Non-myeloablative Stem-Cell Transplantation in the Treatment of Malignant and Non-Malignant Disorders

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Allogeneic stem-cell transplantation is an effective, potentially curative treatment for advanced or high risk hematologic malignancies, as well as for other malignant and non-malignant disorders [1,2]. However, high dose chemoradiotherapy with allogeneic SCT is associated with significant morbidity and mortality due to the toxicity of the preparative regimen, graft-versus-host disease, and the immunodeficient state that accompanies the procedure. Extensive research has been directed towards the development of safer and less toxic approaches to allogeneic transplantation. In recent years it has become apparent that much of the benefit of allogeneic SCT in the treatment of malignant disorders is mediated by an immune-mediated graft-versus-tumor effect [3–6]. The non-myeloablative stem-cell transplantation approach was developed as a means to exploit the GVT effect to cure malignancies by eliminating the need for hazardous and toxic conditioning. NST is an attractive less toxic approach to allow engraftment of donor stem cells to correct genetic and non-malignant disorders, and new indications for allogeneic SCT are continuously being explored. In this review we discuss the rationale for the NST approach and the currently available NST regimens. We report the results from pioneering clinical trials investigating NST in different malignancies and in non-malignant disorders.

How does allogeneic SCT cure malignant and non-malignant disorders?

SCT was initially developed as a means to deliver high dose chemotherapy and radiation for elimination of the underlying disorder [1,2]. Escalation of treatment doses results in better tumor kill but leads to irreversible myelosuppression. SCT was viewed as a supportive-care modality to restore hematopoiesis after treatment. In malignant disorders, the conditioning regimen is directed at elimination of the malignancy and also provides immunosuppression to allow acceptance of donor stem cells and the immune system. In non-malignant disorders, such as bone marrow failure states, phenotypic correction of the hematologic or immune deficiency is achieved by providing new healthy hematopoietic cells. The conditioning regimen allows engraftment and elimination of the pathologic host marrow.

However, it has subsequently become apparent that high dose chemoradiotherapy does not eradicate the underlying malignant disease in many patients and that much of the therapeutic benefit of SCT relates to an associated immune-mediated graft-versus-leukemia or graft-versus-tumor effect [3–6]. Extensive experimental and clinical data support the presence of a GVL effect [3–6]. GVL was documented in animal models as well as in human clinical transplantation. A higher risk of relapse occurs after T cell-depleted or syngeneic transplants. Patients with acute and/or chronic GVHD have a reduced risk of relapse, suggesting a relationship between GVL and GVHD. Elimination of residual disease as detected by cytogenetics or polymerase chain reaction techniques in more indolent malignancies such as chronic myelogenous leukemia may take 6 to 12 months after transplant, presumably due to an ongoing GVL effect. Withdrawal of immunosuppression given for prevention of GVHD can occasionally lead to restoration of remission in patients relapsing after transplant. The most direct evidence of GVL is the observation that infusion of donor lymphocytes can re-induce remission in patients who relapse after allogeneic transplantation [7,8]. This has been most effective against CML. Up to 80% of CML patients relapsing into chronic phase achieve a complete cytogenetic remission after DLI, with the best results reported in early cytogenetic relapse. Acute myelogenous leukemia and myelodysplasia are also subject to GVL; about one-third of the patients respond to DLI but remissions are generally transient. GVT has also been shown in multiple myeloma, chronic lymphocytic leukemia, low grade lymphoma, and some solid tumors.

There are a number of potential antigenic targets for the GVL/GVT effect. GVL may reflect immune reactivity against broadly expressed major and minor histocompatibility antigens, similar to targets of GVHD, which explains the association between these two allogeneic responses. Many patients will achieve an anti-leukemic response to DLI without developing GVHD. Residual benign host hematopoiesis is also often eliminated concomitantly with elimina-

SCT = stem-cell transplantation
GVT = graft-versus-tumor effect
NST = non-myeloablative stem-cell transplantation

GVL = graft versus leukemia
GVHD = graft-versus-host disease
CML = chronic myelogenous leukemia
DLI = donor lymphocyte infusion

tion of residual disease, suggesting that minor histocompatibility antigens restricted to the hematopoietic tissues are involved. This effect has been referred to as the graft-versus-hematopoietic tissue effect. Finally, tumor-specific antigens, or antigens that are over-expressed or abnormally expressed on malignant tissues, may be involved. Molldrem et al. [9] have demonstrated that PR1, a peptide derived from proteinase 3 and presented by HLA A2, is a potential target for CML-specific T cells. There was a strong correlation between the presence of PR1-specific T cells and clinical responses after allogeneic SCT. The existence of such antigens in other malignancies is controversial, but these are the ideal targets for developing specific immunotherapy and extensive research is directed to identify such targets.

