Immunobiology of reduced intensity conditioning in hematopoietic stem cell transplantation

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ABSTRACT

Reduced-intensity and truly nonmyeloablative conditioning regimens followed by allogeneic hematopoietic stem cell transplantation (SCT) have been evaluated in patients with hematological malignancies who were not considered candidates for conventional SCT because of age or medical comorbidities. Reduced intensity conditioning regimens have been aimed at both eliminating host-versus-graft reactions (graft rejection) and producing major anti-tumor effects. Conversely, nonmyeloablative conditioning regimens have relied on optimization of pre- and post-transplant immunosuppression to overcome host-versus-graft reactions, thereby allowing engraftment and eradication of tumors nearly exclusively via immune-mediated graft-versus-tumor effects. Remarkably, a minimally toxic regimen of 2 Gy total body irradiation with or without fludarabine followed by postgrafting immunosuppression with cyclosporine and mycophenolate mofetil has assured engraftment rates almost similar to those after myeloablative conditioning. While nonmyeloablative and reduced-intensity SCT have been associated with reduced regimen-related toxicities and have been curative for many patients with otherwise fatal hematological malignancies, challenges have remained in regard to acute graft-versus-host disease, infections, and disease progression.

Allogeneic hematopoietic stem cell transplantation and graft-versus-tumor effects

Most hematological malignancies display a dose-response susceptibility to radiation therapy and alkylating agents. Because myeloablation is the dose-limiting toxicity of many of these agents, allogeneic hematopoietic stem cell transplantation (SCT) was first developed as a way to rescue patients from severe myelosuppression occurring after administration of high-dose chemoradiotherapy.[1] Despite major improvements in supportive care in the last 40 years, the non-hematopoietic toxicities of high-dose conditioning has restricted its use to patients younger than 55-60 years. Given that median ages at diagnosis for patients with most hematologic malignancies range from 65-70 years,[1] the majority of such patients could not benefit from potentially curative allogeneic SCT. Further, some patients have medical cormorbidities, which exclude them from high-dose conditioning.

Since the late 1970s, it has become apparent that an important part of the efficacy of allogeneic SCT was mediated by immune reactions of the graft itself against recipient tumor cells, termed graft-versus-tumor effects.[2,3] Indeed, patients who developed acute and/or chronic graft-versus-host disease (GVHD) had lower risks of relapse than those who did not.[2-4] In addition, patients given syngeneic SCT and those given T-cell depleted grafts had substantially higher risk of relapse than those receiving unmanipulated grafts from allogeneic donors.[4] Furthermore, a direct evidence of the power of graft-versus-tumor effects came from the observations by Kolb et al. and Slavin et al. that donor lymphocyte infusions (DLI) were able to eradicate the malignancy in a number of patients who relapsed with chronic myeloid leukemia[5] or acute lymphoblastic leukemia (ALL)[6] after allogeneic SCT. Since then, DLI has also proven to be effective in a number of patients with acute myeloid leukemia (AML),[7] multiple myeloma (MM),[8] chronic lymphocytic leukemia (CLL),[9] non-hodgkin lymphoma (NHL),[10] and Hodgkin lymphoma (HL).[11]

Nonmyeloablative and reduced-intensity conditioning

Two key observations in 1997 led to the emergence of nonmyeloablative and reduced-intensity conditioning, respectively. First, Storb *et al.* demonstrated that optimizing post-transplant immunosuppression could not only control GVHD but also graft rejection.[12] Secondly, Giralt *et al.* and Slavin *et al.* showed that administration of purine analogs, such as fludarabine provided sufficient immunosuppression to allow sustained engraftment of HLA-matched allogeneic stem cells.[13,14] Based on these observations, several groups of investigators developed a number of reduced intensity[13-16] or truly nonmyeloablative[17-20] conditioning regimens for allogeneic SCT. Reduced intensity conditioning regimens have been aimed at eliminating host-versus-graft reactions (graft rejections) and producing major anti-tumor effects. Most reduced-intensity conditioning regimens have combined fludara-

