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Non-myeloablative allogeneic hematopoietic stem cell transplants

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Conventional allogeneic hematopoietic stem cell transplantation (HSCT) has been increasingly used over the last 30 years to cure many hematologic diseases.[1] This strategy was founded on the principles of maximal tumor cytoreduction, according to the well-demonstrated dose-response relationship of chemoradiotherapy, and of adequate immunosuppression, to permit engrafment even after non-HLA-genotypically identical donor transplants.[2-4] Despite maximally tolerated doses of chemoradiotherapy, relapse probabilities remain high.[4-7] High-dose chemoradiotherapy is also associated with substantial transplant-related toxicity and a significant incidence of acute and chronic graft-versus-host disease (GvHD).[8] Because of these characteristics, allogeneic HSCT is offered only to relatively young patients (< 55 years old) with optimal organs and general performance status.[1]

There is a large amount of evidence that the donor stem cells may exert not only a repopulating role but also a graft-versus-tumor effect (GvT) due to recognition of malignant host cells.[9-11] Based on these data, attempts have recently been made to diminish transplant-related morbidity and possibly mortality by administering relatively non-toxic, non-myeloablative doses of chemotherapy or radiation therapy prior to allogeneic transplantation,[12-15] thus permitting the treatment of older patients and patients with medical infirmities. The main aim of this new strategy is to create a state in the patient in which the host's and the donor's hematopoietic systems co-exist (mixed chimerism).[12] The induction of mixed chimerism, moreover, may serve as a platform for the development of a graft-versus-tumor effect. To date, many approaches have been proposed in order to achieve this goal demonstrating that while it is possible to achieve a stable mixed chimerism after a non-myeloablative allogeneic transplantation, the effectiveness of these approaches, in terms of disease control, remains to be determined.

This review will briefly describe the preclinical evidence for these non-myeloablative transplant strategies, describe preliminary clinical experience and discuss the rationale for considering such approaches as transplant strategies.

Preclinical data

The observation that in some severe non-malignant disorders such as $-thalassemia, sickle cell disease, aplastic anemia, and autoimmune diseases, persistence or establishment of a state of mixed hematopoietic chimerism conferred an important clinical benefit[16-17] led many centers to investigate whether establishing a state of mixed chimerism would be possible in hematologic malignancies. This would have been the starting point of a strategy to treat malignancy by exploiting an allogeneic graft-versus-tumor effect.

Systematic *in vitro* and *in vivo* studies have been carried out in Seattle and Boston to find possible strategies to achieve a state of stable mixed chimerism.[18-21]

In a murine model, stable mixed lymphohematopoietic chimerism was achieved following low-dose total-body irradiation (TBI) (300 cGy) or cyclophosphamide (200 mg/kg), peri-transplant monoclonal anti-T-cell antibody, thymic irradiation and fully mismatched donor bone marrow transplantation (BMT).[20,21] With the addition of post-transplant cyclosporine (CYA), these mice were completely protected from acute and chronic GvHD. Remarkably, these animals were also resistant to the induction of GvHD following delayed donor lymphocyte

infusion (DLI) (beginning on day +35 post-transplant), despite a potent lymphohematopoietic graft-versus-host response which converted their state of mixed chimerism to one of fully donor hematopoiesis.

Studies performed at the Fred Hutchinson Cancer Research Center in dogs formed the preclinical milestone for a clinical translation of a mixed chimerism approach, based on the premise that a powerful post-graft immunosuppressive regimen would not only prevent GvHD but also host-versus-graft reactions.

