State-of-the-Art Review

Nonmyeloablative Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (HSCT) is the most effective treatment for selected hematological malignancies. Its curative potential is largely mediated by an immune-mediated destruction of malignant cells by donor lymphocytes termed graft-versus-leukemia (GVL) effect. However, because of its toxicity, conventional allogeneic HSCT is restricted to younger and fitter patients. These observations led several groups to set up new (less toxic) transplant protocols (nonmyeloablative stem cell transplantation or NMSCT) based on a two-step approach: first, the use of immunosuppressive (but nonmyeloablative) preparative regimens providing sufficient immunosuppression to achieve engraftment of allogeneic hematopoietic stem cells and, in a second step, destruction of malignant cells by the GVL effect. Preliminary results showed that NMSCT were feasible with a relatively low transplant-related mortality (TRM), even in patients older than 65 years. In addition, strong antitumor responses were observed in several hematological malignancies as well as in some patients with renal cell carcinoma. After discussing the mechanisms and efficacy of the GVL effect as well as the rationale for NMSCT strategies, this article reviews the first results of ongoing clinical trials. Innovative modalities that may permit amplification of the GVL effect while minimizing the risk of GVHD are discussed. Because the benefits of NMSCT over alternative forms of treatment remain to be demonstrated, this strategy should be restricted to patients included in clinical trials.

INTRODUCTION

THE CURATIVE POTENTIAL of allogeneic hematopoietic stem cell transplantation (HSCT) is mediated not only by the eradication of malignant cells by high-dose chemotherapy (and total body irradiation), but also by an immune-mediated graft-versus-leukemia (GVL) effect (1–5). The power of the GVL effect and its apparent mediation by donor lymphocytes led several groups to infuse donor lymphocytes (DLI) in patients with relapsed leukemia after HSCT (6–12). The induction of durable remissions by DLI demonstrated that the GVL effect is capable of erad-

icating hematological malignancies, even in the absence of chemotherapy. This prompted the introduction of new protocols based on the development of a GVL reaction after low-dose (less toxic) nonmyeloablative preparative regimens providing sufficient immunosuppression to achieve engraftment of allogeneic hematopoietic stem cells. Three different approaches are currently investigated: purine analog-based regimens (13–20), low-dose TBI followed by mycophenolate mofetil (MMF) combined with cyclosporine (CsA) (21–22), and a combination of cyclophosphamide, anti-thymocyte globulin (ATG) and thymic irradiation (23,24) (Fig. 1).

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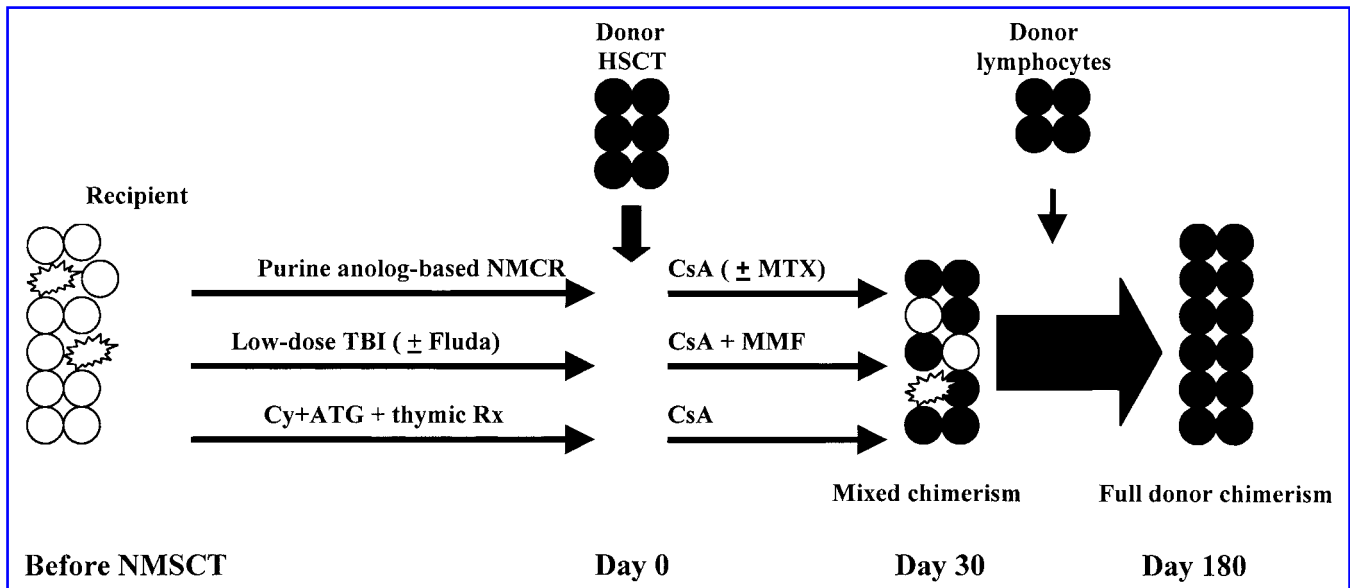
FIG. 1. Schedule of NMSCT. Pretransplant recipient immunosuppression is carried out with either (1) fludarabine-based non-myeloablative conditioning regimen—Houston (13) and Jerusalem approaches (14); (2) low-dose TBI alone—Seattle approach (87); (3) ATG, cyclophosphamide, and thymic irradiation (Rx)—Boston approach (24). Patients receive donor HSC on day 0. Post-grafting immunosuppression is carried out with CsA $\pm$ methotrexate (MTX) for approaches 1 and 3 or with CsA + MMF for approach 2. DLI are given 30–100 days after the transplant in case of mixed chimerism and/or residual disease to obtain full donor chimerism as well as eradication of tumor cells. ($\bigcirc$) Cell of host origin; ($\bullet$) cell of donor origin; ($\oslash$) tumor cell.

THE GVL EFFECT

The existence of a GVL effect in humans was first demonstrated by the Seattle group, which showed a reduced relapse rate in patients with acute (2) and/or chronic (3) GVHD. This was confirmed by other groups who observed an increased risk of relapse after T cell-depleted (TCD) allogeneic HSCT (4,25–27) as well as after syngeneic HSCT (25). The GVL effect was also demonstrated by the evolution of minimal residual disease post-transplantation, which often ceases to be detectable only 6–12 months after HSCT (28) and by the occurrence of GVL activity with or without GVHD after cessation of GVHD prophylaxis for post-transplant relapse (29–31).

