and M. Waer: Crucial role of timing of donor lymphocyte infusion in generating dissociated graft-versus-host and graft-versus-leukemia responses in mice receiving allogeneic bone marrow transplants. *Blood* 100, 1894-1902 (2002)
93. M. Uzunel, J. Mattsson, M. Brune, J. E. Johansson, J. Aschan and O. Ringden: Kinetics of minimal residual disease and chimerism in patients with chronic myeloid leukemia after nonmyeloablative conditioning and allogeneic stem cell transplantation. *Blood* 101, 469-472 (2003)
94. M. L. Sorror, M. B. Maris, B. Storer, B. M. Sandmaier, R. Diaconescu, C. Flowers, D. G. Maloney and R. Storb: 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 (2004)
95. M. Mielcarek, P. J. Martin, W. Leisenring, M. E. Flowers, D. G. Maloney, B. M. Sandmaier, M. B. Maris and R. Storb: Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood* 102, 756-762 (2003)
96. G. R. Hill, T. Teshima, A. Gerbitz, L. Pan, K. R. Cooke, Y. S. Brinson, J. M. Crawford and J. L. Ferrara: Differential roles of IL-1 and TNF-alpha on graft-versus-host disease and graft versus leukemia. *J Clin Invest* 104, 459-467 (1999)
97. J. H. Antin, H. J. Weinstein, E. C. Guinan, P. McCarthy, B. E. Bierer, D. G. Gilliland, S. K. Parsons, K. K. Ballen, I. J. Rimm and G. Falzarano: Recombinant human interleukin-1 receptor antagonist in the treatment of steroid-resistant graft-versus-host disease. *Blood* 84, 1342-1348 (1994)
98. J. H. Antin, D. Weisdorf, D. Neuberg, R. Nicklow, S. Clouthier, S. J. Lee, E. Alyea, C. McGarigle, B. R. Blazar, S. Sonis, R. J. Soiffer and J. L. Ferrara: Interleukin-1 blockade does not prevent acute graft-versus-host disease: results of a randomized, double-blind, placebo-controlled trial of interleukin-1 receptor antagonist in allogeneic bone marrow transplantation. *Blood* 100, 3479-3482 (2002)
99. E. Holler, H. J. Kolb, A. Moller, J. Kempeni, S. Liesenfeld, H. Pechumer, W. Lehmacher, G. Ruckdeschel, B. Gleixner and C. Riedner: Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. *Blood* 75, 1011-1016 (1990)
100. P. Herve, M. Flesch, P. Tiberghien, J. Wijdenes, E. Racadot, P. Bordigoni, E. Plouvier, J. L. Stephan, H. Bourdeau and E. Holler: Phase I-II trial of a monoclonal anti-tumor necrosis factor alpha antibody for the treatment of refractory severe acute graft-versus-host disease. *Blood* 79, 3362-3368 (1992)
101. D. Couriel, R. Saliba, K. Hicks, C. Ippoliti, M. de Lima, C. Hosing, I. Khouri, B. Andersson, J. Gajewski, M. Donato, P. Anderlini, D. P. Kontoyiannis, A. Cohen, T. Martin, S. Giralt and R. Champlin: Tumor necrosis factor-alpha blockade for the treatment of acute GVHD. *Blood* 104, 649-654 (2004)
102. N. Tsukada, T. Kobata, Y. Aizawa, H. Yagita and K. Okumura: Graft-versus-leukemia effect and graft-versus-host disease can be differentiated by cytotoxic mechanisms in a murine model of allogeneic bone marrow transplantation. *Blood* 93, 2738-2747 (1999)
103. C. Schmaltz, O. Alpdogan, S. J. Muriglan, B. J. Kappel, J. A. Rotolo, E. T. Ricchetti, A. S. Greenberg, L. M. Willis, G. F. Murphy, J. M. Crawford and M. R. van den Brink: Donor T cell-derived TNF is required for graft-versus-host disease and graft-versus-tumor activity after bone marrow transplantation. *Blood* 101, 2440-2445 (2003)
104. Y. G. Yang: The role of interleukin-12 and interferon-gamma in GVHD and GVL. *Cytokines Cell Mol Ther* 6, 41-46 (2000)
105. Y. G. Yang, J. Qi, M. G. Wang and M. Sykes: Donor-derived interferon gamma separates graft-versus-leukemia effects and graft-versus-host disease induced by donor CD8 T cells. *Blood* 99, 4207-4215 (2002)
106. T. Kozlowski, T. Sablinski, M. Basker, H. Kitamura, T. R. Spitzer, J. Fishman, M. Sykes, D. K. Cooper and D. H. Sachs: Decreased graft-versus-host disease after haplotype mismatched bone marrow allografts in miniature swine following interleukin-2 treatment. *Bone Marrow Transplant* 25, 47-52 (2000)
107. J. H. Antin, S. J. Lee, D. Neuberg, E. Alyea, R. J. Soiffer, S. Sonis and J. L. Ferrara: A phase I/II double-blind, placebo-controlled study of recombinant human interleukin-11 for mucositis and acute GVHD prevention in allogeneic stem cell transplantation. *Bone Marrow Transplant* 29, 373-377 (2002)
108. 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 (2005)
109. E. S. Morris, K. P. MacDonald, V. Rowe, D. H. Johnson, T. Banovic, A. D. Clouston and G. R. Hill: Donor treatment with pegylated G-CSF augments the generation of IL-10-producing regulatory T cells and promotes transplantation tolerance. *Blood* 103, 3573-3581 (2004)
110. E. S. Morris, K. P. MacDonald and G. R. Hill: Stem cell mobilization with G-CSF analogs: a rational approach to separate GVHD and GVL? *Blood* 107, 3430-3435 (2006)
111. E. S. Morris, K. P. MacDonald, V. Rowe, T. Banovic, R. D. Kuns, A. L. Don, H. M. Bofinger, A. C. Burman, S. D. Olver, N. Kienzle, S. A. Porcelli, D. G. Pellicci, D. I. Godfrey, M. J. Smyth and G. R. Hill: NKT cell-dependent leukemia eradication following stem cell mobilization with potent G-CSF analogs. *J Clin Invest* 115, 3093-3103 (2005)
112. J. F. Apperley, L. Jones, G. Hale, H. Waldmann, J. Hows, Y. Rombos, C. Tsatalas, R. E. Marcus, A. W. Goolden and E. C. Gordon-Smith: Bone marrow transplantation for patients with chronic myeloid leukaemia: T-cell depletion with Campath-1 reduces the incidence of graft-versus-host disease but may increase the risk of leukaemic relapse. *Bone Marrow Transplant* 1, 53-66 (1986)
113. S. D. Nimer, J. Giorgi, J. L. Gajewski, N. Ku, G. J. Schiller, K. Lee, M. Territo, W. Ho, S. Feig and M. Selch: Selective depletion of CD8+ cells for prevention of graft-versus-host disease after bone marrow transplantation. A

randomized controlled trial. *Transplantation* 57, 82-87 (1994)
114. R. Childs, E. Clave, N. Contentin, D. Jayasekera, N. Hensel, S. Leitman, E. J. Read, C. Carter, E. Bahceci, N. S. Young and A. J. Barrett: Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. *Blood* 94, 3234-3241 (1999)
115. E. Orsini, E. P. Alyea, A. Chillemi, R. Schlossman, S. McLaughlin, C. Canning, R. J. Soiffer, K. C. Anderson and J. Ritz: Conversion to full donor chimerism following donor lymphocyte infusion is associated with disease response in patients with multiple myeloma. *Biol Blood Marrow Transplant* 6, 375-386 (2000)
116. W. D. Shlomchik, M. S. Couzens, C. B. Tang, J. McNiff, M. E. Robert, J. Liu, M. J. Shlomchik and S. G. Emerson: Prevention of graft versus host disease by inactivation of host antigen-presenting cells. *Science* 285, 412-415 (1999)
117. T. Teshima, R. Ordemann, P. Reddy, S. Gagin, C. Liu, K. R. Cooke and J. L. Ferrara: Acute graft-versus-host disease does not require alloantigen expression on host epithelium. *Nat Med* 8, 575-581 (2002)
118. C. C. Matte, J. Liu, J. Cormier, B. E. Anderson, I. Athanasiadis, D. Jain, J. McNiff and W. D. Shlomchik: Donor APCs are required for maximal GVHD but not for GVL. *Nat Med* 10, 987-992 (2004)