Rationale for NST

High dose chemoradiotherapy with allogeneic SCT is associated with significant morbidity and mortality due to the toxicity of the preparative regimen, GVHD, and the immunodeficient state that accompanies the procedure. The risk of regimen-related toxicity and GVHD increases with advanced age, limiting standard SCT to younger patients who are in good general condition. Extensive research has been directed towards the development of safer and less toxic approaches to allogeneic transplantation. The discovery of the curative potential of the immune-mediated GVL/GVT effect has led to a novel therapeutic approach. Low dose, relatively non-toxic and tolerable conditioning regimens have been designed – not to eradicate the malignancy but to provide sufficient immunosuppression to achieve engraftment and to allow induction of GVL as the primary treatment [3,4,6]. It is not necessary to physically eliminate the "last tumor cell" by supra-lethal doses of chemotherapy and radiation in order to cure malignancies. The main indication for SCT in this approach is to enable engraftment of donor stem cells and to allow the administration of cellular immunotherapy as the curative approach.

NST does not eliminate all host hematopoiesis and commonly leads to a state of mixed chimerism [10]. Chimerism describes the presence of donor hematopoietic and lymphoid cells in a transplant recipient. Full donor or complete chimerism is defined when all hematopoietic and lymphoid cells in the transplant recipient derive from the donor. Mixed chimerism describes the persistence of donor cells with either benign host hematopoietic cells and/or cells of the underlying malignancy [Figure 1]. Stable long-lived mixed chimerism has been reported in animal models and in patients receiving NST for non-malignant disorders. However, in patients with malignancies, mixed chimerism is a dynamic process that is most often transient, and the conversion to complete chimerism, autologous reconstitution with disappearance of donor cells, or relapse occur either spontaneously or following immune manipulations within the first few months following NST [6,11–13].

The initial non-myeloablative treatment is expected to produce only transient suppression of the underlying malignancy, but it allows time for the immune GVT effect to develop. Patients with benign mixed chimerism or with detectable residual malignancy post-NST may respond to additional immunotherapeutic approaches. Immunosuppressive therapy given post-transplant for prevention of GVHD can also suppress the GVL effect. Early withdrawal of immunosuppressive therapy allows the occurrence of a potent graft-versus-hematopoietic tissue effect, which can potentially eliminate residual disease and host hematopoiesis producing complete chimerism [Figure 1] [10,13,14]. If this does not occur DLI may harness this effect and switch the balance towards complete chimerism. Sometimes a second non-myeloablative or standard transplant may be required. As reported in animal models, this potent graft-versus-hematopoietic tissue effect can occur in the absence of GVHD.

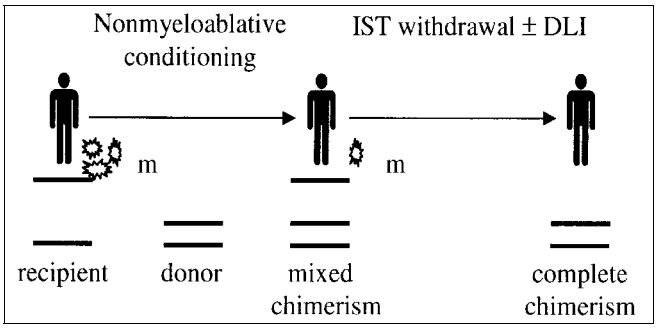

Figure 1. NST program: The initial NST regimen induces mixed chimerism with persistence of both donor and recipient hematopoietic cells. The underlying malignancy (m) is suppressed but not completely eliminated. In the second phase, immune-therapeutic interventions – e.g., withdrawal of immunosuppressive therapy (IST) supplemented if necessary by DLI – induce graft-versus-hematopoietic tissue and graft-versus-tumor effects, eliminating recipient hematopoiesis and the underlying malignancy and converting to complete chimerism.

