REVIEW

Novel conditioning regimens for bone marrow transplantation

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Abstract: Bone marrow transplantation (BMT) has evolved into an effective strategy for the treatment of hematological and oncological disorders. Radiotherapy and chemotherapy are used as conditioning regimens prior to BMT to suppress host immunity and reduce tumor burden. High doses of total body irradiation are conventionally administered along with alkylating agents, ie, the myeloablative regimen, to help ensure rapid engraftment of donor cells and to prevent relapse. However, the toxicity of the myeloablative conditioning regimen and unacceptable nonrelapse mortality rules out this approach for older patients by whom less intense preparative regimens are likely to be better tolerated. The reduced-intensity and nonmyeloablative conditioning regimens have been demonstrated by many investigators to be novel approaches resulting in a lower nonrelapse mortality rate and lower incidence of severe acute graft versus host disease. Here, we review the conditioning regimens employed as a pretreatment for BMT, and focus on the novel conditioning regimens and cutting edge developments.

Keywords: myeloablative conditioning regimen, reduced-intensity conditioning, nonmyeloablative conditioning regimen, relapse, nonrelapse mortality, graft versus host disease

Introduction

Bone marrow transplantation (BMT) was originally developed to treat congenital immunodeficiencies and hematologic disorders.[1,2] BMT has also become a powerful strategy for treating autoimmune and metabolic diseases.[3–7] Diseases frequently encountered in BMT are listed in Table 1. Radiotherapy and/or chemotherapy are prerequisites for recipients of BMT, because these conditioning regimens are essential for successful transplantation. Because the majority of BMT procedures are performed for the treatment of malignant disease, the conditioning regimens could be used to provide tumor cytoreduction and ideally disease eradication. The therapeutic effects of BMT on malignancies are also mediated via induction of the graft versus tumor effect by immunocompetent cells in the graft. Conditioning regimens that can minimize graft versus host disease without jeopardizing engraftment and graft versus tumor effects are being explored.[8]

The intensity of the conditioning regimens varies significantly. Based on the expected duration and reversibility of cytopenia after BMT, Bacigalupo et al classified the conditioning regimens into three categories, ie, myeloablative, reduced-intensity, and nonmyeloablative conditioning regimens.[9] Myeloablative conditioning regimens result in irreversible cytopenia, and stem cell support is required after BMT. The high-dose radiotherapy and chemotherapy used in the myeloablative conditioning regimens

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1

Table 1 Diseases frequently encountered in bone marrow transplantation

Autologous BMT	Allogeneic BMT
Multiple myeloma	Acute myeloid leukemia
Non-Hodgkin lymphoma	Acute lymphoblastic leukemia
Hodgkin disease	Chronic myeloid leukemia
Acute myeloid leukemia	Chronic lymphocytic leukemia
Neuroblastoma	Myeloproliferative disorders
Germ cell tumors	Myelodysplastic syndromes
Autoimmune disorders	Multiple myeloma
	Non-Hodgkin lymphoma
	Hodgkin disease
	Aplastic anemia

Note: Multiple myeloma continues to be the most common indication for autotransplantation and acute myeloid leukemia for allogeneic transplantation.

reduce immunocompetent cells in the recipient, permitting rapid engraftment of even unrelated, mismatched donor bone marrow cells. However, the myeloablative conditioning regimens are associated with considerable morbidity and mortality.[10] Therefore, these approaches have been restricted to young patients without comorbidities, and 50 years is considered an upper age limit.

In contrast with the consensus on a definition of myeloablative conditioning regimens, there are different opinions about reduced-intensity and nonmyeloablative conditioning regimens. Researchers sometimes refer to some of the conventional nonmyeloablative regimens as "reduced-intensity" conditioning regimens.[11–13] "Reduced-intensity conditioning" has also been used instead of "nonmyeloablative" directly.[14] Even though these regimens have been variably named nonmyeloablative conditioning or reduced-intensity conditioning regimens, they share one important characteristic, ie, both result in reversible myelosuppression (usually within 28 days) when given without stem cell support.[12] Above all, these methods use lower doses of cytoreductive treatments and result in low nonhematological toxicity. Some researchers use terms such as "intermediate-intensity" conditioning and semi-intensive conditioning rather than reduced-intensity conditioning.[15,16] Therefore, we have put reduced-intensity and nonmyeloablative conditioning regimens together in this review when discussing those regimens that are not myeloablative and are less toxic.[17–19]

Here, we review the conditioning regimens that are performed as pretreatments for BMT, and focus on some novel conditioning methods (reduced-intensity and nonmyeloablative conditioning regimens) with lower intensity that have expanded the use of BMT to older patients and to those with comorbidities.