119. M. Merad, P. Hoffmann, E. Ranheim, S. Slaymaker, M. G. Manz, S. A. Lira, I. Charo, D. N. Cook, I. L. Weissman, S. Strober and E. G. Engleman: Depletion of host Langerhans cells before transplantation of donor alloreactive T cells prevents skin graft-versus-host disease. *Nat Med* 10, 510-517 (2004)
120. P. Reddy, Y. Maeda, C. Liu, O. I. Krijanovski, R. Korngold and J. L. Ferrara: A crucial role for antigen-presenting cells and alloantigen expression in graft-versus-leukemia responses. *Nat Med* 11, 1244-1249 (2005)
121. A. Choudhury, J. L. Gajewski, J. C. Liang, U. Popat, D. F. Claxton, K. O. Kliche, M. Andreeff and R. E. Champlin: Use of leukemic dendritic cells for the generation of antileukemic cellular cytotoxicity against Philadelphia chromosome-positive chronic myelogenous leukemia. *Blood* 89, 1133-1142 (1997)
122. B. D. Harrison, J. A. Adams, M. Briggs, M. L. Brereton and J. A. Yin: Stimulation of autologous proliferative and cytotoxic T-cell responses by "leukemic dendritic cells" derived from blast cells in acute myeloid leukemia. *Blood* 97, 2764-2771 (2001)
123. L. D. Anderson, Jr., S. Mori, S. Mann, C. A. Savary and C. A. Mullen: Pretransplant tumor antigen-specific immunization of allogeneic bone marrow transplant donors enhances graft-versus-tumor activity without exacerbation of graft-versus-host disease. *Cancer Res* 60, 5797-5802 (2000)
124. J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran and K. Palucka: Immunobiology of dendritic cells. *Annu Rev Immunol* 18, 767-811 (2000)
125. K. Sato, N. Yamashita, N. Yamashita, M. Baba and T. Matsuyama: Regulatory dendritic cells protect mice from murine acute graft-versus-host disease and leukemia relapse. *Immunity* 18, 367-379 (2003)
126. K. Sato, T. Nakaoka, N. Yamashita, H. Yagita, H. Kawasaki, C. Morimoto, M. Baba and T. Matsuyama: TRAIL-transduced dendritic cells protect mice from acute graft-versus-host disease and leukemia relapse. *J Immunol* 174, 4025-4033 (2005)
127. J. P. Radich, J. E. Sanders, C. D. Buckner, P. J. Martin, F. B. Petersen, W. Bensinger, G. B. McDonald, M. Mori, G. Schoch and J. A. Hansen: Second allogeneic marrow transplantation for patients with recurrent leukemia after initial transplant with total-body irradiation-containing regimens. *J Clin Oncol* 11, 304-313 (1993)
128. D. L. Porter, M. S. Roth, C. McGarigle, J. L. Ferrara and J. H. Antin: Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. *N Engl J Med* 330, 100-106 (1994)
129. W. R. Drobyski, C. A. Keever, M. S. Roth, S. Koethe, G. Hanson, P. McFadden, J. L. Gottschall, R. C. Ash, P. van Tuinen and M. M. Horowitz: Salvage immunotherapy using donor leukocyte infusions as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: efficacy and toxicity of a defined T-cell dose. *Blood* 82, 2310-2318 (1993)
130. S. Mackinnon, J. M. Hows and J. M. Goldman: Induction of *in vitro* graft-versus-leukemia activity following bone marrow transplantation for chronic myeloid leukemia. *Blood* 76, 2037-2045 (1990)
131. R. H. Collins, Jr., O. Shpilberg, W. R. Drobyski, D. L. Porter, S. Giralt, R. Champlin, S. A. Goodman, S. N. Wolff, W. Hu, C. Verfaillie, A. List, W. Dalton, N. Ognoskie, A. Chetrit, J. H. Antin and J. Nemunaitis: Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol* 15, 433-444 (1997)
132. F. Dazzi, R. M. Szydlo, N. C. Cross, C. Craddock, J. Kaeda, E. Kanfer, K. Cwynarski, E. Olavarria, A. Yong, J. F. Apperley and J. M. Goldman: Durability of responses following donor lymphocyte infusions for patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. *Blood* 96, 2712-2716 (2000)
133. H. J. Kolb, J. Mittermuller, C. Clemm, E. Holler, G. Ledderose, G. Brehm, M. Heim and W. Wilmanns: Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 76, 2462-2465 (1990)
134. H. J. Kolb, A. Schattenberg, J. M. Goldman, B. Hertenstein, N. Jacobsen, W. Arcese, P. Ljungman, A. Ferrant, L. Verdonck, D. Niederwieser, F. van Rhee, J. Mittermueller, T. de Witte, E. Holler and H. Ansari: Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood* 86, 2041-2050 (1995)
135. W. R. Drobyski, M. S. Roth, S. N. Thibodeau and J. L. Gottschall: Molecular remission occurring after donor leukocyte infusions for the treatment of relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 10, 301-304 (1992)
136. E. Naparstek, R., A. Nagler, G. Cividalli, D. Engelhard, M. Aker, Z. Gimon, N. Manny, T. Sacks and Z. Tochner: T-cell-depleted allogeneic bone marrow transplantation for acute leukaemia using Campath-1 antibodies and post-transplant administration of donor's

peripheral blood lymphocytes for prevention of relapse. *Br J Haematol* 89, 506-515 (1995)
137. R. H. Collins, Jr., S. Goldstein, S. Giralt, J. Levine, D. Porter, W. Drobyski, J. Barrett, M. Johnson, A. Kirk, M. Horowitz and P. Parker: Donor leukocyte infusions in acute lymphocytic leukemia. *Bone Marrow Transplant* 26, 511-516 (2000)
138. J. E. Levine, T. Braun, S. L. Penza, P. Beatty, K. Cornetta, R. Martino, W. R. Drobyski, A. J. Barrett, D. L. Porter, S. Giralt, J. Leis, H. E. Holmes, M. Johnson, M. Horowitz and R. H. Collins, Jr.: Prospective trial of chemotherapy and donor leukocyte infusions for relapse of advanced myeloid malignancies after allogeneic stem-cell transplantation. *J Clin Oncol* 20, 405-412 (2002)
139. K. Matsue, T. Tabayashi, K. Yamada and M. Takeuchi: Eradication of residual bcr-abl-positive clones by inducing graft-versus-host disease after allogeneic stem cell transplantation in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Bone Marrow Transplant* 29, 63-66 (2002)
140. R. Zeiser, H. Bertz, A. Spyridonidis, L. Houet and J. Finke: Donor lymphocyte infusions for multiple myeloma: clinical results and novel perspectives. *Bone Marrow Transplant* 34, 923-928 (2004)
141. R. Champlin, I. Khouri, A. Shimoni, J. Gajewski, S. Kornblau, J. Molldrem, N. Ueno, S. Giralt and P. Anderlini: Harnessing graft-versus-malignancy: non-myeloablative preparative regimens for allogeneic haematopoietic transplantation, an evolving strategy for adoptive immunotherapy. *Br J Haematol* 111, 18-29 (2000)
142. E. P. Alyea, C. Canning, D. Neuberg, H. Daley, H. Houde, S. Giralt, R. Champlin, K. Atkinson and R. J. Soiffer: CD8+ cell depletion of donor lymphocyte infusions using cd8 monoclonal antibody-coated high-density microparticles (CD8-HDM) after allogeneic hematopoietic stem cell transplantation: a pilot study. *Bone Marrow Transplant* 34, 123-128 (2004)
143. A. Whiteway, T. Corbett, R. Anderson, I. Macdonald and H. G. Prentice: Expression of co-stimulatory molecules on acute myeloid leukaemia blasts may effect duration of first remission. *Br J Haematol* 120, 442-451 (2003)
144. L. H. Sehn, E. P. Alyea, E. Weller, C. Canning, S. Lee, J. Ritz, J. H. Antin and R. J. Soiffer: Comparative outcomes of T-cell-depleted and non-T-cell-depleted allogeneic bone marrow transplantation for chronic myelogenous leukemia: impact of donor lymphocyte infusion. *J Clin Oncol* 17, 561-568 (1999)