NST regimens were originally designed to enable treatment of older patients and patients with co-morbidities that preclude standard ablative conditioning. Many hematologic malignancies are more common and have a worse prognosis in the elderly. NST may thus allow the application of a curative therapeutic approach to a much wider patient population. NST regimens are now being explored as ways to reduce toxicity even in younger patients who are eligible for standard ablative therapy, although the indications and the long-term outcome still need to be defined. NST is an attractive approach for patients requiring high risk transplants, such as transplantation of unrelated donor grafts or second transplants in heavily pretreated patients. Although this approach needs to be explored in a larger group of patients, we expect it to become standard therapy in these settings where ablative therapy may have unacceptable toxicity.

NST is a very attractive approach to explore in non-malignant disorders. High dose chemoradiotherapy is unnecessary, and the risks of a standard ablative transplant are unacceptable in this setting. The experience with NST in animal models and patients with malignant disorders has shown that myeloablative conditioning is not required for engraftment of donor cells. Donor marrow can create its own space within the marrow cavity with the aid of the graft-versus-hematopoietic tissue effect, which can occur subclinically in the absence of overt GVHD [15]. In bone marrow failure states, hemoglobinopathies, immune and metabolic states, mixed chimerism may achieve phenotypic correction of the

deficiency [16]. Thus, complete elimination of host hematopoiesis is unnecessary, and even partial replacement of host defective marrow by donor stem cells may suffice. Further elimination of host cells can be achieved, if necessary, with immune manipulations such as DLI [17]. In autoimmune disorders the goal is elimination of host immunity against autoantigens and not necessarily myeloablation. The newly educated immune system, either autologous or allogeneic, may be reset to not recognize these autoantigens.

NST may have additional advantages. In contrast to standard ablative transplants, following NST, autologous reconstitution will generally occur in patients who reject the graft. GVHD is one of the major causes of post-transplant morbidity and mortality. Animal models have shown that the state of mixed chimerism is associated with bilateral transplantation tolerance with graft acceptance and no GVHD [18]. Transplant recipients in these models experience durable engraftment of donor marrow, as well as tolerance of skin or perfused organ allografts from the same donor. The Hadassah University Hospital group has shown that the balance between host and donor in mixed chimera is mediated by veto effects operative in both directions [19]. Host veto cells, particularly $CD8^+$ cells, that survive the NST regimen tolerize allogeneic T cells and prevent GVHD. The opposite phenomenon occurs when donor T cells facilitate engraftment. Mega-doses of stem cells may have similar tolerizing effects [20]. Other mechanisms for bilateral tolerance in the NST regimen may occur. Sykes and co-workers [21] used a regimen based on profound T cell depletion of the host and the graft. Newly formed T lymphocytes acquired central tolerance to host and donor antigens present in the thymus [21]. Interestingly, delayed DLI in this model induced complete lympohematopoietic chimerism with no GVHD. Tolerance could also be induced in this model by using co-stimulation blockade with no need for T cell depletion or any other immunosuppression [22]. Acute GVHD results at least partially from tissue injury and cytokine release secondary to the toxicity of the preparative regimen, amplified by donor immune cells. Use of less toxic conditioning should theoretically limit tissue injury and cytokine release and reduce the incidence and severity of GVHD [23]. Delayed immune manipulations after tissue injury resolve as NST programs are less likely to produce severe GVHD [7]. Theoretically, NST may not completely ablate host immunity and allows at least partial protection from certain infections.

Table 1. NST regimens

	Investigator group [ref]	Indications
F/Bu/ATG	Hadassah, Jerusalem [25]	Hematologic malignancies, non-malignant disorders
FM	MD Anderson, Houston [22]	Hematologic malignancies (mostly myeloid leukemia)
FC	MD Anderson, Houston [28]	Indolent lymphoid malignancies
FC	NCI, Bethesda [14]	Hematologic malignancies, solid tumors
FC (after prior autografting)	Genoa [30]	Hematologic malignancies, solid tumors
Flag/Ida	MD Anderson, Houston [26]	Myeloid malignancies
Low-dose TBI ± F	Seattle cooperative group [13]	Hematologic malignancies, benign disorders
C/ATG/ Thymic XRT	Harvard, Boston [21]	Lymphoid malignancies

F = fludarabine, B = busulfan, ATG = antithymocyte globulin, M = melphalan, C = cyclophosphamide, Flag/Ida = F/cytarabine/ idarubicin.