In an attempt to obtain mixed chimerism in MHC-matched littermates non-lethal doses of 200 cGy TBI and the use of post-transplant CYA alone were not sufficient to establish stable mixed chimerism. The combination of methotrexate (MTX) and CYA was somewhat more effective (with at least 2 out of 6 animals becoming stable mixed chimeras). The most effective combination appeared to be CYA and mycophenolate mofetil (MMF) (11 out of 12 dogs).[19,20]

Preliminary clinical data

With this positive preclinical experience as a background, the clinical investigation of non-myeloablative transplant regimens began in a number of transplant centers. Several published reports have demonstrated the feasibility of achieving allogeneic engraftment following non-myeloablative conditioning therapy.[12-15] These reports indicated the tolerability of most of these regimens, and showed that mixed lymphohematopoietic chimerism can be intentionally induced, even across major HLA barriers. In some cases mixed chimerism led to a potent antitumor response, and that represented a particular important proof of principle in the field of clinical allogeneic stem cell transplantation.

Based on their dog model showing that mixed chimerism is reliably achieved following low-dose total body irradiation (200 cGy) and post-transplant immunosuppression (MMF and CYA), more than 156 patients with hematologic malignancies, ineligible for conventional allografting due to age, prior therapy or organ dysfunction were treated in Seattle. Seventy-three patients were conditioned with 200 cGy TBI alone, and 18% experienced non-fatal graft rejections. With the addition of fludarabine, rejections have become the exception. Most patients did not need platelet transfusions, and a few received red blood cell transfusions. The majority of HSCT were carried out entirely in the outpatient setting. Typical side effects of HSCT, such as alopecia, mucositis, diarrhea, and veno-occlusive disease of the liver, were absent.[22]

There were significantly fewer bacterial infections than seen after conventional HSCT.[23] Grade II-IV acute GvHD occurred in 57% of patients, with 37% having grade II, 13% grade III, and 7% grade IV disease. Chronic GvHD was seen in 65% of patients; however, it responded well to therapy. Fatal progression of underlying diseases occurred in 18% of patients. Non-relapse mortality at 1 year was 20%. With a median follow-up of 220 (range 100-1026) days, 62% of patients were alive, and progression-free survival was 50%. Complete remissions generally occurred slowly over periods of months.

Based on this first experience, the Seattle group started single disease protocols and also the unrelated non-myeloablative program. Among the different diseases, the results achieved in multiple myeloma deserved great consideration by the scientific community. In a multicenter phase II trial 32 patients with previously treated stage II/III myeloma were treated with autologous HSCT followed by a non-myeloablative allogeneic HSCT from HLA-identical siblings according to the Seattle regimen. Thirty-one of the 32 patients received non-myeloablative allogeneic HSCT with medians of 0 days of hospitalization, neutropenia and thrombocytopenia. TRM at day 100 was 6% (one death after autologous HSCT and one from progressive disease after allogeneic HSCT). Forty-five percent of patients developed acute grade II-IV GvHD, and 55% developed chronic GvHD requiring therapy. The response rate was 84% with 53% CR and 31% PR and only two progressions to date. This study provided the rationale for a phase III trial comparing standard autologous HSCT to this two-step allogeneic approach.[24]

The first reports on the unrelated program, despite a not negligible rejection-rate (11%), confirmed the feasibility and safety of the Seattle regimen in this setting of transplants as well. Remarkably, many patients with chemorefractory hematologic disease achieved tumor control after this approach.[25]

Using a similar non-myeloablative preparative regimen to that used in the murine model of Sykes *et al.*,[20,21] the Boston group induced mixed lymphohematopoietic chimerism in patients with chemoradiotherapy-refractory hematologic malignancies.[26] Twenty-eight patients received an HLA-matched donor transplant while 16 received an HLA-mismatched donor transplant. Of 23 evaluable recipients of HLA-matched donor transplantation, 20 have achieved stable mixed lymphohe-

matopoietic chimerism. Ten patients with stable mixed chimerism, who had no evidence of GvHD, received DLI beginning on day +35 post-transplant. Conversion of mixed chimerism to full donor hematopoiesis occurred in six of the ten patients. Full donor T-cell chimerism was not necessary for the development of acute GvHD (or antitumor response). Notable antitumor responses have been seen in the majority of these patients with refractory hematologic malignancies (7/23 evaluable patients with chemorefractory Hodgkin's disease or NHL achieved a partial remission and eight a complete response). Twenty-two patients are reported to be alive. Thirteen of these 22 patients were evaluable for response, and eight were clinically progression free. The incidence of acute GvHD grade # 2 was low (29%) allowing for the early administration of DLI. TRM was 10%. Several patients have had a conversion of chimerism and achieved complete remission without the development of severe GvHD.