Myeloablative conditioning regimens

Total body irradiation was the first conditioning method developed from research of radiation exposure and has been widely used in the conditioning regimens for its powerful immunosuppressive effects and activity against a variety of malignancies. Early myeloablative total body irradiation regimens were carried out using single large fractions of 8–10 Gy. However, such treatment was not tolerated well and was associated with serious toxicity, which resulted in interstitial pneumonitis, and severe nausea/vomiting.[20,21] To reduce the side effects of these high doses of total body irradiation while maintaining or improving efficacy, both fractionation and reductions in dose rates were developed.[22,23]

Host bone marrow can also be ablated with chemotherapy. Furthermore, chemotherapy can reduce or eradicate the tumor burden, while reducing the long-term sequelae of total body irradiation, including cataracts, sterility, and secondary malignancies. The combination of busulfan and cyclophosphamide is currently the most widely used myeloablative conditioning regimen without incorporating total body irradiation to treat malignant and nonmalignant hematological disorders with allogeneic BMT.[24–26] Yet, specific metabolites of cyclophosphamide are known to be associated with increased transplantation-induced mortality after conditioning, especially with busulfan. Therefore, fludarabine, a purine analog, has been used in an attempt to replace the cyclophosphamide in the busulfan and cyclophosphamide combination for myeloablative allogeneic BMT as well as nonmyeloablative transplantation. Fludarabine has considerable efficacy in both immunosuppression and tumor cell killing with minimal extramedullary toxicity. The regimen of busulfan and fludarabine has exhibited lower nonrelapse mortality and higher overall survival in patients with low-risk disease than busulfan and cyclophosphamide.[25,27]

Other possible alkylating agents, such as nitrosoureas (eg, carmustine), melphalan, thiotepa, and etoposide, have been included in the conditioning regimens in some trials. For example, the combination of carmustine, etoposide, cytarabine, and melphalan (BEAM) was designed to provide antilymphoma activity without the toxicity of total body irradiation. BEAM has proven to be an effective preparative regimen for its feasibility and tolerability in patients with lymphoma treated by both autologous and allogeneic BMT.[28,29]

Total body irradiation is often combined with chemotherapy in conditioning regimens and appears to provide benefit over conditioning with chemotherapy alone in many settings.[30–32] The combination of cyclophosphamide and total body irradiation (CyTBI) is considered to be one of the

standard regimens by many transplant centers.[23,26,33,34] Some studies have shown the combination of alkylating agents and total body irradiation to have a number of advantages in the conditioning for treatment of high-risk malignancies and solid tumors but with less toxicity.[35] CyTBI and busulfan and cyclophosphamide are currently the most commonly used regimens in all BMT clinical practice, including myeloablative, reduced-intensity, and nonmyeloablative conditioning regimens.[11,26,34] Comparative studies on clinical therapeutic effects of CyTBI and busulfan and cyclophosphamide have indicated that different regimens and types of malignant diseases may affect the outcome.[36] The myeloablative regimens mentioned above are summarized in Table 2.

Reduced-intensity and nonmyeloablative conditioning regimens
Pros and cons of reduced-intensity and nonmyeloablative conditioning regimens

Although dose intensification of myeloablative conditioning regimens had been shown to be effective in reducing the incidence of relapses, it resulted in unacceptable nonrelapse mortality due to regimen-related toxicity. Many studies have endeavored to identify an ideal conditioning regimen that would provide sufficient disease control to allow for sustained remission but without inducing unacceptable levels of toxicity and nonrelapse mortality. Reduced-intensity and nonmyeloablative conditioning regimens have been offered as alternatives to conventional high-dose radiotherapy and

chemotherapy for older patients undergoing BMT on the basis that the less intense preparative regimens are likely to produce considerably less organ toxicity, so would be better tolerated by such patients. One report compares the outcome of myeloablative and nonmyeloablative conditioning regimens in patients older than 50 years, and suggests that the nonmyeloablative conditioning regimen led to improved overall survival at one year and 2 years. A significantly lower nonrelapse mortality rate was observed in the nonmyeloablative conditioning regimen group than in the myeloablative conditioning group (32% versus 50%).[37] For patients who were heavily pretreated and already refractory to therapy, such as in indolent lymphoma, the majority demonstrated donor engraftment and there was a high rate of complete remission.[13] One third of patients who underwent nonmyeloablative conditioning had failed prior high-dose myeloablative conditioning BMT.[38] Therefore, reduced-intensity and nonmyeloablative conditioning regimens have been used with increasing frequency, particularly in older patients with hematological malignancies and in patients considered at high risk for treatment-related toxicity and mortality associated with high-dose myeloablative conditioning regimens.[39,40]

Secondly, reduced-intensity and nonmyeloablative conditioning regimens may reduce the risk of severe acute graft versus host disease. These regimens cause only limited host damage, which may subsequently translate into less release of inflammatory cytokines which, it has been proposed, provide a proinflammatory milieu for development of graft versus host disease.[41] In addition, development of transient mixed donor-host chimerism after reduced-intensity and nonmyeloablative conditioning regimens may facilitate the establishment of mutual tolerance, which in turn downregulates the activity of graft versus host disease.[42] Residual host T cells also play a role in the suppression of graft versus host disease. Results from the Fred Hutchinson Cancer Research Center showed that the incidence of severe acute graft versus host disease was significantly lower in nonmyeloablative conditioning patients (grades III–IV acute graft versus host disease, 17% in the nonmyeloablative conditioning group versus 35% in the myeloablative conditioning group).[38] There is some controversy regarding the incidence of severe acute graft versus host disease in patients given reduced-intensity and nonmyeloablative conditioning regimens.[37] The timing of onset of acute graft versus host disease after reduced-intensity and nonmyeloablative conditioning regimens is delayed, and may develop after day 100, at a time when chronic graft versus host disease is usually diagnosed after