NST regimens in clinical practice

Over the last few years NST has been widely explored in humans, the kinetics of engraftment and development of GVL are better understood, and the indications and impact on patient outcome are beginning to be defined.

Conditioning regimens form a continuum from the non-myeloablative regimens, through the reduced intensity regimens, to the maximally tolerated ablative doses of standard SCT. Conditioning regimens have been referred to as non-myeloablative if they do not completely eradicate host hematopoiesis and immunity [4]. A few of these regimens have been given with no stem-cell support and allow prompt hematologic recovery. Autologous reconstitution of hematopoiesis is expected if the allograft is rejected. These non-myeloablative regimens have potent immunosuppressive effects but are only mildly myelosuppressive. More intensive regimens have also been developed. These regimens have been referred to as reduced intensity conditioning. They have not been given without stem-cell support and autologous recovery following treatment may be slow. These regimens usually combine immunosuppressive agents with agents exerting moderate myelosuppressive effects (such as busulfan and melphalan). However, dose intensity is reduced compared to that of standard ablative regimen, allowing reduction of toxicity [Table 1].

Purine analogues have been the cornerstone of NST regimens [3,4]. These are well-tolerated agents with potent immunosuppressive effects, in addition to anti-tumor activity against a range of hematologic malignancies. Fludarabine inhibits cytokine-induced activation of STAT1 and STAT1-dependent gene transcription, which may account for its immunosuppressive effects [24]. Purine analogues have synergistic effects with alkylating agents by inhibiting DNA repair systems responsible for repair of cellular damage induced by these agents.

The Hadassah group [25] and the MD Anderson group [26] pioneered the use of purine analogues in NST regimens. At Hadassah, Jerusalem, fludarabine (30 mg/m^2 for 6 days) has been combined with reduced-dose busulfan (a total of 8 mg/kg) administered orally (or more recently, at Sheba Medical Center, intravenously) and antithymocyte globulin (F/Bu/ATG). This reduced intensity regimen was used successfully in more than 150 transplants from related [3,25] and unrelated donors [11] for a variety of malignant and non-malignant disorders. The median age was 38 years (range 3–63). All the patients engrafted. Engraftment was prompt, and the median time for conversion to complete

chimerism was 21 days (range 10–96). The regimen was well tolerated, even in heavily pretreated patients such as those failing a prior autologous transplant [27]. For many patients it could almost be administered on an ambulatory basis. One-third of the patients did not develop aplasia, 10% did not become neutropenic below 0.5 x 10^9/L, and 10% did not require any blood products throughout the post-transplant period. Mucositis was minimal and most patients were able to maintain an oral diet. Severe organ toxicity was uncommon. GVHD was the single major problem in this series. Up to one-third of the patients developed severe GVHD, which was the primary cause of death in 14%. Day 100 mortality among patients with hematologic malignancies was 4%. With a median follow-up of over 3 years, the probabilities of overall survival and disease-free survival for the first 74 patients with various hematologic malignancies who were transplanted from HLA-matched siblings were 68% and 48%, respectively [3]. Relapse occurred in 23% of these patients. DLI was administered to 15 patients with persistent or recurrent disease, and 10 responded.

The MD Anderson group used a similar reduced intensity regimen combining fludarabine with melphalan [12]. This regimen was used in elderly patients and patients considered poor candidates for standard transplants, up to age 70. The 2 year overall survival for all patients was 28% and 49–57% for good risk patients (such as acute myeloid leukemia in remission or CML in chronic phase). These are encouraging results considering the treatment of elderly patients with refractory malignancies.

At Sheba Medical Center we have also used the fludarabine with melphalan regimen in a group of 12 patients aged 45–63 years. Four patients with leukemia were given this regimen due to compromised pulmonary function related to prior therapy. Eight patients had multiple myeloma. All of the patients engrafted and none had sustained significant regimen-related organ toxicity. After a median follow-up of 10 months, 11 are alive and progression-free; one died of chronic GVHD.