Recently the M.D. Anderson Cancer Center group published the results of 3 subsequent trials of reduced intensity conditioning with melphalan (180 mg/m^2) and purine analogs (mostly fludarabine 125 mg/m^2) for the treatment of hematologic malignancies.[27] Eighty-six patients (of whom 8 received cladribine instead of fludarabine) considered ineligible for conventional allogeneic SCT were treated according to that regimen. Forty of these patients received their graft from a matched unrelated donor. The status at transplant was 1st remission (n = 7), untreated 1st relapse or subsequent remissions (n = 16) and refractory disease (n = 63). Eighty patients had donor cell engraftment between 80% and 100% by day 30. Acute GvHD prophylaxis was tacrolimus and methotrexate (5 mg/m^2); the probability of grade II-IV acute GvHD was 0.49 and 16/41 deaths before day 100 were due to GvHD. The risks of developing acute GvHD and dying were higher in the unrelated group (62% vs 41% and 11/40 vs 4/46, respectively). The overall 2-year survival probability was 0.28 for all patients. For patients in 1st CR and for those in untreated 1st relapse or subsequent remissions disease-free survival was 57% and 49%, respectively. TRM at day 100 was 37.4% for the fludarabine-treated group while it was 87.5% for the cladribine group.

Khouri *et al.*[28] treated 20 patients with follicular or small cell lymphocytic lymphoma after relapse from a prior response to conventional chemotherapy. The preparative regimen was fludarabine (25 mg/m^2 given daily for 5 days or 30 mg/m^2 daily for 3 days) and intravenous cyclophosphamide (1 g/m^2 given daily for 2 days or 750 mg/m^2 daily for 3 days). Thirteen patients received peripheral blood stem cell transplants from HLA-identical sibling donors. Nine patients received rituximab in addition to chemotherapy. Hematologic recovery was prompt and sustained in all patients and none developed graft failure. All patients had evidence of donor cell engraftment; the median percentage of donor cells at 1 month after transplantation was 80% (range, 10-100%). The cumulative incidence of acute grade II to IV GvHD was 20%. Chronic GvHD developed in 8 patients. All patients achieved a complete remission. None of the total group has relapsed. The median follow-up period was 21 months (5-46 months). Seventeen patients (85%) remain alive and in complete remission.

Using a busulfan-based preparative regimen, Slavin *et al.*[14] demonstrated excellent tolerability and favorable survival probabilities in 26 patients with hematologic malignancies and four patients with genetic diseases. Preparative therapy consisted of busulfan at a dose of 8 mg/kg, plus fludarabine 180 mg/m^2 and anti-T-lymphocyte globulin. GvHD prophylaxis consisted of CYA. Twenty-five patients received HLA-identical sibling donor transplants. One patient received stem cells from a donor with a single antigen mismatch at the A and C locus. Treatment was generally well tolerated. All patients had evidence of donor engraftment. In 9 of 26 evaluable patients, transient mixed chimerism was observed. Acute GvHD occurred in 12 of 26 patients. Six patients developed grade III-IV GvHD. Limited chronic GvHD developed in 9 of 25 evaluable patients. At a median of eight months post-transplant, 22 of 26 patients (85%) were alive, 21 of whom (81%) were clinically disease free.