bine with relatively high doses of busulfan (8 mg/kg),[21] melphalan (140 mg/m^2),[22] or thiotepa.[16] Conversely, nonmyeloablative conditioning regimens have relied on optimization of pre- and post-transplant immunosuppression to overcome host-versus-graft reactions, thereby allowing engraftment,[12,23] while eradication of tumors has depended nearly exclusively on the graft-versus-tumor effects.[24] Examples of nonmyeloablative conditioning regimens include low-dose (2 Gy) TBI with or without added fludarabine,[18] 8 Gy total lymphoid irradiation with anti-thymocyte globulin (ATG),[25] or fludarabine with cyclophosphamide.[17] Figure 1 shows commonly used conditioning regimens in relation to their immunosuppressive and myelosuppressive properties.

Typical complications of high-dose therapy, such as nausea, vomiting, pancytopenias, mucositis, and new-onset alopecia have been observed with most of reduced-intensity conditioning regimens, and sinusoidal obstructive syndrome has also been seen, although less frequently than after myeloablative conditioning.[21,22,26-29] Conversely, nonmyeloablative regimens produced only mild myelosuppression and few regimen-related toxicities,[30] and have been associated with a low 100-day incidence of nonrelapse mortality, even in elderly patients and those with comorbid conditions.[18] Typically, many transplants following nonmyeloablative conditioning could be carried out entirely in the outpatient setting.[31]

No randomized study thus far has compared nonmyeloablative versus reduced-intensity conditioning regimens. A recent analysis by the Société Française de Greffe de Moelle et de Thérapie Cellulaire (SFGM-TC) retrospectively compared outcomes in patients given grafts after 2 Gy TBI with or without added fludarabine (n=255; nonmyeloablative conditioning) or after fludarabine, busulfan plus ATG (n=465; reduced-intensity conditioning) as treatment for various hematological malignancies.[32] Three-year overall and disease-free survivals were both similar in the two groups.

Engraftment kinetics

By definition, nonmyeloablative and reduced-intensity conditioning regimens usually lead to an initial state of mixed chimerism defined as co-existence of hematopoiesis of host and donor origin.[33] Several factors have been associated with faster donor T-cell engraftment, including higher intensity of the conditioning regimen,[33,34] having received prior myelosuppressive chemotherapy,[35,36] the use of peripheral blood stem cells (PBSC) instead of marrow as a stem cell source,[20,37,38] a high number of CD34$^+$ and T-cells in the graft,[35,37,38] and intense post-grafting immunosuppression.[39]

Further, several authors have demonstrated correlations between engraftment kinetics and clinical events. Specifically, high levels (>50%) of donor T- and NK-cell chimerism 1 month after SCT have each been associated with lower risk of graft rejection.[18,36] While high day 14 to 42 donor T-cell chimerism levels were associated with high incidence of acute GVHD,[36,40] high donor NK cell levels had no such association.[41] Conversely, high donor NK cell[41] and T cell[40,41] chimerism levels early after SCT have each been associated with low relapse risk and good progression-free survival.