145. D. I. Marks, J. M. Bird, K. Vettenranta, L. Hunt, A. Green, J. M. Cornish, N. Goulden, D. H. Pamphilon, C. G. Steward and A. Oakhill: T cell-depleted unrelated donor bone marrow transplantation for acute myeloid leukemia. *Biol Blood Marrow Transplant* 6, 646-653 (2000)
146. F. Keil, O. A. Haas, G. Fritsch, P. Kalhs, K. Lechner, C. Mannhalter, E. Reiter, D. Niederwieser, P. Hoecker and H. T. Greinix: Donor leukocyte infusion for leukemic relapse after allogeneic marrow transplantation: lack of residual donor hematopoiesis predicts aplasia. *Blood* 89, 3113-3117 (1997)
147. B. R. Blazar, C. J. Lees, P. J. Martin, R. J. Noelle, B. Kwon, W. Murphy and P. A. Taylor: Host T cells resist graft-versus-host disease mediated by donor leukocyte infusions. *J Immunol* 165, 4901-4909 (2000)
148. M. R. Pelot, D. A. Pearson, K. Swenson, G. Zhao, J. Sachs, Y. G. Yang and M. Sykes: 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 (1999)
149. H. Sefrioui, A. D. Billiau and M. Waer: Graft-versus-leukemia effect in minor antigen mismatched chimeras given delayed donor leucocyte infusion: immunoregulatory aspects and role of donor T and ASGM1-positive cells. *Transplantation* 70, 348-353 (2000)
150. B. D. Johnson and R. L. Truitt: Delayed infusion of immunocompetent donor cells after bone marrow transplantation breaks graft-host tolerance allows for persistent antileukemic reactivity without severe graft-versus-host disease. *Blood* 85, 3302-3312 (1995)
151. K. S. Peggs, K. Thomson, D. P. Hart, J. Geary, E. C. Morris, K. Yong, A. H. Goldstone, D. C. Linch and S. Mackinnon: Dose-escalated donor lymphocyte infusions following reduced intensity transplantation: toxicity, chimerism, and disease responses. *Blood* 103, 1548-1556 (2004)
152. W. A. Bethge, U. Hegenbart, M. J. Stuart, B. E. Storer, M. B. Maris, M. E. Flowers, D. G. Maloney, T. Chauncey, B. Bruno, E. Agura, S. J. Forman, K. G. Blume, D. Niederwieser, R. Storb and B. M. Sandmaier: Adoptive immunotherapy with donor lymphocyte infusions after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. *Blood* 103, 790-795 (2004)
153. A. M. Raiola, M. T. Van Lint, M. Valbonesi, T. Lamparelli, F. Gualandi, D. Occhini, S. Bregante, C. di Grazia, A. Dominietto, M. Soracco, C. Romagnani, F. Vassallo, M. Casini, B. Bruno, F. Frassoni and A. Bacigalupo: Factors predicting response and graft-versus-host disease after donor lymphocyte infusions: a study on 593 infusions. *Bone Marrow Transplant* 31, 687-693 (2003)
154. S. Shiobara, S. Nakao, M. Ueda, H. Yamazaki, S. Takahashi, S. Asano, H. Yabe, S. Kato, S. Imoto, A. Maruta, T. Yoshida, H. Gondo, Y. Morishima and Y. Kodera: Donor leukocyte infusion for Japanese patients with relapsed leukemia after allogeneic bone marrow transplantation: indications and dose escalation. *Ther Apher* 5, 40-45 (2001)
155. N. Novitzky, R. Rubinstein, J. M. Hallett, C. E. du Toit and V. L. Thomas: Bone marrow transplantation depleted of T cells followed by repletion with incremental doses of donor lymphocytes for relapsing patients with chronic myeloid leukemia: a therapeutic strategy. *Transplantation* 69, 1358-1363 (2000)
156. D. Rondelli, G. Bandini, M. Cavo, F. Re, M. Motta, B. Senese, G. Leopardi, M. Stanzani and S. Tura: Discrepancy between serological complete remission and concomitant new bone lytic lesions after infusion of escalating low doses of donor lymphocytes in multiple myeloma: a case report. *Bone Marrow Transplant* 24, 685-687 (1999)
157. H. M. Lokhorst, A. Schattenberg, J. J. Cornelissen, L. L. Thomas and L. F. Verdonck: Donor leukocyte infusions are effective in relapsed multiple myeloma after allogeneic bone marrow transplantation. *Blood* 90, 4206-4211 (1997)
158. A. Bacigalupo, M. Soracco, F. Vassallo, M. Abate, M. T. Van Lint, F. Gualandi, T. Lamparelli, D. Occhini, N.

Mordini, S. Bregante, O. Figari, F. Benvenuto, M. Sessarego, G. Fugazza, P. Carlier and M. Valbonesi: Donor lymphocyte infusions (DLI) in patients with chronic myeloid leukemia following allogeneic bone marrow transplantation. *Bone Marrow Transplant* 19, 927-932 (1997)
159. B. R. Dey, S. McAfee, C. Colby, R. Sackstein, S. Saidman, N. Tarbell, D. H. Sachs, M. Sykes and T. R. Spitzer: 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 (2003)
160. P. Bader, H. Kreyenberg, W. Hoelle, G. Dueckers, R. Handgretinger, P. Lang, B. Kremens, D. Dilloo, K. W. Sykora, M. Schrappe, C. Niemeyer, A. Von Stackelberg, B. Gruhn, G. Henze, J. Greil, D. Niethammer, K. Dietz, J. F. Beck and T. Klingebiel: Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukemia after allogeneic stem-cell transplantation: possible role for pre-emptive immunotherapy? *J Clin Oncol* 22, 1696-1705 (2004)
161. P. Bader, H. Kreyenberg, W. Hoelle, G. Dueckers, B. Kremens, D. Dilloo, K. W. Sykora, C. Niemeyer, D. Reinhardt, J. Vormoor, B. Gruhn, P. Lang, J. Greil, R. Handgretinger, D. Niethammer, T. Klingebiel and J. F. Beck: Increasing mixed chimerism defines a high-risk group of childhood acute myelogenous leukemia patients after allogeneic stem cell transplantation where pre-emptive immunotherapy may be effective. *Bone Marrow Transplant* 33, 815-821 (2004)
162. D. I. Marks, R. Lush, J. Cavenagh, D. W. Milligan, S. Schey, A. Parker, F. J. Clark, L. Hunt, J. Yin, S. Fuller, E. Vandenberghe, J. Marsh, T. Littlewood, G. M. Smith, D. Culligan, A. Hunter, R. Chopra, A. Davies, K. Towlson and C. D. Williams: The toxicity and efficacy of donor lymphocyte infusions given after reduced-intensity conditioning allogeneic stem cell transplantation. *Blood* 100, 3108-3114 (2002)
163. D. I. Marks, A. Parker and S. P. Robinson: Donor lymphocyte infusions after reduced intensity conditioning allogeneic transplantation: what we need to know. *Blood* 104, 295-296 (2004)
164. F. Dazzi, R. M. Szydlo, C. Craddock, N. C. Cross, J. Kaeda, A. Chase, E. Olavarria, F. van Rhee, E. Kanfer, J. F. Apperley and J. M. Goldman: Comparison of single-dose and escalating-dose regimens of donor lymphocyte infusion for relapse after allografting for chronic myeloid leukemia. *Blood* 95, 67-71 (2000)
165. R. L. Truitt and A. A. Atasoylu: Contribution of CD4+ and CD8+ T cells to graft-versus-host disease and graft-versus-leukemia reactivity after transplantation of MHC-compatible bone marrow. *Bone Marrow Transplant* 8, 51-58 (1991)
166. J. P. OKunewick, D. L. Kociban, L. L. Machen and M. J. Buffo: The role of CD4 and CD8 T cells in the graft-versus-leukemia response in Rauscher murine leukemia. *Bone Marrow Transplant* 8, 445-452 (1991)
167. B. D. Johnson, E. E. Becker and R. L. Truitt: Graft-vs.-host and graft-vs.-leukemia reactions after delayed infusions of donor T-subsets. *Biol Blood Marrow Transplant* 5, 123-132 (1999)