Bacigalupo[29] explored a regimen with thiotepa (10 mg/kg × 1 day) and cyclophosphamide (50 mg/kg × 2 days) in 33 patients with a median age of 52 years (range 4-60) transplanted for hematologic disease from identical siblings. The source of hematopoietic stem cells was bone marrow (n=17) or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood (PB) (n=16). GvHD prophylaxis consisted of CYA and a short course of methotrexate. Acute grade III-IV GvHD occurred in 3% of patients. Chronic GvHD was seen in 45% of patients, with a significant difference between PB (69%) and BM transplants (23%) (p=0.009). For BM grafts the actuarial 2-year TRM was 6%, relapse was 56% and survival 87%; for PB grafts, these figures were, 27%, 33%, and 68%, respectively. Twenty-five patients are alive at a median follow-up of 762 days (range

216-1615) and 20 patients (60%) remain disease-free. Thirteen patients (39%) received donor lymphocyte infusion (DLI) either for persisting or relapsing disease and 6 patients had complete remission. Corradini *et al.*[30] described the engraftment kinetics after non-myeloablative therapy with thiothepa (10 mg/kg ×1 day), cyclophosphamide (30 mg/kg/d ×2 days) and fludarabine (25 mg/m^2/d ×2 days) and HLA-matched or one-antigen mismatched donor blood stem cell transplantation in 45 patients with hematologic malignancies. Patients who did not achieve clinical and molecular remission were eligible for monthly escalating doses of DLI. GvHD prophylaxis consisted of CYA and methotrexate. All patients engrafted. The probability of grades II-IV and III-IV acute GvHD was 47% and 13%, respectively. The probability of non-relapse mortality, progression-free survival, and overall survival was 13%, 57%, and 53%, respectively.

Recently another Italian group published[31] the results of a combined transplant approach autografting followed by a non-myeloablative allograft in 15 patients with advanced resistant Hodgkin's and non-Hodgkin's lymphoma. At a median of 61 days after autotransplant patients were conditioned with cyclophosphamide (300 mg/m^2/day) and fludarabine (30 mg/m^2/day) for 3 days and received peripheral stem cells from HLA identical sibling. CYA and MTX were given as GvHD prophylaxis. All patients promptly engrafted. Seven patients developed acute GvHD and 2 extensive chronic GvHD; TRM was 13%. Five patients were in continuous CR at the time of the report. The combined approach was reported to be safe and have a high response rate.

Solid tumors

Because of the significant TRM following allogeneic HSCT, few investigators have performed allogeneic transplants in non-hematologic malignancies. Two groups investigated the use of allogeneic HSCT in breast cancer.[32,33] In both cases evidence of a graft-versus-breast cancer effect was reported. These preliminary reports suggested the existence of a GvT effect also in solid tumors but the toxicity of the approach stopped further investigations in this field. Non-myeloablative allogeneic transplants with their low-toxicity profile offer possibilities for this hypothesis to be explored in greater detail.

Childs *et al.*[34] achieved regression of tumor in 10/19 (53%) patients with metastatic renal cell carcinoma who were treated with an HLA-identical sibling allogeneic HSCT. TRM was 10%. The median interval of four months from pretransplantation preparative chemotherapy to the first signs of disease regression, the observation that regression occurred only after complete donor T-cell chimerism had been established, and the association of graft-versus-host disease with regression of metastases were all consistent with the occurrence of an antitumor effect that was mediated by the donor's T-cells. These results led many centers to start non-myeloablative allogeneic HSCT protocols in solid tumors. Some preliminary data have been published in abstract form and most of them confirm the existence of a GvT effect also in solid tumors.

Other applications of non-myeloablative preparative regimens for allogeneic stem cell transplantation

Given the excellent tolerance of these non-myeloablative regimens and the high rates of alloengraftment, even following transplants from HLA-mismatched donors, there has been considerable interest in extending these transplant strategies to patients with non-malignant disease.[14,35,36] These include the genetic diseases described by Slavin *et al.* ($-thalassemia major, Fanconi's anemia, Blackfan Diamond anemia and Gaucher's disease).[14] In a separate report[35] the Hadassah University Hospital transplant group reported the case of a child with Fanconi's anemia and leukemic transformation who underwent successful transplantation following a non-myeloablative conditioning regimen consisting of fludarabine, cyclophosphamide and ATG. Two patients with primary T-cell immunodeficiency have been described who received only post-transplant immunosuppression with MMF and CYA.[37] Stable multilineage mixed chimerism was seen in both patients. Both patients developed grade II acute GvHD that responded to prednisone therapy. Studies of immune reconstitution in one patient showed a significant increase in the numbers of T-cells, T-cell subsets and T-cell proliferative responses *in vitro*.