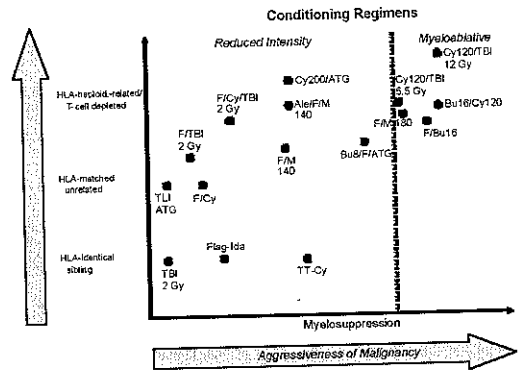

**Figure 1. Commonly used conditioning regimens in relation to their immunosuppressive and myelosuppressive properties.[83] Please note that this classification is not based on direct experimentation, and is thus hypothetical. Ale, alemtuzumab; ATG, antithymocyte globulin; Bu8, busulfan 8 mg/kg; Bu16, busulfan 16 mg/kg; Cy, cyclophosphamide; Cy120, cyclophosphamide 120 mg/kg; Cy200, cyclophosphamide 200 mg/kg; F, fludarabine; Flag-Ida, fludarabine/cytosine arabinoside/idarubicin; M, melphalan; M 140, melphalan 140 mg/m^2; M 180, melphalan 180 mg/m^2; TBI, total body irradiation; TLI, total lymphoid irradiation; TT, thiotepa. *(From Sandmaier BM, Storb R. "Reduced-intensity conditioning followed by hematopoietic cell transplantation for hematologic malignancies." In: Appelbaum FR, Forman SJ, Negrin RS, Blume KG (eds): Thomas' Hematopoietic Cell Transplantation. Oxford, UK: Wiley-Blackwell, p. 1043-1058, 2009. Used with permission from the authors.*

Non-myeloablative versus myeloablative conditioning

In order to determine the relative contributions of conditioning intensity and graft-versus-host reactions to transplant related complications, several retrospective studies compared transplant-related toxicities after nonmyeloablative versus myeloablative SCT. Not surprisingly, the hematological changes after nonmyeloablative conditioning were milder than those seen after myeloablative conditioning.[42] Specifically, neutrophil counts remained above 500 throughout the transplant period in a high proportion of patients given nonmyeloablative conditioning, while temporary grade IV neutropenia was the rule in patients given reduced-intensity or myeloablative conditioning. Further, patients given nonmyeloablative or reduced-intensity conditioning required less platelet and red blood cell transfusions than those given myeloablative conditioning.[43] Similarly, liver, kidney, gastro-intestinal, and lung toxicities were significantly reduced with nonmyeloablative conditioning.[44-47]

Three large retrospective studies from the European Group for Blood and Marrow Transplantation (EBMT) compared SCT outcomes of patients given various myeloablative versus various reduced-intensity/nonmyeloablative conditioning regimens as treatment for AML,[48] myelodysplastic syndrome (MDS),[49] or CLL[50] (Figure 2). Obviously, these studies were limited by the fact that fitter patients were probably more often proposed myeloablative regimens, while older and sicker patients were probably more often given nonmyeloablative or reduced-intensity conditioning. Nevertheless,

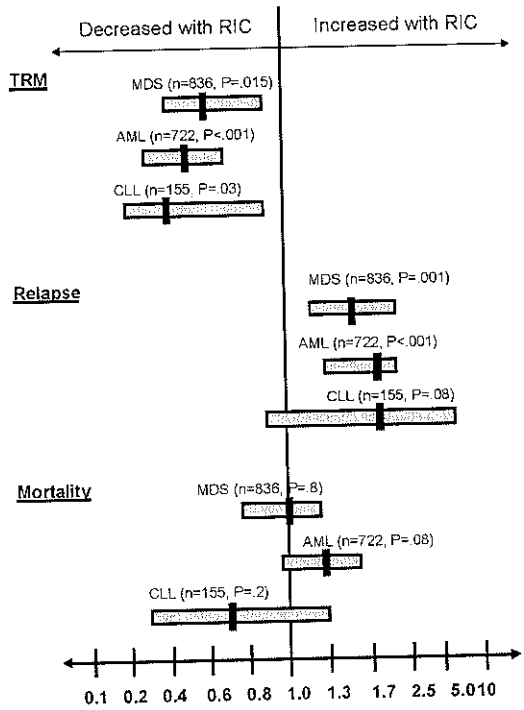

Figure 2. Retrospective studies comparing SCT outcomes after reduced-intensity (RIC) or myeloablative conditioning as treatment for myelodysplastic syndrome (MDS),[49] acute myeloid leukemia (AML),[48] or chronic lymphocytic leukemia (CLL).[50] TRM, transplant related mortality. The black bars show the hazard ratio and the grey bars the 95% confidence intervals.