Finally, the apparent power of the GVL effect and its probable mediation by donor lymphocytes led several groups to infuse DLI in patients with relapsed leukemia after HSCT (6,8,9,12,32). The induction of durable remissions by DLI demonstrated that the GVL effect is capable of eradicating hematological malignancies even in the absence of chemotherapy. DLI induce a complete remission in about 65% of the cases in chronic myelogenous leukemia (CML) and in 20–30% of the cases in acute myeloid leukemia (AML) or myelodysplasic syndromes (MDS) (12). In patients with CML, the response rate is highest when lymphocytes are infused in early cytogenetic relapse (79%) and lowest in the accelerated phase or blast crisis (19%) Table 1) (8,9,33). It has been speculated that the better response of chronic-phase CML may be explained by its low level of evolution and by the fact that dendritic cells, the most potent antigen-presenting cells, are part of the leukemic clone in CML (33) and are capable of inducing a strong T cell response (34). In contrast, the malignant cells present in accelerated-phase CML or in acute leukemia may be less appropriate antigen-presenting cells and may lead to the induction of anergy rather than an anti-leukemic T cell response (35). Some patients with acute lymphoblastic leukemia (ALL) (36), chronic lymphocytic leukemia (CLL) (37), Hodgkin's disease (38), lymphoma (39,40), as well as multiple myeloma (MM) (41,42) have also responded to DLI or discontinuation of immunosuppressive therapy. Finally, the GVL effect mediated by DLI needs time: the median time to achieve a cytogenetic remission was 85 (range 28–241) days for patients with CML (the time to achieve molecular remission can be prolonged) and 34 (range 16–99) days for patients with AML (9).

Complications of DLI include acute and chronic graft-versus-host disease (GVHD) and transient marrow aplasia. Acute GVHD occurs in about 60% of the patients (grade 3 or 4 in about 20%) and is significantly correlated with complete remission (9). Chronic GVHD also occurs in about 60% of the patients (extensive in 30%) and also correlates with response (9,43). However complete remissions (CR) may be observed in the absence of GVHD, suggesting that the GVL response may be inde-

TABLE 1. EVIDENCE FOR GVL AND GVt EFFECTS

Malignancy (reference)	Response to DLI CR (%)	Other evidence
CML (12,16,25)	64	Increased risk of relapse after T cell-depleted HSCT, reduced risk of relapse in patients with GVHD, efficacy of NMSCT
Cytogenetic relapse	79	
Hematologic relapse	74	
Advanced phase	19	
AML (12,25)	20	Increased risk of relapse with identical twin HSCT, reduced risk of relapse in patients with GVHD, efficacy of NMSCT
MDS (12,16)	38	Efficacy of NMSCT
ALL (12,25)	10	Reduced risk of relapse in patients with GVHD
MM (12,16)	29	Efficacy of NMSCT
NHL (12,15)	13	Efficacy of NMSCT
HD (181)	NR (PR reported)	Efficacy of NMSCT
CLL (15,37)	Yes (low numbers)	Efficacy of NMSCT
Breast cancer (72,143)	NR	Tumor response during acute GVHD, efficacy of NMSCT
Renal cell carcinoma (16)	NR	Efficacy of NMSCT
Melanoma (16)	NR	Occasional response after NMSCT
Ovarian cancer (70)	NR	Tumor response during acute GVHD

NR, not reported.

pendent of the clinical development of GVHD (9,20,33,44). It is possible to reduce the risk of GVHD without impairing the GVL effect by CD8 depletion of DLI (11,44,45,48) or by starting with a low dose of T cells and increasing the dose in a stepwise fashion in case of no response (46,47).

In the European Group for Blood and Marrow Transplantation (EBMT) study, survival after DLI for relapsed CML was as good as that after transplantation: survival probabilities for patients with hematological and cytogenetic relapses were 58% at 8 years and 80% at 6 years, respectively (49). For AML, remissions of more than 2–4 years occurred (33). In the North American study, survival after DLI-induced remission of CML was 87%, 76%, and 73% at 1, 2, and 3 years, respectively (50). For other diseases, survival probabilities at 1 and 2 years were 77% and 65%, respectively. However, unlike patients with early-stage CML or AML, patients with MM do not enjoy durable responses.

MHC-restricted $CD4^+$ and $CD8^+$ T cells, natural killer (NK) cells, macrophages, as well as dendritic cells are probably all involved in the process of both GVL and GVHD (51–57). It is likely that cells implicated in the GVL effect vary as a function of the hematologic malignancies involved, depending upon their major histocompatibility complex (MHC) expression (58) and the nature of antigens presented. However, the time required to achieve a clinical response after DLI suggests that a specific response involving T cells and human leukocyte antigen (HLA)-restricted antigens is important (35). Finally, the efficacy of CD8-depleted DLI (11,44,59) sug-

gests that $CD4^+$ cells are essential for the GVL reaction, or that they recruit $CD8^+$ T cells in the patients.

DLI are associated with conversion from mixed chimerism before infusion to complete donor hematopoiesis after DLI (9,44,46,60,61). Infusion of donor lymphocytes in patients who do not show donor hematopoiesis before DLI induces severe marrow aplasia that may be resolved by the infusion of donor stem cells (8,33). Moreover, DLI can displace residual host stem cells when given for recurrence of nonmalignant diseases after allogeneic HSCT (62,63). In addition, donor-derived cytotoxic T cells (CTL) from allogeneic chimeras recognize both normal and leukemic host hematopoietic cells (64,65). Take together, these observations suggest that the effect of DLI is probably directed against allo-specific antigens [such as minor histocompatibility antigens (mHA)] rather than disease-specific targets. However, aberrantly expressed or overexpressed cellular components, such as proteinase 3 (66–68) or WT-1 (69), could also be target antigens in the GVL effect.

A graft-versus-tumor (GVt) effect has also been demonstrated in breast cancer and in renal cell carcinoma, and possibly in ovarian (70) and non-small cell lung carcinomas (71) (Table 1). Tumor regression associated with acute GVHD has been reported in patients receiving an allogeneic HSCT for metastatic breast cancer (72,73). Moreover, mHA-specific as well as MHC class I antigen-specific CTL recognizing breast carcinoma target cells were isolated from the blood of one such patient (72). Simultaneous GVt and GVL effects were reported after allo-HSCT and DLI in a patient with concurrent

breast cancer and AML (74). Childs et al. recently reported evidence for a GVt effect in patients with metastatic renal cell carcinoma (RCC) undergoing nonmyeloablative stem cell transplantation (NMSCT) (16,75,76). They observed partial and complete responses 2–7 months after transplantation, preceded by achievement of full donor T cell chimerism and accompanying GVHD (16,76).