168. D. van der Harst, E. Goulmy, J. H. Falkenburg, Y. M. Kooij-Winkelaar, S. A. Luxemburg-Heijs, H. M. Goselink and A. Brand: Recognition of minor histocompatibility antigens on lymphocytic and myeloid leukemic cells by cytotoxic T-cell clones. *Blood* 83, 1060-1066 (1994)
169. Y. Z. Jiang and A. J. Barrett: Cellular and cytokine-mediated effects of CD4-positive lymphocyte lines generated *in vitro* against chronic myelogenous leukemia. *Exp Hematol* 23, 1167-1172 (1995)
170. Y. Z. Jiang, D. Mavroudis, S. Dermime, N. Hensel, D. Couriel, J. Molldrem and A. J. Barrett: Alloreactive CD4+ T lymphocytes can exert cytotoxicity to chronic myeloid leukaemia cells processing and presenting exogenous antigen. *Br J Haematol* 93, 606-612 (1996)
171. D. L. Porter and J. H. Antin: The graft-versus-leukemia effects of allogeneic cell therapy. *Annu Rev Med* 50, 369-386 (1999)
172. L. M. Faber, S. A. Luxemburg-Heijs, W. F. Veenhof, R. Willemze and J. H. Falkenburg: Generation of CD4+ cytotoxic T-lymphocyte clones from a patient with severe graft-versus-host disease after allogeneic bone marrow transplantation: implications for graft-versus-leukemia reactivity. *Blood* 86, 2821-2828 (1995)
173. E. P. Alyea, R. J. Soiffer, C. Canning, D. Neuberg, R. Schlossman, C. Pickett, H. Collins, Y. Wang, K. C. Anderson and J. Ritz: Toxicity and efficacy of defined doses of CD4(+) donor lymphocytes for treatment of relapse after allogeneic bone marrow transplant. *Blood* 91, 3671-3680 (1998)
174. R. Champlin, W. Ho, J. Gajewski, S. Feig, M. Burnison, G. Holley, P. Greenberg, K. Lee, I. Schmid and J. Giorgi: Selective depletion of CD8+ T lymphocytes for prevention of graft-versus-host disease after allogeneic bone marrow transplantation. *Blood* 76, 418-423 (1990)
175. A. Shimoni, J. A. Gajewski, M. Donato, T. Martin, S. O'Brien, M. Talpaz, A. Cohen, M. Korbling, R. Champlin and S. Giralt: Long-Term follow-up of recipients of CD8-depleted donor lymphocyte infusions for the treatment of chronic myelogenous leukemia relapsing after allogeneic progenitor cell transplantation. *Biol Blood Marrow Transplant* 7, 568-575 (2001)
176. F. Baron, J. Siquet, N. Schaaf-Lafontaine, E. Baudoux, J. P. Hermanne, G. Fillet and Y. Beguin: Pre-emptive immunotherapy with CD8-depleted donor lymphocytes after CD34-selected allogeneic peripheral blood stem cell transplantation. *Haematologica* 87, 78-88 (2002)
177. M. Zeis, L. Uharek, G. Hartung, B. Glass, J. Steinmann and N. Schmitz: Graft-vs-leukemia activity and graft-vs-host disease induced by allogeneic Th1- and Th2-type CD4+ T cells in mice. *Hematol J* 2, 136-144 (2001)
178. D. H. Fowler and R. E. Gress: Th2 and Tc2 cells in the regulation of GVHD, GVL, and graft rejection: considerations for the allogeneic transplantation therapy of leukemia and lymphoma. *Leuk Lymphoma* 38, 221-234 (2000)
179. D. Montagna, E. Yvon, V. Calcaterra, P. Comoli, F. Locatelli, R. Maccario, A. Fisher and M. Cavazzana-Calvo: Depletion of alloreactive T cells by a specific anti-interleukin-2 receptor p55 chain immunotoxin does not

impair *in vitro* antileukemia and antiviral activity. *Blood* 93, 3550-3557 (1999)
180. J. Michalek, R. H. Collins and E. S. Vitetta: Clinical-scale selective depletion of alloreactive T cells using an anti-CD25 immunotoxin. *Neoplasma* 50, 296-299 (2003)
181. M. Cavazzana-Calvo, C. Fromont, F. Le Deist, M. Lusardi, L. Coulombel, J. M. Derocq, I. Gerota, C. Griscelli and A. Fischer: Specific elimination of alloreactive T cells by an anti-interleukin-2 receptor B chain-specific immunotoxin. *Transplantation* 50, 1-7 (1990)
182. M. Cavazzana-Calvo, J. L. Stephan, S. Sarnacki, S. Chevret, C. Fromont, C. de Coene, F. Le Deist, D. Guy-Grand and A. Fischer: Attenuation of graft-versus-host disease and graft rejection by *ex vivo* immunotoxin elimination of alloreactive T cells in an H-2 haplotype disparate mouse combination. *Blood* 83, 288-298 (1994)
183. S. R. Solomon, S. Mielke, B. N. Savani, A. Montero, L. Wisch, R. Childs, N. Hensel, J. Schindler, V. Ghetie, S. F. Leitman, T. Mai, C. S. Carter, R. Kurlander, E. J. Read, E. S. Vitetta and A. J. Barrett: Selective depletion of alloreactive donor lymphocytes: a novel method to reduce the severity of graft-versus-host disease in older patients undergoing matched sibling donor stem cell transplantation. *Blood* 106, 1123-1129 (2005)
184. I. Andre-Schmutz, F. Le Deist, S. Hacein-Bey, Y. Hamel, E. Vitetta, J. Schindler, A. Fischer and M. Cavazzana-Calvo: Donor T lymphocyte infusion following *ex vivo* depletion of donor anti-host reactivity by a specific anti-interleukin-2 receptor P55 chain immunotoxin. *Transplant Proc* 34, 2927-2928 (2002)
185. P. J. Amrolia, G. Mucioli-Casadei, H. Huls, H. E. Heslop, J. Schindler, P. Veys, E. S. Vitetta and M. K. Brenner: Add-back of allodepleted donor T cells to improve immune reconstitution after haplo-identical stem cell transplantation. *Cytotherapy* 7, 116-125 (2005)
186. P. J. Amrolia, G. Muccioli-Casadei, E. Yvon, H. Huls, U. Sili, E. D. Wieder, C. Bollard, J. Michalek, V. Ghetie, H. E. Heslop, J. J. Molldrem, C. M. Rooney, J. Schlinder, E. Vitetta and M. K. Brenner: Selective depletion of donor alloreactive T cells without loss of antiviral or antileukemic responses. *Blood* 102, 2292-2299 (2003)
187. P. J. Amrolia, G. Muccioli-Casadei, H. Huls, S. Adams, A. Durett, A. Gee, E. Yvon, H. Weiss, M. Cobbold, H. B. Gaspar, C. Rooney, I. Kuehnle, V. Ghetie, J. Schindler, R. Krance, H. E. Heslop, P. Veys, E. Vitetta and M. K. Brenner: Adoptive immunotherapy with allodepleted donor T-cells improves immune reconstitution after haploidentical stem cell transplant. *Blood* ., (2006)
188. S. L. Martins, L. S. St John, R. E. Champlin, E. D. Wieder, J. McMannis, J. J. Molldrem and K. V. Komanduri: Functional assessment and specific depletion of alloreactive human T cells using flow cytometry. *Blood* 104, 3429-3436 (2004)
189. M. Guimond, A. Balassy, M. Barrette, S. Brochu, C. Perreault and D. C. Roy: P-glycoprotein targeting: a unique strategy to selectively eliminate immunoreactive T cells. *Blood* 100, 375-382 (2002)
190. W. R. Godfrey, M. R. Krampf, P. A. Taylor and B. R. Blazar: *Ex vivo* depletion of alloreactive cells based on CFSE dye dilution, activation antigen selection, and dendritic cell stimulation. *Blood* 103, 1158-1165 (2004)
191. J. Michalek, R. H. Collins, H. P. Durrani, P. Vaclavkova, L. E. Ruff, D. C. Douek and E. S. Vitetta: Definitive separation of graft-versus-leukemia- and graft-versus-host-specific CD4+ T cells by virtue of their receptor beta loci sequences. *Proc Natl Acad Sci U S A* 100, 1180-1184 (2003)
192. E. Bachar-Lustig, S. Reich-Zeliger and Y. Reisner: Anti-third-party veto CTLs overcome rejection of hematopoietic allografts: synergism with rapamycin and BM cell dose. *Blood* 102, 1943-1950 (2003)