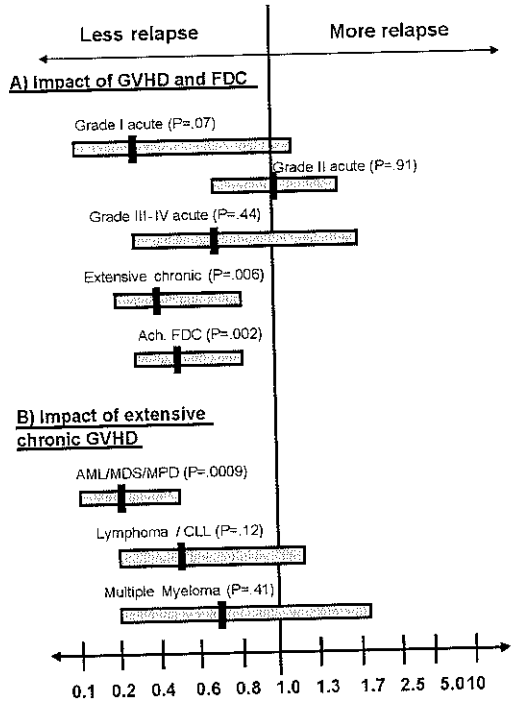

Figure 3. (A) Impact of acute and chronic GVHD and of achievement of full donor T-cell chimerism (Ach. FDC) on relapse risk in 322 patients reported in ref. 24 given grafts after 2 Gy TBI with or without fludarabine. (B) Impact of chronic GVHD on relapse risk according to disease group in the same study. The black bars show the hazard ratio and the grey bars the 95% confidence intervals.

these studies found similar disease-free and overall survival in the two groups of patients, since nonrelapse mortality was lower in nonmyeloablative patients, but relapse rate was lower in myeloablative recipients.[48-50]

Further, Scott *et al.* compared efficacy of SCT after nonmyeloablative (with 2 Gy TBI with or without added fludarabine; n=38) or myeloablative conditioning (with busulfan [16 mg/kg, targeted to 800 to 900 ng/mL] and cyclophosphamide [120 mg/kg; n=112]) in patients with MDS over 40 years of age.[51] In multivariate analyses adjusted for IPSS group and comorbidity at SCT, 3-year overall survival [(HR=1.2, p=0.56), progression-free survival (HR=1.1, p=0.60), progression rate (HR=1.3, p=0.43), and nonrelapse mortality (HR=1.0, p=0.94)] did not differ significantly between the two groups of patients.

More recently, Sorror *et al.* compared outcomes among patients with lymphoma or CLL given either nonmyeloablative (n = 152, consisting of 2 Gy TBI with or without added fludarabine) or myeloablative (n = 68) conditioning.[52] Outcomes were stratified by the hematopoietic cell transplantation (HCT)-specific comorbidity index (HCT-CI).[53,54] Among patients without comorbidity at SCT (HCT-CI = 0), survival and nonrelapse mortality were comparable for patients given nonmyeloablative or myeloablative conditioning (p=0.7 and p=0.7, respectively). In contrast, among patients with comorbidities (HCT-CI score ≥1) at SCT, the use of nonmyeloablative conditioning was associated with lower nonrelapse mortality (HR: 0.19; p<0.001) and better overall survival (HR: 0.33; p=0.007) in multivariate analyses. These observations suggest that the HCT-CI score might help in determining which patients might benefit from nonmyeloablative or reduced-intensity conditioning, and which others could safely receive myeloablative regimens.

Graft-versus-host disease and graft-versus-tumor effects after nonmyeloablative conditioning

Graft-versus-host disease

Several studies have shown slightly lower day-100 incidence of acute GVHD after nonmyeloablative than after myeloablative conditioning.[48,51,55,56] In contrast, the incidence of chronic GVHD has been comparable in patients given nonmyeloablative or myeloablative conditioning.[48,51,55,56]