ROLES OF THE CONDITIONING REGIMEN

Elimination of host tumor cells

Allogeneic HSCT was first considered to deliver supralethal doses of chemotherapy and total body irradiation (77,78). The major demonstration of the anti-tumor efficacy of supralethal chemo-radiotherapy is contributed by the superiority of autologous HSCT over conventional chemotherapy in various hematological malignancies (79–82). However, pretransplant high-dose therapy is unable to eradicate the malignancy in many patients. Attempts to improve disease-free survival by increasing the intensity of the conditioning regimen were usually accompanied by an increase in transplant-related mortality (TRM) and overall as well as disease-free survival remained unchanged or worsened (83,84).

Making space for donor cells

Immature progenitor cells occupy defined niches within the marrow stroma to obtain the necessary support for proliferation and differentiation (85,86). To allow access for donor cells to these niches, it was commonly believed that host stem cells must be eradicated by the conditioning regimen (87). However, Storb et al. recently demonstrated that the graft itself, most likely through subclinical GVH reactions, is capable to create these marrow spaces in the absence of both chemotherapy and bone marrow irradiation (88).

Eradication of the host's immune responses

It is necessary to abolish host defense prior to transplantation to avoid immune-mediated graft rejection caused by alloreactive cytotoxic host lymphocytes or by HLA-specific antibodies (89). The risk of graft rejection increases in the case of HLA disparities or prior host presensitization via administration of multiple blood products before HSCT (89). Both the conditioning regimen and donor T lymphocytes (and particularly donor CD8 lymphocytes) (90) are implicated in the destruction of the host immune system. Therefore, TCD of the graft as a method to prevent GVHD may have deleterious effects

on engraftment (27,91). Unfortunately, the use of more intensive conditioning regimens also increases organ toxicity and infection rates. Recently, the Seattle group demonstrated that optimizing postgrafting immunosuppression can also control the host-versus-graft (HVG) reaction (92,93). Thus, contrary to TCD of the graft that prevents GVHD but increases the risk of graft rejection, optimal postgrafting immunosuppression reduces both GVHD and rejection incidences.

NONMYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION

Mixed hematopoietic chimerism

NMSCT usually results in mixed hematopoietic chimerism (MC) that can be defined as the presence of 1–95% hematopoietic cells of donor origin (Fig. 1). This state is characterized by mutual donor-host tolerance (and thus control of both GVH and HVG reactions without continued use of immunosuppressive agents) while immune responses against other antigens remain normal. The mechanisms involved include central thymic deletion of both donor- and host-reactive T cells (because both donor and host dendritic cells are present in the thymus of mixed chimera) and peripheral tolerance due to suppressor T cells (94).

Preclinical and clinical data suggest that stable mixed chimerism may be useful to alleviate clinical symptoms in genetic diseases such as thalassemia (95,96), sickle cell disease (97), or congenital immunodeficiencies (98,99), to control autoreactivity in autoimmune diseases (100–102), or to prevent graft rejection in organ transplantation (103–105).

The first trials of NMSCT for sickle cell disease are currently ongoing. Initial results suggest that acute GVHD may be particularly frequent and severe in this group of patients (106). Two recent reports have shown that NMSCT may be an ideal treatment to achieve cures in congenital immunodeficiencies (98,99). Among 18 patients treated by these two groups, 14 were alive and well 8–26 months after the transplant despite of 4/14 patients remaining mixed chimera (98,99).

Several reports have shown that life-threatening autoimmune diseases can be stabilized or cured by autologous HSCT (102,107–109). However, initial failures as well as relapses are relatively frequent. Several animal studies as well as some observations in humans suggest that allogeneic HSCT (after conventional or nonmyeloablative conditioning) may be more efficient (102,110). Because of its high toxicity, conventional allogeneic HSCT has so far been restricted to patients with autoimmune disease who developed a coincident hematologic disorder (102). For others, a nonmyeloablative approach

of allogeneic HSCT may become particularly useful in the future.

The potential role of mixed hematopoietic chimerism in organ transplantation was recently demonstrated by the Boston group, who reported the induction of renal allograft tolerance by combined kidney and peripheral blood stem cell (PBSC) transplantation from the same HLA-identical sibling donor after a nonmyeloablative conditioning regimen in a patient with MM and renal failure (105). Remarkably, the patient accepted the kidney graft without any immunosuppression for at least 2 years (94).

For the treatment of hematologic malignancies, mixed donor chimerism (MC) is not expected to be always curative (111–114). It is now well demonstrated that MC is associated with relapse in patients with CML receiving TCD HSCT (111). More recently, Roman et al. studied the incidence and the significance of minimal residual disease and MC in CML patients treated with standard unmanipulated allogeneic bone marrow transplantation (BMT) (113). In this study, relapse occurred in 1/39 patients with full donor chimerism (FC) versus 6/9 patients with MC ($p < 0.0001$) (113). Moreover, 3 of the 6 patients who relapsed experienced low-level MC that was restricted to T cells while they remained BCR-ABL negative (113). For those patients with hematologic malignancies, MC can be converted to FC by DLI (16,23,24,115,116) (Fig. 2).
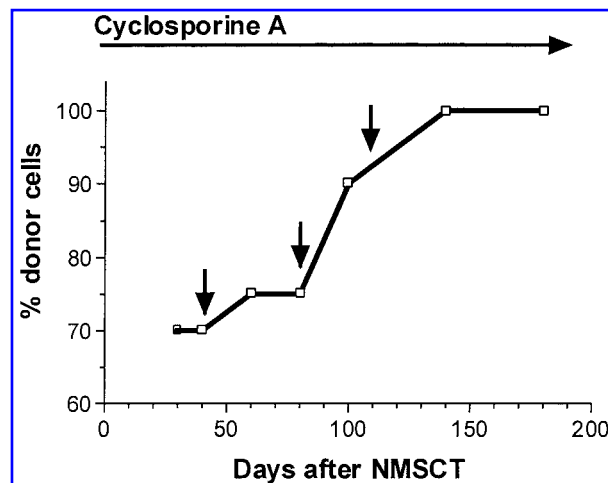
FIG. 2. Conversion of mixed chimerism to full donor chimerism by DLI. The patient received a CD34-selected graft from an HLA-identical sibling donor after a nonmyeloablative conditioning regimen combining cyclophosphamide and fludarabine (183). Analysis of his white blood cell chimerism on day 30 evidenced mixed chimerism. CD8-depleted DLI (black arrows) were given in incremental doses (10, 50, and 50 × 10⁶ CD3⁺ cells/kg recipient) on days 40, 80, and 120, respectively, and achieved full donor chimerism without GVHD.