193. F. D. Arditti, S. Aviner, B. Dekel, R. Krauthgamer, J. Gan, A. Nagler, A. Tabilio, M. Martelli, A. Berrebi and Y. Reisner: Eradication of B-CLL by autologous and allogeneic host nonreactive anti-third-party CTLs. *Blood* 105, 3365-3371 (2005)
194. B. E. Anderson, J. McNiff, J. Yan, H. Doyle, M. Mamula, M. J. Shlomchik and W. D. Shlomchik: Memory CD4+ T cells do not induce graft-versus-host disease. *J Clin Invest* 112, 101-108 (2003)
195. B. J. Chen, X. Cui, C. Liu and N. J. Chao: Prevention of graft-versus-host disease while preserving graft-versus-leukemia effect after selective depletion of host-reactive T cells by photodynamic cell purging process. *Blood* 99, 3083-3088 (2002)
196. Y. Zhang, G. Joe, E. Hexner, J. Zhu and S. G. Emerson: Alloreactive memory T cells are responsible for the persistence of graft-versus-host disease. *J Immunol* 174, 3051-3058 (2005)
197. W. R. Drobyski, M. Gendelman, S. Vodanovic-Jankovic and J. Gorski: Elimination of leukemia in the absence of lethal graft-versus-host disease after allogenic bone marrow transplantation. *J Immunol* 170, 3046-3053 (2003)
198. C. Bonini, G. Ferrari, S. Verzeletti, P. Servida, E. Zappone, L. Ruggieri, M. Ponzoni, S. Rossini, F. Mavilio, C. Traversari and C. Bordignon: HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. *Science* 276, 1719-1724 (1997)
199. E. Litvinova, S. Maury, O. Boyer, S. Bruel, L. Benard, G. Boisserie, D. Klatzmann and J. L. Cohen: Graft-versus-leukemia effect after suicide-gene-mediated control of graft-versus-host disease. *Blood* 100, 2020-2025 (2002)
200. P. Hari, B. Logan and W. R. Drobyski: Temporal discordance between graft-versus-leukemia and graft-versus-host responses: a strategy for the separation of graft-versus-leukemia/graft-versus-host reactivity? *Biol Blood Marrow Transplant* 10, 743-747 (2004)
201. P. Tiberghien, C. Ferrand, B. Lioure, N. Milpied, R. Angonin, E. Deconinck, J. M. Certoux, E. Robinet, P. Saas, B. Petracca, C. Juttner, C. W. Reynolds, D. L. Longo, P. Herve and J. Y. Cahn: Administration of herpes simplex-thymidine kinase-expressing donor T cells with a T-cell-depleted allogeneic marrow graft. *Blood* 97, 63-72 (2001)
202. S. Verzeletti, C. Bonini, S. Marktel, N. Nobili, F. Ciceri, C. Traversari and C. Bordignon: Herpes simplex virus thymidine kinase gene transfer for controlled graft-versus-host disease and graft-versus-leukemia: clinical follow-up and improved new vectors. *Hum Gene Ther* 9, 2243-2251 (1998)
203. C. Berger, M. E. Flowers, E. H. Warren and S. R. Riddell: Analysis of transgene-specific immune responses that limit the *in vivo* persistence of adoptively transferred

HSV-TK-modified donor T cells after allogeneic hematopoietic cell transplantation. *Blood* 107, 2294-2302 (2006)
204. D. C. Thomis, S. Marktel, C. Bonini, C. Traversari, M. Gilman, C. Bordignon and T. Clackson: A Fas-based suicide switch in human T cells for the treatment of graft-versus-host disease. *Blood* 97, 1249-1257 (2001)
205. K. Junker, U. Koehl, S. Zimmerman, S. Stein, D. Schwabe, T. Klingebiel and M. Grez: Kinetics of cell death in T lymphocytes genetically modified with two novel suicide fusion genes. *Gene Ther* 10, 1189-1197 (2003)
206. F. Carlotti, A. Zaldumbide, P. Martin, K. E. Boulukos, R. C. Hoeben and P. Pognonec: Development of an inducible suicide gene system based on human caspase 8. *Cancer Gene Ther* 12, 627-639 (2005)
207. P. Moss and A. Rickinson: Cellular immunotherapy for viral infection after HSC transplantation. *Nat Rev Immunol* 5, 9-20 (2005)
208. F. M. Kloosterboer, S. A. Luxemburg-Heijs, R. A. van Soest, A. M. Barbui, H. M. van Egmond, M. P. Strijbosch, M. G. Kester, W. A. Marijt, E. Goulmy, R. Willemze and J. H. Falkenburg: Direct cloning of leukemia-reactive T cells from patients treated with donor lymphocyte infusion shows a relative dominance of hematopoiesis-restricted minor histocompatibility antigen HA-1 and HA-2 specific T cells. *Leukemia* 18, 798-808 (2004)
209. P. Fontaine, G. Roy-Proulx, L. Knafo, C. Baron, D. C. Roy and C. Perreault: Adoptive transfer of minor histocompatibility antigen-specific T lymphocytes eradicates leukemia cells without causing graft-versus-host disease. *Nat Med* 7, 789-794 (2001)
210. R. E. Clark, I. A. Dodi, S. C. Hill, J. R. Lill, G. Aubert, A. R. Macintyre, J. Rojas, A. Bourdon, P. L. Bonner, L. Wang, S. E. Christmas, P. J. Travers, C. S. Creaser, R. C. Rees and J. A. Madrigal: Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. *Blood* 98, 2887-2893 (2001)
211. K. Cathcart, J. Pinilla-Ibarz, T. Korontsvit, J. Schwartz, V. Zakhaleva, E. B. Papadopoulos and D. A. Scheinberg: A multivalent bcr-abl fusion peptide vaccination trial in patients with chronic myeloid leukemia. *Blood* 103, 1037-1042 (2004)
212. P. Yotnda, H. Firat, F. Garcia-Pons, Z. Garcia, G. Gourru, J. P. Vernant, F. A. Lemonnier, V. Leblond and P. Langlade-Demoyen: Cytotoxic T cell response against the chimeric p210 BCR-ABL protein in patients with chronic myelogenous leukemia. *J Clin Invest* 101, 2290-2296 (1998)
213. E. Zorn, E. Orsini, C. J. Wu, B. Stein, A. Chillemi, C. Canning, E. P. Alyea, R. J. Soiffer and J. Ritz: A CD4+ T cell clone selected from a CML patient after donor lymphocyte infusion recognizes BCR-ABL breakpoint peptides but not tumor cells. *Transplantation* 71, 1131-1137 (2001)
214. J. L. Schultze, A. A. Cardoso, G. J. Freeman, M. J. Seamon, J. Daley, G. S. Pinkus, J. G. Gribben and L. M. Nadler: Follicular lymphomas can be induced to present alloantigen efficiently: a conceptual model to improve their tumor immunogenicity. *Proc Natl Acad Sci U S A* 92, 8200-8204 (1995)
215. R. Buhmann, A. Nolte, D. Westhaus, B. Emmerich and M. Hallek: CD40-activated B-cell chronic lymphocytic leukemia cells for tumor immunotherapy: stimulation of allogeneic versus autologous T cells generates different types of effector cells. *Blood* 93, 1992-2002 (1999)
216. S. A. Xue, L. Gao, D. Hart, R. Gillmore, W. Qasim, A. Thrasher, J. Apperley, B. Engels, W. Uckert, E. Morris and H. Stauss: Elimination of human leukemia cells in NOD/SCID mice by WT1-TCR gene-transduced human T cells. *Blood* 106, 3062-3067 (2005)
217. S. Hacein-Bey-Abina, C. von Kalle, M. Schmidt, M. P. McCormack, N. Wulffraat, P. Leboulch, A. Lim, C. S. Osborne, R. Pawliuk, E. Morillon, R. Sorensen, A. Forster, P. Fraser, J. I. Cohen, B. G. de Saint, I. Alexander, U. Wintergerst, T. Frebourg, A. Aurias, D. Stoppa-Lyonnet, S. Romana, I. Radford-Weiss, F. Gross, F. Valensi, E. Delabesse, E. Macintyre, F. Sigaux, J. Soulier, L. E. Leiva, M. Wissler, C. Prinz, T. H. Rabbitts, F. Le Deist, A. Fischer and M. Cavazzana-Calvo: LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 302, 415-419 (2003)
218. C. Bonini, M. Grez, C. Traversari, F. Ciceri, S. Marktel, G. Ferrari, M. Dinauer, M. Sadat, A. Aiuti, S. Deola, M. Radrizzani, A. Hagenbeek, J. Apperley, S. Ebeling, A. Martens, H. J. Kolb, M. Weber, F. Lotti, A. Grande, E. Weissinger, J. A. Bueren, M. Lamana, J. H. Falkenburg, M. H. Heemskerk, T. Austin, S. Kornblau, F. Marini, C. Benati, Z. Magnani, S. Cazzaniga, S. Toma, C. Gallo-Stampino, M. Introna, S. Slavin, P. D. Greenberg, M. Bregni, F. Mavilio and C. Bordignon: Safety of retroviral gene marking with a truncated NGF receptor. *Nat Med* 9, 367-369 (2003)