Some reduced-intensity conditioning regimens have used *in vivo* T-cell depletion of the grafts (with either anti-thymocyte globulin (ATG) or alemtuzumab) in order to decrease the incidence of acute and chronic GVHD. While these strategies achieved their goal,[15,57] increased incidences of both infections (due to slower

immune recovery)[58] and disease relapse[59] were observed, especially when high doses of these agents were used.[32] Based on murine experiments, the Stanford University group investigated an innovative nonmyeloablative regimen that favored the presence of a high proportion of regulatory NK-T cells.[60] This regimen combined total lymphoid irradiation (TLI, 8 Gy) with ATG (Thymoglobulin, 7.5 mg/kg total dose), and post-grafting immunosuppression with mycophenolate mofetil and cyclosporine. Initial results in 37 patients with various hematological malignancies indicated that this regimen was indeed associated with a low incidence of acute and chronic GVHD, while graft-versus-tumor effects were apparently preserved,[25] some late relapses did occur.[61]

Association between graft-versus-host disease and graft-versus-tumor effects

There has been a close relationship between GVHD and graft-versus-tumor responses after myeloablative conditioning.[24,62,63] We investigated whether such a relationship existed for patients given nonmyeloablative conditioning in 322 patients given grafts from HLA-matched related (n=192) or unrelated (n=130) donors after 2 Gy TBI with or without added fludarabine.[24] Grade II, III and IV acute GVHD occurred in 44%, 11% and 3% of the patients, respectively, while extensive chronic was seen in 56% of the patients. While grades II and III-IV acute GVHD had no significant impact on relapse/progression, they were associated with increased non-relapse mortality and decreased progression-free survival. Conversely, extensive chronic GVHD was associated with decreased relapse/progression (p=0.006; Figure 3), and better progression-free survival (p=0.003). The greatest reduction of relapse risk in patients with chronic GVHD was observed in the subgroup of patients with AML or MDS (p<0.001; Figure 3), while a similar trend was observed in patients with lymphoma or CLL (p=0.12). In contrast, the study failed to show a significant association between GVHD and relapse risk in patients with MM (p=0.41).

Confirming these observations, several groups of investigators observed a significant association between occurrence of chronic GVHD and low relapse risk and high progression-free survival in patients with AML or MDS. Specifically, Blaise *et al.* analyzed outcomes of 33 patients with AML in first complete remission (CR) receiving allogeneic SCT from HLA-identical siblings following reduced-intensity conditioning with fludarabine, busulfan and ATG.[64] In a landmark analysis starting on day 100, occurrence of chronic GVHD was associated with a lower risk of relapse (0% versus 44%, p=0.007) and better disease-free survival (95% versus 53%, p=0.007). More recently, Valcarcel *et al.* studied data from 93 patients with AML (n=59) or MDS (n=34) given allogeneic SCT after a reduced intensity conditioning consisting of fludarabine and busulfan, and post-grafting immunosuppression with cyclosporine plus methotrexate or mycophenolate mofetil.[65] The 4-year overall and disease-free survival rates were 45% and 43%, respectively. The 4-year cumulative incidence of chronic GVHD was 53% (45% extensive), and its development was the major factor associated with lower relapse incidence and improved overall and disease-free survival. In contrast, acute GVHD was not associated with a lower risk of relapse.