A variety of other non-ablative regimens has been developed. The combination of fludarabine and cyclophosphamide was pioneered at MD Anderson for the treatment of lymphoid malignancies without stem-cell support. It was subsequently used for NST in patients with indolent lymphoid malignancies [28]. The National Cancer Institute used a similar regimen with larger doses of cyclophosphamide in a variety of hematologic malignancies and solid tumors [14,29]. Carella et al. [30] used a two-phase approach: high dose chemotherapy and autologous transplantation for cytoreduction and as an immunosuppressive platform for a subsequent NST with low doses of fludarabine with melphalan. Similarly the Flag/Ida regimen was developed initially at MD Anderson for the treatment of acute myeloid leukemia and was subsequently used as a non-ablative regimen for NST in elderly patients with myeloid malignancies [6,26]. This regimen was used successfully in patients up to age 75 [31].

Following the results obtained from their pre-clinical canine model, the Seattle cooperative group designed a regimen consisting of low dose TBI (200 cGy) and pre- and post-transplant immunosuppression with cyclosporine and mycophenolate-mofetil to prevent the host-versus-graft and GVH reactions. This is a very tolerable regimen and allows ambulatory treatment and treatment of elderly patients [13].

Following their murine NST model, Sykes et al. [21] explored a regimen in men consisting of cyclophosphamide, thymic radiation, and antithymocyte globulin both pre- and post- NST for profound T cell depletion of the host and allograft. This regimen allowed acceptance of HLA-mismatched grafts with minimal GVHD.

The selection of the appropriate regimen for a patient depends on several factors, including the specific indication, age, general medical condition, immune competence of the recipient, and genetic disparity between the patient and donor. However, perhaps the most important determining factor is the aggressiveness and chemosensitivity of the underlying malignancy.

The non-myeloablative regimens intentionally induce a state of mixed chimerism. These regimens reduce toxicity by the use of less intensive, non-toxic conditioning. Also, mixed chimerism describes a state of bilateral tolerance with the potential of limiting the occurrence of GVHD. Theoretically, bilateral tolerance may also delay the development of potent GVL/GVT, allowing rapid progression of active malignancies. These regimens are therefore more suited for indolent malignancies or for diseases in remission at the time of NST. These regimens rely in many patients on immune manipulations to convert to complete donor chimerism or eliminate residual disease. Close monitoring of chimerism by sensitive chimerism testing is critical to guide the timely introduction of immune therapeutic interventions [10]. Early withdrawal of immunosuppressive therapy followed if necessary by DLI can eliminate residual host hematopoiesis and malignancy; however it also predisposes the NST recipient to GVHD. The late administration of DLI may reduce the incidence and severity of GVHD. Patients with mixed chimerism and especially those with low level donor chimerism may develop graft rejection and autologous reconstitution despite these immune therapeutic manipulations [6,10,13]. Early intervention is needed in face of decreasing donor chimerism after NST in order to prevent rejection, but it may be delayed in patients with a high degree of donor chimerism and no residual disease in order to reduce the risks [10]. Some of these regimens may not be immunosuppressive enough to consistently allow engraftment of unrelated or mismatched donor grafts and they carry a substantial risk of graft rejection [6,13].

Reduced intensity conditioning regimens have a more intense myelosuppressive effect and rapidly induce complete chimerism and anti-tumor responses, but they are more toxic and may be associated with higher risk for GVHD. Still, toxicity is reduced in comparison with standard ablative regimens. These regimens are a more appropriate approach for aggressive malignancies such as acute myeloid leukemia, especially when not in remission. In this setting, rapid achievement of complete chimerism and transient disease control is needed to induce GVL. In indolent malignancies GVL may occur slowly, even in mixed chimeras, allowing further reduction of toxicity with less intensive regimens. The risk of early graft rejection or late autologous reconstitution with reduced intensity conditioning regimens is minimal, allowing engraftment of unrelated donor grafts [11].

NST in the treatment of different malignancies

There are major differences between different malignancies in their sensitivity to the GVT effect and hence their susceptibility to the NST approach.