Given the therapeutic dilemmas that have surrounded the application of allogeneic BMT for conditions such as sickle cell anemia and thalassemia major, particularly the early transplant-related mortality risk among a group of patients who may have prolonged survival with medical therapy alone, these non-myeloablative transplant approaches may have particular benefit. The advantages include a low risk of transplant-related mortality, the development of stable mixed erythroid chimerism and the lack of a need to enhance a GvL effect by giving delayed DLI, all of which make this strategy particularly attractive for non-malignant disorders.

Remaining questions and future directions

The feasibility of achieving a state of stable mixed lymphohematopoietic chimerism, even following transplants from HLA-mismatched donors, and the use of this mixed chimerism as a platform for subsequent adoptive cellular immunotherapy, are the first important questions regarding non-myeloablative allogeneic stem cell transplant strategies answered by several centers. However, it is likely that just as many questions remain regarding the utility of these approaches as have been answered. The optimal non-myeloablative regimen has not been determined and it is unlikely that a single conditioning regimen will prove to be superior to others or applicable to all situations. Rapidly progressing hematologic malignancies will, in most situations, require initial cytoreduction of the tumor (thus probably requiring a reasonably aggressive chemotherapeutic preparation) in order to test whether a later GvL effect (such as, for example, that induced or potentiated by later DLI) will be operative. On the other hand, indolent hematologic malignancies (for instance, early CLL) or non-malignant disease may be optimally managed with conditioning regimens of lesser intensity. Long-term follow-up will also be required to determine the toxicity of these regimens.

Non-myeloablative HSCTs appear to be associated with significantly less transplant-related morbidity, and possibly mortality.[14,20-22,24-30] The incidence of acute GvHD still represents an important issue. The prophylaxis of this complication as well as its duration need to be studied and defined. Furthermore mixed chimerism may create an important platform for the administration of adoptive cellular immunotherapy (DLI) and for the optimization of the GvL effect; however this procedure is still too toxic and non-specific. The identification of tumor antigens for donor-vaccination or adoptive transfer of tumor-specific cytotoxic T-lymphocytes will, in the future, represent possible ways for reducing these limitations.[37] Regarding the optimal source of stem cells, both bone marrow and growth factor-mobilized peripheral blood stem cells have been used. There is a suggestion that immunologic recovery is faster following transplants in which peripheral blood stem cells are used than in those in which bone marrow is grafted.[38,39] However, hematologic recovery is usually rapid following non-myeloablative preparative regimens, regardless of the stem cell source. Thus, a comparison of antitumor efficacy, when bone marrow or peripheral blood stem cells are used, needs to be performed. Theoretically, the markedly increased number of T-cells in a peripheral blood stem cell allograft could promote earlier and more complete donor chimerism and obviate the potential platform for delivering DLI.

The published series of non-myeloablative stem cell transplants have primarily involved patients with refractory hematologic malignancies or those who were otherwise poor candidates for conventional allogeneic HSCT. As questions about feasibility and safety were answered, testing this approach in patients with less advanced disease and prospective randomized trials will be necessary to determine safety and efficacy in other diseases such as multiple myeloma and CML. Further studies of the efficacy of non-myeloablative transplant strategies for HLA-mismatched donor transplantation will also be required. These studies will be important, both because of the frequent need for alternative donor sources and because of the enhanced antitumor (i.e., GvL) effect that may occur in the setting of HLA incompatibility.

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