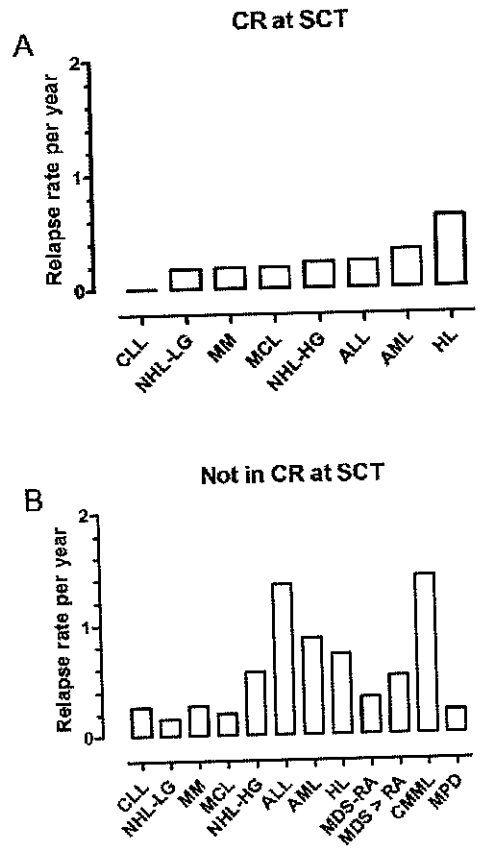

Figure 4. Susceptibility of disease groups to graft-versus-tumor effects. Relapse rates per patient year during the first 2 years after SCT corrected for follow-up and competing nonrelapse mortality in a cohort of 834 consecutive patients reported in ref 69 given grafts after 2 Gy TBI with or without added fludarabine: (A) in patients in complete remission (CR) at SCT. (B) In patients not in CR at SCT. CLL, chronic lymphocytic leukemia; NHL-LG, low-grade non-Hodgkin lymphoma; MM, multiple myeloma; MCL, mantle cell lymphoma; NHL-HG, high-grade NHL; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; HL, Hodgkin lymphoma; MDS-RA, myelodysplastic syndrome with refractory anemia; MPD, myeloproliferative disease; CMML, chronic myelomonocytic leukemia.

The impact of chronic GVHD on relapse risk in patients with MM has been more controversial. Indeed, two studies found lower risk of relapse and better progression-free survival in MM patients who developed chronic GVHD in time-dependent Cox analyses,[59,66] while two others failed to find such an association.[67,68] Finally, a recent analysis from the EBMT demonstrated in a landmark analysis that Hodgkin lymphoma patients who had chronic GVHD before 9 months after SCT had lower risk of relapse than patients who did not (p=0.008).[11]

Disease susceptibility to graft-versus-tumor effects

In order to assess the susceptibility of the different hematological malignancies to graft-versus-tumor effects, Kahl *et al.* analyzed the relapse risk according to

disease characteristics in a cohort of 834 consecutive patients (median age, 55 years; range, 5 to 74 years). They were given related (n=498) or unrelated (n=336) grafts after 2 Gy TBI alone (n=171) or combined with fludarabine (90 mg/m^2; n=663).[69] Besides the variation in tumor burden, which had a major impact on the relapse risk, graft-versus-tumor effects were most impressive in patients with indolent NHL, CLL and mantle cell lymphoma. Specifically, relapse rates per patient year (PY) during the first 2 years after SCT (corrected for follow-up and competing nonrelapse mortality) were between 0.0 and 0.24 in patients with CLL and MM in CR (most of whom received tandem autologous/allogeneic SCT), low-grade or mantle cell NHL in CR or in partial remission (PR), and high-grade NHL in CR (Figure 4). In contrast, patients with advanced myeloid and advanced aggressive lymphoid malignancies had rates of more than 0.50. Patients with lymphoproliferative diseases not in CR (except Hodgkin lymphoma and high-grade NHL) and myeloid malignancies in CR had rates of 0.26 to 0.37. The exact reasons for the variable graft-versus-tumor effects are unclear, but might include variability in kinetics of tumor cell growth, presentation and density of minor-histocompatibility (and perhaps tumor-specific) target antigens on tumor cells, susceptibility of tumor cells to cytotoxic cell kill, or access of tumor to donor cytotoxic cells.

Nonmyeloablative or reduced-intensity conditioning for cord blood or HLA-haploidentical stem cell transplantation