219. B. R. Dey, S. McAfee, C. Colby, K. Cieply, M. Caron, S. Saidman, F. Preffer, J. Shaffer, N. Tarbell, R. Sackstein, D. Sachs, M. Sykes and T. R. Spitzer: 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 (2005)
220. M. T. Rubio, Y. M. Kim, T. Sachs, M. Mapara, G. Zhao and M. Sykes: Antitumor effect of donor marrow graft rejection induced by recipient leukocyte infusions in mixed chimeras prepared with nonmyeloablative conditioning: critical role for recipient-derived IFN-gamma. *Blood* 102, 2300-2307 (2003)
221. M. T. Rubio, T. I. Saito, K. Kattleman, G. Zhao, J. Buchli and M. Sykes: Mechanisms of the antitumor responses and host-versus-graft reactions induced by recipient leukocyte infusions in mixed chimeras prepared with nonmyeloablative conditioning: a critical role for recipient CD4+ T cells and recipient leukocyte infusion-derived IFN-gamma-producing CD8+ T cells. *J Immunol* 175, 665-676 (2005)
222. V. Kumar and M. E. McNerney: A new self: MHC-class-I-independent natural-killer-cell self-tolerance. *Nat Rev Immunol* 5, 363-374 (2005)
223. P. Parham and K. L. McQueen: Alloreactive killer cells: hindrance and help for haematopoietic transplants. *Nat Rev Immunol* 3, 108-122 (2003)
224. H. G. Ljunggren and K. Karre: In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today* 11, 237-244 (1990)

225. M. Zijlstra, H. Auchincloss, Jr., J. M. Loring, C. M. Chase, P. S. Russell and R. Jaenisch: Skin graft rejection by beta 2-microglobulin-deficient mice. *J Exp Med* 175, 885-893 (1992)
226. M. Vales-Gomez, H. T. Reyburn, M. Mandelboim and J. L. Strominger: Kinetics of interaction of HLA-C ligands with natural killer cell inhibitory receptors. *Immunity* 9, 337-344 (1998)
227. L. L. Lanier: Natural killer cell receptor signaling. *Curr Opin Immunol* 15, 308-314 (2003)
228. D. Pende, C. Cantoni, P. Rivera, M. Vitale, R. Castriconi, S. Marcenaro, M. Nanni, R. Biassoni, C. Bottino, A. Moretta and L. Moretta: Role of NKG2D in tumor cell lysis mediated by human NK cells: cooperation with natural cytotoxicity receptors and capability of recognizing tumors of nonepithelial origin. *Eur J Immunol* 31, 1076-1086 (2001)
229. D. Cosman, J. Mullberg, C. L. Sutherland, W. Chin, R. Armitage, W. Fanslow, M. Kubin and N. J. Chalupny: ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* 14, 123-133 (2001)
230. V. Groh, S. Bahram, S. Bauer, A. Herman, M. Beauchamp and T. Spies: Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc Natl Acad Sci U S A* 93, 12445-12450 (1996)
231. M. Vitale, C. Bottino, S. Sivori, L. Sanseverino, R. Castriconi, E. Marcenaro, R. Augugliaro, L. Moretta and A. Moretta: NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis. *J Exp Med* 187, 2065-2072 (1998)
232. S. Sivori, M. Vitale, L. Morelli, L. Sanseverino, R. Augugliaro, C. Bottino, L. Moretta and A. Moretta: p46, a novel natural killer cell-specific surface molecule that mediates cell activation. *J Exp Med* 186, 1129-1136 (1997)
233. C. Cantoni, C. Bottino, M. Vitale, A. Pessino, R. Augugliaro, A. Malaspina, S. Parolini, L. Moretta, A. Moretta and R. Biassoni: NKp44, a triggering receptor involved in tumor cell lysis by activated human natural killer cells, is a novel member of the immunoglobulin superfamily. *J Exp Med* 189, 787-796 (1999)
234. A. Pessino, S. Sivori, C. Bottino, A. Malaspina, L. Morelli, L. Moretta, R. Biassoni and A. Moretta: Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. *J Exp Med* 188, 953-960 (1998)
235. D. Pende, S. Parolini, A. Pessino, S. Sivori, R. Augugliaro, L. Morelli, E. Marcenaro, L. Accame, A. Malaspina, R. Biassoni, C. Bottino, L. Moretta and A. Moretta: Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. *J Exp Med* 190, 1505-1516 (1999)
236. F. Aversa, A. Terenzi, A. Tabilio, F. Falzetti, A. Carotti, S. Ballanti, R. Felicini, F. Falcinelli, A. Velardi, L. Ruggeri, T. Aloisi, J. P. Saab, A. Santucci, K. Perruccio, M. P. Martelli, C. Mecucci, Y. Reisner and M. F. Martelli: Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. *J Clin Oncol* 23, 3447-3454 (2005)
237. F. Aversa, A. Tabilio, A. Terenzi, A. Velardi, F. Falzetti, C. Giannoni, R. Iacucci, T. Zei, M. P. Martelli, C. Gambelunghe and .: Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. *Blood* 84, 3948-3955 (1994)
238. F. Aversa, A. Tabilio, A. Velardi, I. Cunningham, A. Terenzi, F. Falzetti, L. Ruggeri, G. Barbabietola, C. Aristei, P. Latini, Y. Reisner and M. F. Martelli: Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *N Engl J Med* 339, 1186-1193 (1998)
239. Y. Reisner, E. Bachar-Lustig, H. W. Li, F. Aversa, A. Velardi and M. F. Martelli: The role of megadose CD34+ progenitor cells in the treatment of leukemia patients without a matched donor and in tolerance induction for organ transplantation. *Ann N Y Acad Sci* 872, 336-348 (1999)
240. L. Ruggeri, M. Capanni, E. Urbani, K. Perruccio, W. D. Shlomchik, A. Tosti, S. Posati, D. Rogaia, F. Frassoni, F. Aversa, M. F. Martelli and A. Velardi: Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 295, 2097-2100 (2002)
241. S. Giebel, F. Locatelli, T. Lamparelli, A. Velardi, S. Davies, G. Frumento, R. Maccario, F. Bonetti, J. Wojnar, M. Martinetti, F. Frassoni, G. Giorgiani, A. Bacigalupo and J. Holowiecki: Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. *Blood* 102, 814-819 (2003)
242. D. W. Beelen, H. D. Ottinger, S. Ferencik, A. H. Elmaagacli, R. Peceny, R. Trenschel and H. Grosse-Wilde: Genotypic inhibitory killer immunoglobulin-like receptor ligand incompatibility enhances the long-term antileukemic effect of unmodified allogeneic hematopoietic stem cell transplantation in patients with myeloid leukemias. *Blood* 105, 2594-2600 (2005)
243. A. Romanski, G. Bug, S. Becker, M. Kampfmann, E. Seifried, D. Hoelzer, O. G. Ottmann and T. Tonn: Mechanisms of resistance to natural killer cell-mediated cytotoxicity in acute lymphoblastic leukemia. *Exp Hematol* 33, 344-352 (2005)
244. M. Bornhauser, R. Schwerdtfeger, H. Martin, K. H. Frank, C. Theuser and G. Ehninger: Role of KIR ligand incompatibility in hematopoietic stem cell transplantation using unrelated donors. *Blood* 103, 2860-2861 (2004)
245. F. H. Claas and M. Oudshoorn: Role of NK cells in mismatched unrelated haematopoietic stem-cell transplantation: fact or fiction? *Tissue Antigens* 65, 515-518 (2005)
246. S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh and M. Toda: 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 (1995)

247. S. Sakaguchi: Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 6, 345-352 (2005)
248. K. J. Wood and S. Sakaguchi: Regulatory T cells in transplantation tolerance. *Nat Rev Immunol* 3, 199-210 (2003)