Chronic myelogenous leukemia

CML cells are highly sensitive to the GVL effect. As discussed above, relapse rates following T cell depletion or syngeneic transplants are very high, but on the other hand patients relapsing post-SCT into cytogenetic or chronic phase very often achieve durable molecular remissions with DLI. Much of the curative potential of allogeneic transplantation in CML is therefore related to the GVL effect and this makes CML a very attractive disease for the NST approach. A total of 21 patients with CML in the first or second stable chronic phase underwent NST at the Hadassah University Hospital with the F/Bu/ATG regimen and matched related donors [32]. All patients achieved rapid and stable engraftment with minimal toxicity. The 4 year actuarial overall survival and disease-free survival were 87% and 81% respectively. NST was also feasible and tolerable with unrelated donors. Results were inferior in patients with CML in accelerated or blastic phase. Pre-transplantation treatment with STI-571 is an attractive new option for patients with advanced-phase CML. At Sheba Medical Center we have recently used this approach in several patients with good preliminary results (Shimoni A, Nagler A, in preparation). Further studies are needed to confirm the efficacy of NST in comparison with ablative SCT and to better define the optimal approach to patients with advanced stage disease.

Acute myeloid and lymphoid leukemia

Standard ablative SCT has an established role in the treatment of acute leukemia. Acute myeloid leukemia is more common with increased age, and is associated with poor prognostic factors in the elderly. Elderly patients and patients with medical problems that developed during prior therapy, such as organ dysfunction or opportunistic infections, are not eligible for ablative SCT but may still be cured with the more tolerable NST approach [6,12]. Preliminary results show that NST is more effective in patients who are in remission at the time of transplant. The leukemia is suppressed for sufficient time for the GVL effect to develop and eliminate minimal residual disease [6]. Results have been disappointing in refractory leukemia [33]. In these patients the leukemia often recurs rapidly after NST, outpacing the GVL response. The EBMT registry has reported that overall survival, treatment-related mortality, and relapse rate were 67%, 17%, and 21% in patients in CR1 or CR2 compared to 24%, 68%, and 46% in patients with more advanced disease, respectively. In patients with advanced disease the NST approach was still associated with significant toxicity. New strategies to reduce the high relapse rates, such as judicious use of preemptive DLI, need to be explored.

Acute lymphoid leukemia appears to be least susceptible to GVL, although a few successful cases have been reported. It has been suggested that acute lymphoid leukemia blasts lack co-stimulatory molecules necessary to stimulate an effective immune response. The EBMT analysis failed to document benefit from NST in acute lymphoid leukemia at any stage.

Non-Hodgkin's lymphoma

Indolent lymphoid malignancies such as follicular lymphoma and chronic lymphocytic leukemia are attractive diseases for the NST approach. Potent GVT occurs against these disorders and their indolent nature allows the exploitation of GVT with no need of high dose chemotherapy for disease control. Most patients are also elderly and unable to tolerate high dose chemotherapy with allogeneic SCT. The MD Anderson group has reported very encouraging results with NST using a combination of fludarabine and cyclophosphamide. Treatment-related toxicity was minimal, with potent GVT responses, although the observation period is limited [28].

NST in aggressive non-Hodgkin's lymphoma and in Hodgkin's disease is less established. High dose chemotherapy and autologous stem-cell transplantation has become the standard of care for patients with chemosensitive relapse of these diseases, and it is also explored as a consolidation of first remission in high risk patients. The relapse rate after ASCT is substantial. Conventional ablative allogeneic transplantation is associated with a lesser risk of relapse but with an unacceptable high frequency of treatment-related mortality in this group. With the wide administration of ASCT, more patients now present with post-ASCT relapse and no good standard therapies are available. NST is therefore a reasonable approach to investigate.

Nagler and colleagues [34] have reported encouraging results with NST in a group of 23 patients, 19 with non-Hodgkin's lymphoma (intermediate and high grade by the working formulation) and 4 with Hodgkin's disease. Twelve had resistant disease and all patients were heavily pretreated, including 5 patients who had a prior autologous bone marrow transplant. After a median of 22.5 months, 10 patients are alive and disease-free and the projected 3 year overall and disease-free survival was 40%. Transplant-related toxicity was moderate in this heavily pretreated group. Overall, seven patients died of treatment-related causes. Patients with overt relapse did not respond to DLI, but two patients with persistent minimal residual disease achieved its complete elimination with DLI; both developed chronic GVHD. These results support the potential of graft-versus-tumor effect in aggressive lymphoma and Hodgkin's disease. Treatment of these very high risk patients with grim prognosis was feasible, and although toxicity was higher than often observed with NST, a significant fraction of patients was cured. The EBMT registry of 115 patients with NST for lymphoma also disclosed a relatively high treatment-related mortality of 28%, and 44% were disease-free at 1 year (29% of patients with aggressive non-Hodgkin's, 58% with Hodgkin's disease) [35].