Assessment of hematopoietic chimerism

The assessment of hematopoietic chimerism requires more sensitive techniques than conventional cytogenetic analyses because of the availability of only small numbers of dividing cells. The most current techniques are fluorescent in situ hybridization (FISH) with X- and Y-specific probes in case of sex-mismatched transplant (14) and PCR-based assays of polymorphic mini- or microsatellite markers in case of sex-matched transplant (16,115,117,118). Other techniques based on restriction fragment length polymorphism (RFLP) are also used (15).

The evolution of myeloid and lymphoid chimerism after nonmyeloablative HSCT may be discordant. Achievement of full donor T cell chimerism is associated with disease regression (16). Moreover, the Seattle group recently showed that the level of T-cell chimerism on day 28 predicted for both graft failure and acute GVHD (21), underlying the importance of lineage-specific chimerism analysis (Fig. 3).

Nonmyeloablative conditioning regimens (NMCR) (Table 2)

Because of its toxicity, conventional allogeneic HSCT is restricted to younger patients (<55 years for allograft procedures with HLA-identical siblings and <50 years for unrelated donor transplants) without significant organ impairment. Unfortunately, the majority of malignancies potentially cured by allogeneic HSCT and for which a GVL effect has been demonstrated are more frequent in older patients (Fig. 4). The median age at diagnosis for CML, AML, MM, and CLL varied from 55 to 65 years (119). Thus, it may be important to develop less toxic approaches to allografting that can also be extended to older patients or patients with pre-existing organ impairment.

In 1997, Giralt et al. (13) reported the engraftment of HLA-identical allogeneic HSC after nonmyeloablative chemotherapy based on purine analogs. The rationale for using purine analogs (fludarabine or 2-CDA) was their capacity to inhibit the mixed lymphocyte reaction in vitro and to produce lymphopenia and substantial immunosuppression in vivo. Other pilot trials by the same group confirmed these preliminary results and achieved durable engraftment and remissions in some patients with AML (120), CML (18), as well as lymphoid malignancies (15), with a relatively low TRM. The Jerusalem's group developed another nonmyeloablative purine analog-based protocol combining fludarabine, ATG, and low-dose oral busulfan (14). This NMCR allowed the achievement of engraftment and full donor chimerism in the majority of the patients with a low TRM. However, it should be emphasized that many patients included in this study would be considered eligible for conventional allogeneic HSCT.
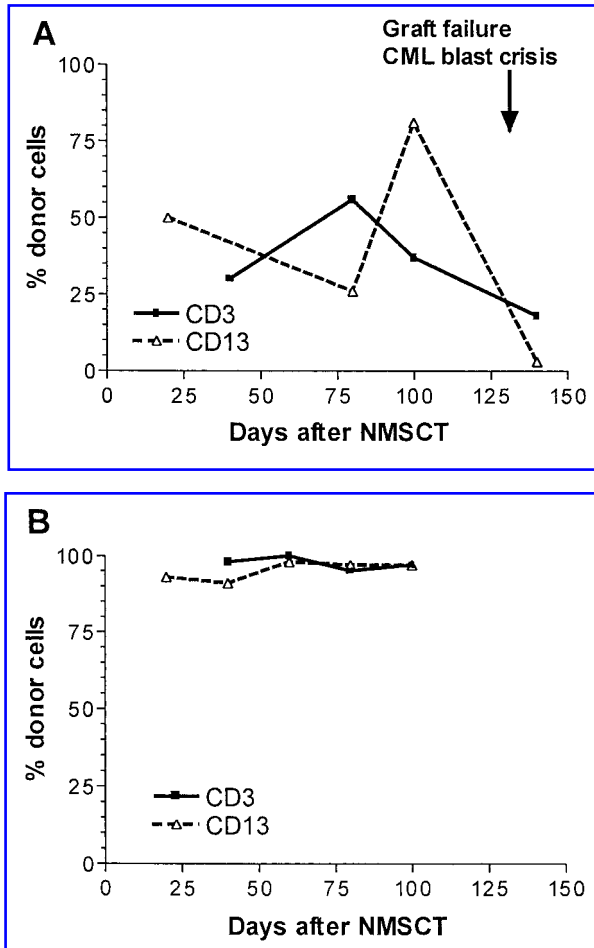
FIG. 3. Illustration of the influence of the underlying disease and previous therapy on engraftment after a nonmyeloablative conditioning regimen consisting of 2 Gy TBI alone. (**A**) T cell (CD3) and myeloid (CD13) chimerism in a patient transplanted for chronic-phase CML. (**B**) T cell (CD3) and myeloid (CD13) chimerism in a patient transplanted for poor-prognosis Hodgkin's disease after a failed autologous transplant.

The feasibility of fludarabine-based nonmyeloablative transplant protocols has also been confirmed more recently by several others groups (16,17,19,121–124).

In an elegant canine allogeneic transplant model, the Seattle group demonstrated that stable mixed chimerism could be achieved using pretransplant low-dose total body irradiation (TBI) combined with post-grafting immunosuppression with a combination of CsA and MMF and that post-grafting immunosuppression can serve to control both HVG and GVH reactions (92,93). Complete chimerism was achieved through DLI. Initial experience in humans showed the feasibility and safety of this approach (21,22,93). Moreover, major disease responses were observed in more than 70% of the patients who had measurable disease pretransplant and achieved sustained engraftment (21).

Finally, the Boston's group demonstrated in a murine model (125) and then in humans that mixed chimerism could be induced in HLA-matched (24) or two or three loci-mismatched (23) allogeneic HSCT by a nonmyeloablative conditioning regimen combining cyclophosphamide, thymic irradiation, and ATG.