249. J. L. Cohen, A. Trenado, D. Vasey, D. Klatzmann and B. L. Salomon: CD4(+)CD25(+) immunoregulatory T Cells: new therapeutics for graft-versus-host disease. *J Exp Med* 196, 401-406 (2002)
250. P. A. Taylor, C. J. Lees and B. R. Blazar: The infusion of *ex vivo* activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. *Blood* 99, 3493-3499 (2002)
251. P. Hoffmann, J. Ermann, M. Edinger, C. G. Fathman and S. Strober: Donor-type CD4(+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. *J Exp Med* 196, 389-399 (2002)
252. A. Trenado, F. Charlotte, S. Fisson, M. Yagello, D. Klatzmann, B. L. Salomon and J. L. Cohen: 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 (2003)
253. S. C. Jones, G. F. Murphy and R. Korngold: Post-hematopoietic cell transplantation control of graft-versus-host disease by donor CD425 T cells to allow an effective graft-versus-leukemia response. *Biol Blood Marrow Transplant* 9, 243-256 (2003)
254. Q. Tang, K. J. Henriksen, M. Bi, E. B. Finger, G. Szot, J. Ye, E. L. Masteller, H. McDevitt, M. Bonyhadi and J. A. Bluestone: *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J Exp Med* 199, 1455-1465 (2004)
255. P. Hoffmann, R. Eder, L. A. Kunz-Schughart, R. Andreesen and M. Edinger: Large-scale *in vitro* expansion of polyclonal human CD4(+)CD25high regulatory T cells. *Blood* 104, 895-903 (2004)
256. O. Annacker, R. Pimenta-Araujo, O. Burlen-Defranoux, T. C. Barbosa, A. Cumano and A. Bandeira: CD25+ CD4+ T cells regulate the expansion of peripheral CD4 T cells through the production of IL-10. *J Immunol* 166, 3008-3018 (2001)
257. S. Slavin, Z. Fuks, H. S. Kaplan and S. Strober: Transplantation of allogeneic bone marrow without graft-versus-host disease using total lymphoid irradiation. *J Exp Med* 147, 963-972 (1978)
258. A. Salam and M. Waer: Graft-versus-host reactivity and graft-versus-leukemia effect in murine allogeneic bone marrow chimeras conditioned with total body irradiation or total lymphoid irradiation. *Transplantation* 61, 826-830 (1996)
259. D. Zeng, D. Lewis, S. Dejbakhsh-Jones, F. Lan, M. Garcia-Ojeda, R. Sibley and S. Strober: Bone marrow NK1.1(-) and NK1.1(+) T cells reciprocally regulate acute graft versus host disease. *J Exp Med* 189, 1073-1081 (1999)
260. F. Lan, D. Zeng, M. Higuchi, J. P. Higgins and S. Strober: Host conditioning with total lymphoid irradiation and antithymocyte globulin prevents graft-versus-host disease: the role of CD1-reactive natural killer T cells. *Biol Blood Marrow Transplant* 9, 355-363 (2003)
261. M. Kuwatani, Y. Ikarashi, A. Iizuka, C. Kawakami, G. Quinn, Y. Heike, M. Yoshida, M. Asaka, Y. Takaue and H. Wakasugi: Modulation of acute graft-versus-host disease and chimerism after adoptive transfer of *in vitro*-expanded invariant Valpha14 natural killer T cells. *Immunol Lett* 106, 82-90 (2006)
262. D. Hashimoto, S. Asakura, S. Miyake, T. Yamamura, L. Van Kaer, C. Liu, M. Tanimoto and T. Teshima: Stimulation of host NKT cells by synthetic glycolipid regulates acute graft-versus-host disease by inducing Th2 polarization of donor T cells. *J Immunol* 174, 551-556 (2005)
263. Y. Ilan, M. Ohana, O. Pappo, M. Margalit, G. Lalazar, D. Engelhardt, E. Rabbani and A. Nagler: Alleviation of acute and chronic graft-versus-host disease in a murine model is associated with glucocerebroside-enhanced natural killer T lymphocyte plasticity. *Transplantation* 83, 458-467 (2007)
264. R. Lowsky, T. Takahashi, Y. P. Liu, S. Dejbakhsh-Jones, F. C. Grumet, J. A. Shizuru, G. G. Laport, K. E. Stockerl-Goldstein, L. J. Johnston, R. T. Hoppe, D. A. Bloch, K. G. Blume, R. S. Negrin and S. Strober: Protective conditioning for acute graft-versus-host disease. *N Engl J Med* 353, 1321-1331 (2005)
265. Z. X. Zhang, L. Yang, K. J. Young, B. DuTemple and L. Zhang: Identification of a previously unknown antigen-specific regulatory T cell and its mechanism of suppression. *Nat Med* 6, 782-789 (2000)
266. K. J. Young, L. S. Kay, M. J. Phillips and L. Zhang: Antitumor activity mediated by double-negative T cells. *Cancer Res* 63, 8014-8021 (2003)
267. H. Sefrioui, A. D. Billiau, L. Overbergh, O. Rutgeerts and M. Waer: Influence of the additional injection of host-type bone marrow on the immune tolerance of minor antigen-mismatched chimeras: possible involvement of double-negative (natural killer) T cells. *Transplantation* 68, 1560-1567 (1999)
268. V. Bronte, E. Apolloni, A. Cabrelle, R. Ronca, P. Serafini, P. Zamboni, N. P. Restifo and P. Zanovello: Identification of a CD11b(+)/Gr-1(+)/CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. *Blood* 96, 3838-3846 (2000)
269. B. Almand, J. I. Clark, E. Nikitina, J. van Beynen, N. R. English, S. C. Knight, D. P. Carbone and D. I. Gabrilovich: Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J Immunol* 166, 678-689 (2001)
270. D. I. Gabrilovich, M. P. Velders, E. M. Sotomayor and W. M. Kast: Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. *J Immunol* 166, 5398-5406 (2001)
271. D. Gabrilovich: Mechanisms and functional significance of tumour-induced dendritic-cell defects. *Nat Rev Immunol* 4, 941-952 (2004)
272. V. Bronte and P. Zanovello: Regulation of immune responses by L-arginine metabolism. *Nat Rev Immunol* 5, 641-654 (2005)
273. A. D. Billiau, S. Fevery, O. Rutgeerts, W. Landuyt and M. Waer: Transient expansion of Mac1+Ly6-G+Ly6-

C+ early myeloid cells with suppressor activity in spleens of murine radiation marrow chimeras: possible implications for the graft-versus-host and graft-versus-leukemia reactivity of donor lymphocyte infusions. *Blood* 102, 740-748 (2003)
274. I. Rasmusson, O. Ringden, B. Sundberg and K. Le Blanc: Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. *Exp Cell Res* 305, 33-41 (2005)
275. W. T. Tse, J. D. Pendleton, W. M. Beyer, M. C. Egalka and E. C. Guinan: Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 75, 389-397 (2003)
276. K. Le Blanc and O. Ringden: Mesenchymal stem cells: properties and role in clinical bone marrow transplantation. *Curr Opin Immunol* 18, 586-591 (2006)
277. O. Ringden, M. Uzunel, I. Rasmusson, M. Remberger, B. Sundberg, H. Lonnies, H. U. Marschall, A. Dlugosz, A. Szakos, Z. Hassan, B. Omazic, J. Aschan, L. Barkholt and K. Le Blanc: Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation* 81, 1390-1397 (2006)
278. K. R. Cooke, A. Gerbitz, J. M. Crawford, T. Teshima, G. R. Hill, A. Tesolin, D. P. Rossignol and J. L. Ferrara: LPS antagonism reduces graft-versus-host disease and preserves graft-versus-leukemia activity after experimental bone marrow transplantation. *J Clin Invest* 107, 1581-1589 (2001)
279. P. Reddy, Y. Maeda, K. Hotary, C. Liu, L. L. Reznikov, C. A. Dinarello and J. L. Ferrara: Histone deacetylase inhibitor suberoylanilide hydroxamic acid reduces acute graft-versus-host disease and preserves graft-versus-leukemia effect. *Proc Natl Acad Sci U S A* 101, 3921-3926 (2004)