Mantle cell lymphoma is another attractive disease for NST, due to its aggressive nature and the potent GVT in this disease. NST is more effective when administered earlier in the course, when the disease is chemosensitive and of low tumor bulk and when the patients are less heavily treated. This approach merits further investigation in a larger patient group and earlier in the disease course.

ASCT = autologous stem-cell transplantation

Multiple myeloma

Allogeneic SCT in patients with multiple myeloma is associated with exceptionally high risks of morbidity and mortality. Many patients are only considered for allogeneic SCT after failure of a prior autologous SCT and extensive prior therapy, and are therefore poor candidates for standard ablative SCT. GVT has been documented in myeloma, and some patients with post-SCT relapse have been successfully re-induced into remission with DLI. Giralt and associates [36] have pioneered an NST approach using the combination of fludarabine and melphalan with related and unrelated donor grafts. Of 18 patients treated, 15 achieved a response, which was complete in 8. One year overall survival in this very high risk patient group was 33%. Earlier administration of this treatment and transplantation in chemosensitive, low tumor bulk state may improve results. We used the same approach in 10 patients relapsing after ASCT. After a median one year follow-up, four are alive in complete remission, one achieved stable partial remission, three are too early to assess, one died of post-SCT relapse, and one died of organ toxicity (Hardan I et al., in preparation).

Solid tumors

Childs and colleagues [29] reported exciting results in the treatment of metastatic renal cell cancer with NST. Nineteen patients with refractory metastatic renal cell carcinoma were treated with fludarabine and cyclophosphamide and allogeneic NST. Overall, 10 patients responded. In three patients complete regression of pulmonary metastasis was well documented late in the course after conversion to complete donor chimerism. Two died of transplant-related complications. Anecdotal patients with documentation of graft-versus-breast cancer or other solid tumors have also been reported. Further development of this strategy will rely on identification of specific tumor antigens. These antigens can be used to generate and expand specific cytotoxic T lymphocyte clones for more specific adoptive cellular immunotherapy [37].

NST in the treatment of non-malignant disorders

As discussed above, NST is an attractive approach for the treatment of non-malignant disorders and preliminary results are starting to accumulate. Or et al. [16] reported the Hadassah experience with fludarabine-containing NST in genetic disorders. Six patients with thalassemia major were included in that series. In four, thalassemia was corrected, but in two patients mixed chimerism switched to autologous reconstitution, with return of thalassemic phenotype. This observation led the researchers to increase the busulfan dose in pediatric patients with thalassemia in an attempt to prevent this occurrence. Nagler and co-workers [38] recently reported their very successful experience with the NST approach in seven patients with Fanconi's anemia. Patients with Fanconi's anemia are extremely sensitive to alkylating agents and were therefore given fludarabine combined with minute doses of cyclophosphamide. Six of the seven patients are alive and well; one patient who was transplanted from a mismatched unrelated donor died of graft failure. Nagler et al. [17] reported a successful case of a boy with chronic granulomatous disease who was cured of the disease with allogeneic NST after receiving DLI for conversion from mixed to complete donor chimerism. Another adult patient with chronic granulomatous disease and severe pulmonary compromise, who was by no means a candidate for standard ablative therapy, achieved marked improvement of pulmonary and performance status after NST. Patients with a variety of other genetic disorders have been treated, including osteopetrosis, combined immunodeficiency, Blackfan-Diamond syndrome, adrenoleukodystrophy, and Gaucher's disease [16,25]. Most of the patients were children but some were young adults.

Life-threatening autoimmune disorders can be treated successfully with SCT. Most of the experience has been gained with autologous SCT, where the rationale is to provide intensive immunosuppression with the intent that the reconstituting immune system will be tolerant to autoantigens. Far less experience has been gained with allogeneic SCT. Slavin et al. [39] reported a patient with chronic myelogenous leukemia and psoriasis who achieved resolution of the psoriatic lesions upon developing GVHD after NST. This observation, as well as data from animal models, supports a graft-versus-autoimmunity effect. This effect is probably related to elimination of host immunity by donor immunity, which is tolerant to the autoantigens driving the autoimmune process.