Toxicity, TRM, and engraftment

Generally, NMCR are well tolerated, inducing little or no grade 3–4 toxicity, even in patients older than 65 years or with concomitant comorbidities (15,126,127). However, there are important discrepancies among the different studies, due to the intensity of the NMCR used, the age of the patients, as well as the type of transplant (sibling versus unrelated, HLA-identical versus mismatch) (Tables 2 and 3). The 200-day TRM varied from 4% in the Seattle study (21) (using low-dose TBI alone as conditioning regimen in HLA-identical sibling transplants) to 37% in the Houston's study (18) (using melphalan and purine analog-containing preparative regimens in related or unrelated graft recipients ineligible for conventional transplants). In the EBMT study reporting on 256 NMSCT for various hematologic malignancies, the 1-year probability of TRM was 13% for patients in CR at the time of transplant versus 36% for patients in more advanced disease (128). Moreover, age was also significantly associated with TRM in some studies (20). The primary causes of nonrelapse mortality in 4 major studies are given in Table 4.

The engraftment rate was also related to the intensity of the NMCR as well as the type of transplant. Generally, more intensive conditioning regimens resulted in higher engraftment rates: graft failure rates ranged from 0% to 20% of the cases in the Jerusalem study and in the Seattle study, respectively (14,21). Moreover, the immune status of the recipient also appeared to be important for engraftment. For example, a high incidence of graft rejection was observed by the Seattle group in previously untreated CML patients (129), inducing them to add fludarabine in their "TBI only" protocol for such patients.

Acute and chronic GVHD

In both animal and human studies, the use of less severe conditioning (130,131), as well as the initial presence of host hematopoietic cells (132,133), decreased the severity of acute GVHD. These observations predict that acute GVHD may be reduced by the use of NMCR because of their low intensity and the high incidence of mixed chimerism achieved.

Indeed, preliminary data suggest that acute GVHD is relatively mild and generally controllable after NMCR (21,120). Moreover, acute GVHD is usually delayed and

TABLE 2. NMSCT REGIMENS CURRENTLY UNDER INVESTIGATION

Center (reference)	Preparative regimen	Rejection/GVHD prophylaxis	Stem cell source, HLA match
MD Anderson (18)	Fludarabine 25 mg/m² (or 2-CDA 12 mg/m²) × 5 Melphalan 70–90 mg/m² × 2	FK506 + MTX	PBSC (6/6 or 5/6 or UD)
United Kingdom (17)	Fludarabine 30 mg/m² × 5 Melphalan 140 mg/m² CAMPATH-1H 20 mg × 5	CsA + CAMPATH-1H	PBSC (6/6 or 5/6) BM (UD)
Jerusalem (14)/Marseille (19)	Fludarabine 30 mg/m² × 6 Busulfan (p.o.) 8 mg/kg ATG 10 mg/kg × 4	CsA	PBSC or BM (6/6 or 5/6 or UD)
Dresden (124)	Fludarabine 30 mg/m² × 6 Busulfan (i.v.) 66 mg/kg	CsA ± MTX OR MMF	PBSC (6/6)
USA (99)	Fludarabine 25 mg/m² × 5 Cyclophosphamide 60 mg/kg × 2 ATG 40 mg/kg × 4	CsA TCD	PBSC (6/6)
National Institutes of Health (16)	Fludarabine 25 mg/m² × 5 Cyclophosphamide 60 mg/kg × 2	CsA	PBSC (6/6 or 5/6)
Genoa (121)	Fludarabine 30 mg/m² × 3 Cyclophosphamide 300 mg/m² × 3	CsA + MTX	PBSC (6/6)
Boston (24)	Cyclophosphamide 50 mg/kg × 3–4 ATG 30 mg/kg × 2 Thymic irradiation 700 cGy	CsA	BM (6/6 or 5/6)
Seattle (22)	Fludarabine 30 mg/m² × 3 TBI 200 cGy	CsA + MMF	PBSC or BM (UD)
Seattle (21)	TBI 200 cGy	CsA + MMF	PBSC (6/6)

2-CDA, Cladribine; FK506, tacrolimus; MTX, methotrexate; BM, bone marrow; 6/6, HLA-identical sibling donor; 5/6, 1 mismatch sibling donor; UD, unrelated donor.
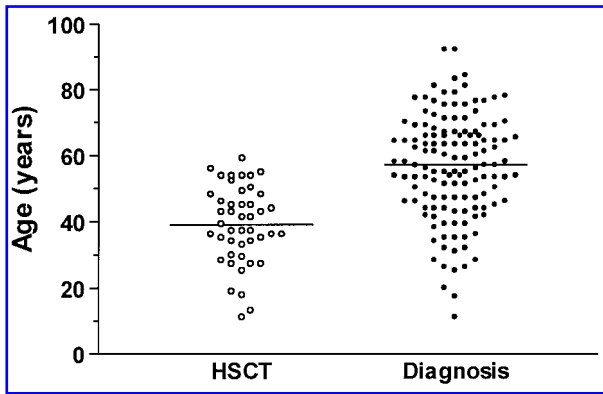
FIG. 4. Age distribution of patients diagnosed with CML (●) or transplanted for CML (○) in Liège.

occurs after patients have recovered from conditioning-related toxicities (16,21). However, there are relatively large discrepancies among the different studies. This variability probably relates to differences in the source of stem cells (bone marrow versus PBSC), type of transplant (related versus unrelated), GVHD prophylaxis, use of ATG, as well as age of the patient. However, acute GVHD is still the leading cause of nonrelapse mortality (Table 4).

The optimal type and duration of post-transplant immunosuppressive therapy are uncertain. After conventional transplantation, high-dose immunosuppressive therapy given soon after HSCT to prevent GVHD also increases the risk of relapse (134). On the other hand, the French study recently evidenced a significant influence of GVHD prophylaxis on overall survival (better with longer prophylaxis) and TRM (higher with shorter prophylaxis) after NMSCT (123).

Additional DLI are significantly associated with increased risks of both GVHD (24) and TRM (123). However, the time of infusion as well as the dose of lymphocytes given play a major role. This is illustrated by the observation of powerful GVL effects without significant GVHD of single DLI of 10^7 CD3$^+$ cells/kg given on day 35, contrasting with the high incidence of severe acute GVHD after repeated DLI on days 35 and 56 (24).

Because of short follow-up, the incidence and severity of chronic GVHD is still uncertain. However, preliminary trials reported the occurrence of severe chronic GVHD in some cases (15). Moreover, despite such short follow-up, the risk of chronic GVHD was already 74% in the Seattle's study (21) and 68% in the Houston's report (18).

Antitumor efficacy

Although data are too early to assess antitumor effects definitively, preliminary results clearly demonstrated the

occurrence of major disease responses in patients with hematological as well as some solid tumors.