280. O. I. Krijanovski, G. R. Hill, K. R. Cooke, T. Teshima, J. M. Crawford, Y. S. Brinson and J. L. Ferrara: Keratinocyte growth factor separates graft-versus-leukemia effects from graft-versus-host disease. *Blood* 94, 825-831 (1999)
281. K. Hattori, T. Hirano, K. Oshimi, H. Yagita and K. Okumura: A metalloproteinase inhibitor prevents acute graft-versus-host disease while preserving the graft-versus-leukaemia effect of allogeneic bone marrow transplantation. *Leuk Lymphoma* 38, 553-561 (2000)
282. K. Sun, L. A. Welniak, A. Panoskaltsis-Mortari, M. J. O'Shaughnessy, H. Liu, I. Barao, W. Riordan, R. Sitcheran, C. Wysocki, J. S. Serody, B. R. Blazar, T. J. Sayers and W. J. Murphy: Inhibition of acute graft-versus-host disease with retention of graft-versus-tumor effects by the proteasome inhibitor bortezomib. *Proc Natl Acad Sci U S A* 101, 8120-8125 (2004)
283. K. Sun, D. E. Wilkins, M. R. Anver, T. J. Sayers, A. Panoskaltsis-Mortari, B. R. Blazar, L. A. Welniak and W. J. Murphy: Differential effects of proteasome inhibition by bortezomib on murine acute graft-versus-host disease (GVHD): delayed administration of bortezomib results in increased GVHD-dependent gastrointestinal toxicity. *Blood* 106, 3293-3299 (2005)
284. M. H. Hsieh, G. Varadi, N. Flomenberg and R. Korngold: Leucyl-leucine methyl ester-treated haploidentical donor lymphocyte infusions can mediate graft-versus-leukemia activity with minimal graft-versus-host disease risk. *Biol Blood Marrow Transplant* 8, 303-315 (2002)
285. T. Imado, T. Iwasaki, T. Kuroiwa, H. Sano and H. Hara: Effect of FK506 on donor T-cell functions that are responsible for graft-versus-host disease and graft-versus-leukemia effect. *Transplantation* 77, 391-398 (2004)
286. B. R. Blazar, P. A. Taylor, A. Panoskaltsis-Mortari and D. A. Vallera: Rapamycin inhibits the generation of graft-versus-host disease- and graft-versus-leukemia-causing T cells by interfering with the production of Th1 or Th1 cytotoxic cytokines. *J Immunol* 160, 5355-5365 (1998)
287. L. Weiss, S. Reich and S. Slavin: Effect of deoxyspergualin on graft-versus-host disease and graft-versus-leukemia in mice. *Bone Marrow Transplant* 17, 789-792 (1996)
288. M. Y. Shapira, E. Hirshfeld, L. Weiss, M. Zeira, J. Kasir, R. Or, I. B. Resnick and S. Slavin: Mycophenolate mofetil does not suppress the graft-versus-leukemia effect or the activity of lymphokine-activated killer (LAK) cells in a murine model. *Cancer Immunol Immunother* 54, 383-388 (2005)
289. Y. M. Kim, T. Sachs, W. Asavaroengchai, R. Bronson and M. Sykes: Graft-versus-host disease can be separated from graft-versus-lymphoma effects by control of lymphocyte trafficking with FTY720. *J Clin Invest* 111, 659-669 (2003)
290. R. S. Lee, C. S. Kuhr, G. E. Sale, E. Zellmer, W. J. Hogan, R. Storb and M. T. Little: FTY720 does not abrogate acute graft-versus-host disease in the dog leukocyte antigen-nonidentical unrelated canine model. *Transplantation* 76, 1155-1158 (2003)
291. F. M. Uckun, B. A. Roers, B. Waurzyniak, X. P. Liu and M. Cetkovic-Cvrlje: Janus kinase 3 inhibitor WHI-P131/JANEX-1 prevents graft-versus-host disease but spares the graft-versus-leukemia function of the bone marrow allografts in a murine bone marrow transplantation model. *Blood* 99, 4192-4199 (2002)
292. J. Ohata, J. Sakurai, K. Saito, K. Tani, S. Asano and M. Azuma: Differential graft-versus-leukaemia effect by CD28 and CD40 co-stimulatory blockade after graft-versus-host disease prophylaxis. *Clin Exp Immunol* 129, 61-68 (2002)
293. B. R. Blazar, P. A. Taylor, M. W. Boyer, A. Panoskaltsis-Mortari, J. P. Allison and D. A. Vallera: CD28/B7 interactions are required for sustaining the graft-versus-leukemia effect of delayed post-bone marrow transplantation splenocyte infusion in murine recipients of myeloid or lymphoid leukemia cells. *J Immunol* 159, 3460-3473 (1997)
294. E. C. Guinan, V. A. Boussiotis, D. Neuberg, L. L. Brennan, N. Hirano, L. M. Nadler and J. G. Gribben: Transplantation of anergic histoincompatible bone marrow allografts. *N Engl J Med* 340, 1704-1714 (1999)
295. F. S. Hodi, M. C. Mihm, R. J. Soiffer, F. G. Haluska, M. Butler, M. V. Seiden, T. Davis, R. Henry-Spires, S. MacRae, A. Willman, R. Padera, M. T. Jaklitsch, S. Shankar, T. C. Chen, A. Korman, J. P. Allison and G. Dranoff: Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc Natl Acad Sci U S A* 100, 4712-4717 (2003)

296. A. Ribas, L. H. Camacho, G. Lopez-Berestein, D. Pavlov, C. A. Bulanhagui, R. Millham, B. Comin-Anduix, J. M. Reuben, E. Seja, C. A. Parker, A. Sharma, J. A. Glaspy and J. Gomez-Navarro: Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206. *J Clin Oncol* 23, 8968-8977 (2005)
297. K. Sanderson, R. Scotland, P. Lee, D. Liu, S. Groshen, J. Snively, S. Sian, G. Nichol, T. Davis, T. Keler, M. Yellin and J. Weber: Autoimmunity in a phase I trial of a fully human anti-cytotoxic T-lymphocyte antigen-4 monoclonal antibody with multiple melanoma peptides and Montanide ISA 51 for patients with resected stages III and IV melanoma. *J Clin Oncol* 23, 741-750 (2005)
298. P. Attia, G. Q. Phan, A. V. Maker, M. R. Robinson, M. M. Quezado, J. C. Yang, R. M. Sherry, S. L. Topalian, U. S. Kammula, R. E. Royal, N. P. Restifo, L. R. Haworth, C. Levy, S. A. Mavroukakis, G. Nichol, M. J. Yellin and S. A. Rosenberg: Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. *J Clin Oncol* 23, 6043-6053 (2005)
299. B. R. Blazar, P. A. Taylor, A. Panoskaltsis-Mortari, A. H. Sharpe and D. A. Vallera: Opposing roles of CD28:B7 and CTLA-4:B7 pathways in regulating *in vivo* alloresponses in murine recipients of MHC disparate T cells. *J Immunol* 162, 6368-6377 (1999)
300. B. R. Blazar, B. S. Kwon, A. Panoskaltsis-Mortari, K. B. Kwak, J. J. Peschon and P. A. Taylor: Ligation of 4-1BB (CDw137) regulates graft-versus-host disease, graft-versus-leukemia, and graft rejection in allogeneic bone marrow transplant recipients. *J Immunol* 166, 3174-3183 (2001)
301. C. Schmaltz, O. Alpdogan, B. J. Kappel, S. J. Muriglan, J. A. Rotolo, J. Ongchin, L. M. Willis, A. S. Greenberg, J. M. Eng, J. M. Crawford, G. F. Murphy, H. Yagita, H. Walczak, J. J. Peschon and M. R. van den Brink: T cells require TRAIL for optimal graft-versus-tumor activity. *Nat Med* 8, 1433-1437 (2002)
302. C. Hao, J. H. Song, B. Hsi, J. Lewis, D. K. Song, K. C. Petruk, D. L. Tyrrell and N. M. Kneteman: TRAIL inhibits tumor growth but is nontoxic to human hepatocytes in chimeric mice. *Cancer Res* 64, 8502-8506 (2004)
303. R. L. Vanoosten, J. M. Moore, A. T. Ludwig and T. S. Griffith: Depsipeptide (FR901228) enhances the cytotoxic activity of TRAIL by redistributing TRAIL receptor to membrane lipid rafts. *Mol Ther* 11, 542-552 (2005)

Key Words: Graft-versus-leukemia, Graft-Versus-Host Disease, Allogeneic Hematopoietic Stem Cell Transplantation, Review

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