Another exciting new indication for NST is the induction of tolerance to solid organ transplantation. This concept has been established in animal models. Spitzer et al. [40] reported a patient with multiple myeloma who underwent bone marrow and renal transplantation simultaneously from the same living HLA-matched donor. Induction of chimerism allowed acceptance of renal allograft, with no rejection after withdrawal of immunosuppressive therapy. In the future, NST may be used to facilitate solid organ transplantation and, with improvement in NST techniques, even across HLA and perhaps even species barriers.

Conclusions and future perspectives

NST is becoming a widely accepted method for allogeneic SCT. Vast experience has been gained, and the biology, indications and limitations are becoming clearer. Non-myeloablative conditioning allows consistent engraftment of allografts from related and unrelated donors. NST has been able to reduce the toxicity of allogeneic SCT. The better immediate outcome may produce better disease-free survival. NST has already succeeded in widening the indications for allogeneic transplantation. NST is feasible in elderly patients with almost no upper age limit [31], as well as in patients with organ dysfunction or other co-morbidities precluding standard ablative conditioning. Since leukemia in the elderly is more common than in the young and is associated with poor outcome, NST is a promising method to investigate in these patients. NST has also reduced the regimen-related toxicity of allogeneic SCT in a high risk setting, such as SCT in heavily pretreated patients or following failure of a prior transplant procedure, and in the unrelated setting [11,27]. In certain malignancies such as non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma, standard ablative SCT has been reported to result in exceptionally high treatment-related mortality, and NST is being investigated as a more reasonable alternative. NST is also rapidly developing for the treatment of various non-malignant disorders [3,16]. It may also reduce the

toxicity of the procedure even in younger patients who are eligible for ablative SCT, however the long-term impact on patient outcome in this group is not yet established. NST may ultimately replace ablative SCT in certain settings in young patients, such as chronic phase CML or acute leukemia in remission. In most hematologic malignancies, allogeneic SCT reduces relapse rates, however overall survival is offset by treatment-related mortality, and thus SCT is often delayed to a more advanced stage of the disease. NST is associated with better outcome when applied earlier in the disease and in the minimal chemosensitive disease state. If NST proves as efficient in preventing disease recurrence as ablative SCT, but safer, it may result in improved survival, and the timing and disease indications may ultimately change. Prospective comparative trials are needed to establish the role of NST in all these transplant settings.

The optimal preparative regimens still need to be defined. Ultimately less chemotherapy will be used and more specific immunomodulation, rather than intense non-specific immunosuppression, will be used to achieve host-versus-graft tolerance. Animal models have been developed such that the prevention of graft rejection is achieved with no chemotherapy, e.g., using co-stimulation blockade alone [22].

Although much progress has been made with consistent achievement of engraftment using NST, graft-versus-host disease and disease recurrence remain major obstacles to successful treatment. NST may limit the incidence and severity of GVHD. Limitation of regimen-related toxicity and bilateral transplantation tolerance afforded by mixed chimerism are believed to have a major role in limiting GVHD. Nonetheless, GVHD remains the primary cause of treatment-related mortality. The development of techniques to separate GVHD and GVL is essential for further improvement of NST outcome. Better understanding of the biology and targets of GVHD and GVL may allow the elimination of alloreactive T cells responsible for GVHD from the graft while retaining T cells with GVL and infection-control potential. Recurrence of the underlying malignancy is a major complication when NST is attempted in patients with chemorefractory diseases and with high tumor bulk. NST serves as a platform for cellular immunotherapy. Judicious use of preemptive DLI needs to be explored. DLI may be amplified by activation of donor lymphocytes with interleukin-2 or *in vivo* administration of IL-2. Identification of tumor antigens will lead the way to *ex vivo* generation and expansion of tumor-specific cytotoxic T lymphocytes to be used as potent immunotherapy without the hazards of GVHD.

The goal of allogeneic transplantation is rapidly changing from that of administration of supra-lethal doses of chemotherapy and radiation in the attempt to physically eliminate the "last tumor cell" – to the more subtle and tolerated sophisticated immunotherapy, focusing on specific induction of host-versus-graft tolerance followed by induction of tumor-specific GVT effect to cure the underlying malignancy.

IL = interleukin

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