CLL and lymphoma: Durable complete responses were observed in several patients with refractory non-Hodgkin's lymphoma (NHL), Hodgkin's disease (HD), or CLL (15,23,135). The Boston group reported the evolution of 16 patients treated with NMSCT after a conditioning regimen combining cyclophosphamide, ATG, and thymic irradiation for refractory NHL, HD, or CLL. Complete responses were observed in 7/16 patients (4/11 patients with NHL, 2/3 patients with HD, and 1/2 patients with CLL). Similarly, the Jerusalem group reported on a group of 23 heavily treated high-risk malignant lymphomas (136). Ten of the 23 patients were alive in CR 15–37 months after the transplant and the 3-year probability of disease-free survival was 40%. Kottaridis et al. reported on 13 patients with HD or NHL in partial remission or with refractory disease (17). The NMCR consisted of fludarabine, melphalan, and CAMPATH-1H. Four out of the 13 patients experienced a complete response, and stabilization occurred in 7 other patients. However, in a retrospective study of 115 lymphoma patients (most of them receiving an HLA-identical sibling transplant after a fludarabine-based NMCR), the EBMT encountered a 38% rate of TRM at 1 year, mostly due to a high incidence of severe GVHD (137).

CML: Complete cytogenetic or molecular remissions were obtained in more than 75% CML patients transplanted in chronic phase (16–18,21,138) (Table 5). Moreover, some patients with more advanced-phase disease also achieved molecular remission (21,124). The EBMT recently summarized the results of 58 CML patients, most of them receiving an HLA-identical sibling transplant after a fludarabine-based NMCR (139). The overall 1-year survival was 87% for patients grafted in first chronic phase ($n = 32$) versus 58% for patients transplanted in more advanced phase. The 1-year disease-free survivals were 75% and 46%, respectively.

Multiple myeloma: Durable (>1 year) partial and complete responses were also observed in some patients with MM (17,21,140). Badros et al. (140) studied 16 MM patients receiving a NMSCT after conditioning with melphalan 100 mg/m^2. After a median follow-up of 1 year, 5 patients achieved and sustained CR, 3 near CR, and 4 partial response (PR). Two patients died of progressive disease and 3 died of GVHD without active disease. The EBMT retrospectively collected data from 54 patients who received NMSCT for good- (CR, PR1, or PR2, $n = 36$) or poor- ($n = 18$) risk MM. (141). In this study, the 1-year rates of survival, TRM, and relapse for the good-risk group were 83%, 13%, and 11%, respectively. The figures were 25%, 68%, and 20% for poor-risk patients.

TABLE 3. NMSCT IN 337 PATIENTS WITH MALIGNANT OR NONMALIGNANT DISORDERS

Center name (reference) Regimen (dose in mg/m^2)	Number of patients (median age)	Diagnosis (number of patients)			Graft failure (%)	Day 200 TRM (%)	Acute GVHD		Chronic GVHD (%)	1-Year OS (%)
		CML CP/ AML CR/ NHL CR	Other malignant disease	Non-malignant disease			Grade 2–4 (%)	Grade 3–4 (%)		
MD Anderson (18) Fluda 125+Mel 180	86 (52 yrs)	12	74	0	2	37	49	29	68	35
United Kingdom (17) Fluda 150+Mel 140+C1H	44 (41 yrs)	16	28	0	2	9	4	0	2	73
Jerusalem (sibling) (14) Fluda 180+Bu 8+ATG	26 (31 yrs)	15	7	4	0	15	46	23	42	85
Jerusalem (unrelated) (138) Fluda 180+Bu 8+ATG	16 (34 yrs)	8	8	0	0	12	44	19	0	75
Marseille (19) Fluda 180+Bu 8+ATG	21 (52 yrs)	7	14	0	0	10	32	10	NR	NR
Dresden (124) Fluda 150+Bu 6.6	24 (47 yrs)	6	18	0	0	8	25	0	33	63
USA (99) Fluda 125+Cy 120 mg/kg+ATG	10 (15 yrs)	0	0	10 (GD)	20	0	30	10	20	90
National Institutes of Health (16) Fluda 125+Cy 120 mg/kg	15 (51 yrs)	2	13	0	7	8	60	20	NR	NR
National Institutes of Health (145) Fluda 125+Cy 120 mg/kg	19 (48 yrs)	0	19 (RCC)	0	0	11	53	16	21	47
Genoa (121) Fluda 90+Cy 900	23 (36 yrs)	0	23	0	9	NR	43	13	NR	72
Boston (24) Cy 200 mg/kg + ATG + Thymic Rx	21 (44 yrs)	0	21	0	17	10	52	14	NR	52
Seattle (21) TBI 200 cGy	45 (56 yrs)	15	30	0	20	4	47	11	74	65

RCC, renal cell carcinoma; GD, granulomatous disease; OS, overall survival; NR, not reported.

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TABLE 4. CAUSES OF NONRELAPSE MORTALITY IN FOUR MAJOR STUDIES

Author	Number of nonrelapse deaths/number of transplants	Causes of death: number (%)			
		GVHD	Toxicity	Infection	Other
Giralt (18)	29/86	16 (55)	3 (10)	9 (31)	1 (3)
McSweeney (21)	7/45	4 (57)[a]	0 (0)	3 (43)	0 (0)
Sykes (24)	2/21	2 (100)[a]	0 (0)	0 (0)	0 (0)
Lalancette (128)	32/115	18 (56)	5 (16)	9 (28)	0 (0)
Total	70/267	40 (57)	8 (11)	21 (30)	(1)

[a]After DLI.

AML, ALL, and MDS: Storb recently reported the results of 17 AML patients treated with related NMSCT after conditioning with 2 Gy TBI ± fludarabine (90 mg/m^2) (22). Eight of 10 patients grafted in CR remained in CR after 5–18 months. Moreover, 2/3 patients with primary refractory disease were in remission at more than 20 months. Prolonged remissions in refractory AML patients were also reported by other groups (18,138). The EBMT recently reported data on 154 patients treated with NMSCT for AML, ALL, or MDS (142). For AML patients in CR1 or CR2, the 1-year actuarial overall survival, TRM, and relapse rates were 67%, 17%, and 21%, respectively, compared to 24%, 68%, and 46% for patients in more advanced disease (142). For ALL patients, the figures were 15%, 72%, and 55%, respectively, and results in CR1-2 were the same as in more advanced disease, suggesting that, in contrast with AML patients, ALL patients did not benefit from NMSCT (142). Finally, in the same study, 3/3 patients with refractory anemia survived in CR more than 1 year after transplant and patients with more advanced MDS experienced 48% TRM and 33% relapse rates at 1 year (142).