Given that HLA-matched donors can only be found for 50-80% of patients, depending on their ethnic group, there has been a considerable interest to extend the use of nonmyeloablative or reduced-intensity conditioning to cord blood or HLA-haploidentical SCT. Due to greater degrees of histoincompatibility, the use of such alternative donors has been associated with increased risks of both graft rejection and GVHD. Brunstein *et al.* investigated the feasibility of unrelated cord blood transplantation after nonmyeloablative conditioning consisting of fludarabine (200 mg/m^2), cyclophosphamide (50 mg/kg), and 2 Gy TBI.[70] Data from 110 patients (median age 51 [range, 17-69] years) with hematologic malignancies (n=106) or aplastic anemia (n=4) given one (n=17) or two (n=93) unrelated cord blood units have been recently analyzed.[70] Thirty-nine patients not given myelosuppressive chemotherapy in the 6 months preceding SCT were also given ATG. Postgrafting immunosuppression consisted of MMF and CSP. Cord blood units were predominantly 1- or 2-HLA-antigen mismatched with the recipient. Primary neutrophil recovery occurred in 92% of patients at a median of 12 days (range, 0-32) after SCT, while the cumulative incidence of sustained donor engraftment was 85%. The cumulative incidence of grade II-IV and grade III-IV acute GVHD were 59% and 22%, respectively, while chronic GVHD was seen in 23% of patients. Three-year overall and progression-free survivals were 45% and 38%, respectively. The most frequent causes of death were disease relapse/progression (n=27), infection (n=12) and GVHD (n=6).

O'Donnell *et al.* investigated feasibility of unmanipulated HLA-haploidentical marrow transplantation after nonmyeloablative conditioning combining fludarabine (150 mg/m^2), cyclophosphamide (29 mg/kg), and 2 Gy TBI in 68 patients with advanced hematologic malignancies (n=67), or paroxysmal nocturnal hemoglobinuria (n=1).[71] Postgrafting immunosuppression consisted of MMF, tacrolimus, and cyclophosphamide, the latter given at a dose of 50 mg/kg on day 3 or on days 3 and 4 after SCT. Nine patients (13%) had graft rejection, while 59 achieved sustained donor engraftment. The 200-day cumulative incidences of grades II-IV and III-IV acute GVHD were 34% and 6%, respectively, while chronic GVHD occurred in less than 30% of the patients. With a median follow-up of 2 years, 2-year overall and disease-free survivals were 36% and 26%, respectively.

Conclusions and perspectives

Nonmyeloablative and reduced-intensity conditioning regimens have allowed older patients, those who had failed a prior myeloablative SCT,[72] and those with comorbidity to benefit from the potentially curative graft-versus-tumor effects. While graft-versus-tumor effects were most impressive in patients with CLL, indolent NHL or mantle cell lymphoma, patients with chemosensitive aggressive lymphoma or Hodgkin lymphoma, and those with MDS or myeloprolifarative disorders with less than 5% marrow blasts, or acute leukemias in complete remission also had encouraging results following nonmyeloablative or reduced-intensity conditioning.[25] Anti-tumor responses in some disease types required extended periods, with some patients achieving a CR more than 1 year after SCT.[18,24] Occurrence of chronic GVHD has been associated with lower risk of relapse and better progression-free survival in several studies, and particularly so in patients with AML or MDS.

For patients with non-malignant diseases, ongoing efforts are directed at further decreasing the intensity of the conditioning regimen needed to achieve sustained donor engraftment. Promising strategies aimed at reducing the intensity of host immune responsiveness include pre-transplant administration of donor cells together with T-cell costimulation blocking agents,[73,74] or pre-transplant administration of radiolabeled monoclonal antibodies directed against T-cell receptors.[75]

For patients with hematological malignancies, ongoing efforts are directed at better preventing acute GVHD, better treating extensive chronic GVHD, and preventing relapse in patients with aggressive malignancies by reducing tumor burden at the time of SCT, through preceding cytoreductive autologous SCT in the cases of multiple myeloma,[76] or the addition of radiolabeled monoclonal antibodies against CD20 or CD45 in case of advanced non-Hodgkin lymphoma,[77] or myeloid malignancies.[78] The addition of disease-targeted therapy, such as imatinib, thalidomide, bortezomib, lenalidomide or monoclonal antibodies after transplant seems also promising.[9,78-81] Finally, recent progress in large scale identification of minor-histocompatibility antigens[82] might allow the adoptive transfer of T-cell populations specifically directed against recipient minor-histocompatibility antigens expressed only by hematopoietic (including tumor) recipient cells, thus increasing graft-versus-tumor effects without inducing GVHD.

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