Solid tumors: In patients with solid tumors, responses were partial and transient in patients with breast cancer (143,144) or melanoma (16,145), whereas some patients with RCC achieved durable complete responses (16,75,76).

Childs et al. has recently reported the evolution of 19 patients treated with NMSCT after conditioning with fludarabine and cyclophosphamide for metastatic RCC (76). Ten of the 19 patients enjoyed major responses, including 3 patients with sustained (>20 months) complete response. These responses occurred 3–6 months after the transplant and usually after cyclosporine discontinuation. Acute GVHD was associated with disease response but, interestingly, one patient had a complete response in the absence of acute GVHD (76).

The same group explored the same NMSCT approach in 15 patients with advanced metastatic melanoma (145). Four of the 15 patients had partial responses that occurred soon after transplant (before CsA withdrawal and before the development of acute GVHD), suggesting that these responses were related to the conditioning regimen rather than to a GVt effect. All other patients progressed. Surface analysis of renal and melanoma tumor cell lines obtained from transplanted patients evidenced that most of the RCC cells expressed MHC class I, whereas several lines obtained from melanoma patients did not (Barrett et al., EBMT 2001, educational book). This finding could partially explain the lack of sensitivity of melanoma cells to the GVt effect. However, other mechanisms of tumor

TABLE 5. RESULTS IN CHRONIC-PHASE CML

Author (reference)	Number of patients	Number of patients with CCR	Number of patients with graft rejection
McSweeney (21)	6	4	2
Giralt (18)	5	2	1
Kottaridis (17)	1	1	0
Barrett (16)	3	2	1
Slavin (182)	21	19	0
Total	36	28	4

CCR, continuous CR.

escape from immune destruction, including the loss of adhesion or costimulatory molecules, secretion of inhibitory cytokines or expression of fas ligand, may also probably play a major role in the NMSCT setting (146,147).

Secondary malignancies after autologous or allogeneic HCT: Treatment options for patients who relapse or develop secondary malignancies after autologous or allogeneic HCT are limited. In these patients, results of a second alloHSCT are generally poor, primarily because of a high rate of TRM. Recently, the Jerusalem group studied the feasibility of a second allogeneic HSCT after a nonmyeloablative conditioning regimen (148). Among the 12 patients included, only one died of procedure-related complications, suggesting that NMCR significantly reduce TRM associated with second transplants. Moreover, the actuarial disease-free survival at 34 months was 50%. These findings were confirmed by Kottaridis et al., who reported a 14% TRM associated with an allogeneic NMSCT for disease relapses occurring after standard autologous or allogeneic HSCT (17).

Autologous HSCT followed by NMSCT: Previous attempts of immunotherapy after autologous HSCT by induction of autologous GVHD (149,150) or by interleukin (IL-2) (151) did not show significant antitumor efficacy. For patients with a high tumor burden, the Genoa's group studied the feasibility of conventional autologous HSCT followed by NMCST 1–3 months later (121) (Fig. 5). The rationale for high-dose therapy followed by autologous HSCT was debulking and the rationale for NMSCT was to induce immune-mediated antitumor effects. The rationale for separating high-dose therapy from allogeneic transplantation was to reduce the TRM and the risk of

acute GVHD (see above). Preliminary results evidenced the feasibility of this approach with a low TRM (121,144,152).

PERSPECTIVES

Manipulation of donor cells to separate the GVL effect from GVHD

Escalating doses of DLI: It is well demonstrated that the risk of GVHD correlates with the dose of lymphocytes infused (8,33,46). In an elegant article, Mackinnon et al. (46) showed that it was possible to reduce the risk of GVHD without impairing the GVL effect by starting with a low dose of T cells and increasing the dose in a stepwise fashion in case of no response. Their observations were recently confirmed by another study that compared the efficacy and safety of a single infusion of relatively large doses of donor lymphocytes (bulk dose regimen, BDR) versus infusion of smaller doses repeated as necessary at 3-month intervals (escalating dose regimen, EDR) in CML patients relapsing after conventional allografting (47). The CR rate at 2 years was higher (but not statistically significant) and the risk of both acute and chronic GVHD was significantly lower in patients allocated to the EDR regimen, even when the total number of cells administered was similar. This approach is currently investigated in the NMSCT setting.

CD8-depletion of the graft and/or DLI: Contrary to pan T cell depletion of donor marrow that increases the risk of relapse (particularly in patients with CML), selective $CD8^+$ T cell depletion of the graft significantly reduces the risk of GVHD without affecting the GVL ef-
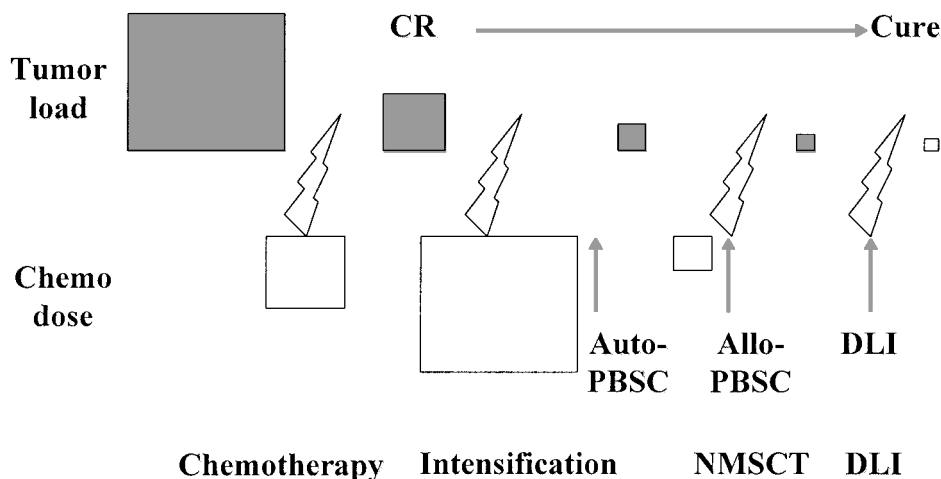
FIG. 5. The Genoa approach. Schedule of high-dose therapy and autologous HSCT followed by moderate immunosuppression and allogeneic NMSCT.

fect (153,154). Similarly, several studies demonstrated that CD8-depletion of therapeutic DLI (44,59,155) reduces the risk of DLI-induced GVHD without impairing the GVL effect. The potential role of CD8-depletion should be examined in NMSCT.

T cell depletion of the graft followed by T cell add-back: It is now well demonstrated that a conditioning regimen-related cytokine storm plays a major role in the pathogenesis of GVHD (156). Moreover, in the NMSCT setting, it is well demonstrated that donor lymphocytes given several weeks after the transplant in mixed chimera induce significantly less GVHD than a similar dose of donor T cells given together with the transplant, without reducing their antitumor efficacy (125). Recently, we have reported that transplantation of CD34-selected allogeneic PBSC after a myeloablative preparative regimen followed by pre-emptive CD8-depleted DLI significantly decreases the incidence of acute and severe chronic GVHD as compared with unmanipulated BMT (157). We also investigated the feasibility and efficacy of NMSCT with CD8-depleted or CD34-selected PBSC followed by pre-emptive CD8-depleted DLI given in incremental doses on days 40 and 80 (depleted group). None of the 10 patients included in the depleted group versus 3/4 recipients of unmanipulated PBSC and DLI experienced grade II–IV acute GVHD. Most of the patients included in the depleted group were mixed chimera on day 30 but became full-donor chimera after CD8-depleted DLI (Fig. 2).

In vivo T cell depletion using CAMPATH-1H: Kottaridis et al. (17) recently investigated a novel nonmyeloablative conditioning regimen consisting in CAMPATH-1H, fludarabine (150 mg/m^2) and melphalan (140 mg/m^2). They observed a high engraftment rate (>97%), but most of the patients analyzed were mixed chimera. The incidence of GVHD was exceptionally low (5% of grade II–IV acute GVHD) (Table 3). The authors explain this observation by the use of in vivo CAMPATH-1H (achieving in vivo T cell depletion of the graft because of its prolonged half-life in humans) and by the high incidence of mixed chimerism (known to reduce the incidence and severity of GVHD) (133). However, because mixed chimerism may diminish the GVL effect seen in the allograft setting, longer follow-up is needed to clarify whether this approach respects the GVL effect.

Infusion of donor lymphocytes transfected with a suicide gene: Another interesting approach consists in in vitro insertion of a suicide gene, the herpes simplex virus thymidine kinase (HSV-tk) gene, which selectively phosphorylates gancyclovir (GCV) leading to its incorporation into DNA and causing cell death into lymphocytes, and allowing their selective elimination by GCV if se-

vere GVHD develops after DLI (158–160). A first clinical study using this approach demonstrated antitumor responses and efficient elimination of infused cells by GCV in case of GVHD (159). Unfortunately, induction of a strong immune response against genetically modified cells and partial resistance to ganciclovir-mediated elimination of transduced cells in chronic GVHD were observed (161,162). Another trial of 23 patients (14 with CML) was recently reported (163). No toxicity or GVHD was observed even with cumulative doses >2 × 10^8 CD^3/kg recipient. However, only 2 patients (2 with CML) achieved CR, suggesting that the GVL effect was impaired by the transduction procedure. Whether this approach will be applicable in the nonmyeloablative setting remains to be determined.

Infusion of tumor-specific CTL: Donor-derived CTL have been used successfully for the treatment of cytomegalovirus (CMV) infections (164) or for the prevention or treatment of Epstein-Barr virus (EBV)-associated lymphoma after allogeneic HSCT (165). Remarkably, no significant toxicity nor GVHD were observed with this early post-transplant cell immunotherapy. Recently, the Leiden's group reported the achievement of CR in a patient with accelerated-phase CML by treatment with leukemia-reactive CTL (35). The infusion of donor-derived specific CTL against specific antigens such as mHA preferentially expressed in hematopoietic system (166,167), tumor-specific antigens (168,169), or antigens overexpressed in tumor cells, such as proteinase 3 (66–68) or WT-1 (69), all represent promising methods of immune cell therapy. Combining these approaches with CD8-depletion, CD34-selection, or other forms of in vitro TCD or with the use of in vivo CAMPATH-1H in the future may permit to increase the GVL effect while minimizing the risk of GVHD after NMSCT.

Combination of NMSCT with other approaches

Recombinant human (rh) IL-2 or interferon-α in conjunction with DLI: Several approaches have been developed to increase the efficacy of DLI (170). First, Slavin et al. showed that rhIL-2 activated DLI can induce CR in several patients with hematologic malignancies refractory to unmanipulated DLI (171,172). In RCC, Childs et al. have demonstrated that interferon-α (IFN-α) could increase the antitumor effect of DLI, even in patients previously refractory to IFN-α (76).

Combination of STI-571 and NMSCT/DLI: STI-571 is a specific inhibitor of the BCR-ABL tyrosine kinase (173). Preliminary results indicate that STI-571 induces complete cytogenetic responses in the majority of CML patients in the chronic phase as well as in some patients with CML in blast crisis or Phi-positive acute leukemia

(174–176). Unfortunately, responses were often transient, and resistance to STI-571 occurred in the majority of patients with blast crisis or Phi-positive acute leukemia (176–178). Because the outcome of DLI is better in early-phase CML than in more advanced relapse, prior reduction of marrow blasts may be an useful step before NMSCT or before DLI for CML in blast crisis or for Phi-positive acute leukaemia. Recently, we have reported the successful treatment by STI-571 and DLI of Phi-chromosome positive acute leukaemia relapsing after a standard unrelated HSCT (179). Similarly, Olavarria et al. recently showed that STI-571 alone could induce mixed chimerism in CML patients relapsing in blast crisis after allogeneic HSCT (180). Taken together, these observations suggest that the combination of STI-571 and NMSCT may be an effective strategy for CML blast crisis patients.

CONCLUSION

In conclusion, NMSCT is feasible and can lead to molecular responses. This transplant strategy offers several advantages over conventional HSCT: (1) TRM is reduced; (2) acute GVHD could be less frequent and less severe than after myeloablative HSCT; and (3) NMSCT is possible in patients older than 55 or with concomitant comorbidities. Further clinical trials are needed to define more effective strategies to separate GVL effects from GVHD and to compare the relative efficacy of this approach to conventional treatment. Because the benefits of NMSCT over alternative forms of treatment remain to be demonstrated, this strategy should be restricted to patients included in clinical trials.

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