Table 2 Summary of frequently used myeloablative regimens

Regimen	Radiotherapy and chemotherapy	Total dose	Reference
BuCy	Busulfan	po: 16 mg/kg or	22–26,37,
	Cyclophosphamide	iv: 12.8–16 mg/kg	38
		iv: 120 mg/kg or	
		3.6 g/m^2	
BuFlu	Busulfan	po: 16 mg/kg or	25,27
	Fludarabine	iv: 12.8 mg/kg	
		iv: 180–200 mg/m^2	
CyTBI	Cyclophosphamide	iv: 120 mg/kg or	23,26,33,
		3.6 g/m^2	34,38
	TBI	8–15.75 Gy	
BEAM	Carmustine	iv: 300 mg/m^2	28,29
	Etoposide	iv: 400–800 mg/m^2	
	Cytarabine	iv: 800–1600 mg/m^2	
	Melphalan	iv: 140 mg/m^2	
CyATG	Cyclophosphamide	iv: 200 mg/kg	63
	ATG	iv: 90 mg/kg	

Abbreviations: po, per os; iv, intravenous; TBI, total body irradiation; ATG, antithymocyte globulin.

the myeloablative conditioning regimens.[42] More recently developed nonmyeloablative conditioning regimens have shifted some or all of the burden of killing tumor cells from the conditioning regimen to the graft versus tumor effects.[43] Therefore, donor lymphocyte infusion, which has been used as a helpful tool for inducing a sustained complete response of malignancies but which is always followed by serious graft versus host disease in myeloablative conditioning regimens, could replace high-dose cytotoxic therapy because of its graft versus tumor effects in reduced-intensity and nonmyeloablative conditioning regimens.[44] Donor lymphocyte infusion performed after these conditioning regimens has shown promising results, even in the treatment of solid malignancies in both animal and clinical studies.[17,45]

Thirdly, the defense provided by the host's immune system is partly protected because the reduced-intensity and nonmyeloablative conditioning regimens do not immediately and completely eliminate host-derived immunocompetent cells, and the level of host neutropenia is reduced. This is extremely important for early immunity after transplantation, and infectious complications may be reduced.[46]

Recent advances with reduced-intensity and nonmyeloablative conditioning regimens have significantly decreased early mortality and acute graft versus host disease, while enabling robust and prompt engraftment, and hence enhancing the therapeutic benefits of BMT.[47] However, there are also potential disadvantages of using these condition regimens, disease relapse being a primary cause of treatment failure for patients receiving them. In one study, a higher rate of relapse (albeit not statistically significant) was observed in patients with myelodysplastic syndrome or acute myeloid leukemia in a nonmyeloablative conditioning group than in the myeloablative conditioning group (46% versus 30%, $P = 0.052$).[37] Similar results have been reported by other groups, and greater intensity leads to less relapse, although possibly at the expense of higher nonrelapse mortality.[48,49] Chronic graft versus host disease is another disadvantage of reduced-intensity and nonmyeloablative conditioning regimens. The incidence and times of onset of chronic extensive graft versus host disease were similar between myeloablative and reduced-intensity and nonmyeloablative conditioning regimens.[38]

Candidate patients for reduced-intensity and nonmyeloablative conditioning regimens often have adverse characteristics, including advancing age, higher risk diseases, and higher pretransplantation comorbidity scores. However, despite the potential disadvantages, considering these unfavorable factors and the improvements in nonrelapse mortality, acute

graft versus host disease suppression, progression-free survival, and overall survival, the overall outcome of these conditioning regimens is encouraging, and the number of BMT operations performed using them for a variety of hematological conditions is increasing dramatically.[11,37]

Examples of reduced-intensity and nonmyeloablative conditioning regimens

Low-dose (2–3 Gy) total body irradiation alone is an easy and convenient nonmyeloablative conditioning regimen. Its intensity is, to the best of our knowledge, the lowest in use today. Fludarabine is added in low doses in an attempt to reduce the risk of graft rejection. Low-dose total body irradiation, with or without fludarabine 90 mg/m^2, is a minimally toxic regimen developed for allogeneic BMT to treat patients with advanced hematological malignancies who are older or have comorbid conditions. It is one of the most widely used regimens by clinical centers.[11,38,42–44,50,51] Prospective clinical allogeneic BMT trials have shown that, in patients aged 60–75 years treated with this regimen, 5-year overall and 5-year progression-free survival rates were 35% and 32%, respectively.[52]

Other chemotherapy drugs, especially alkylating agents, are often combined with fludarabine. FAI is a regimen consisting of fludarabine, cytarabine, and idarubicin, and busulfan and fludarabine is a regimen used in myeloablative conditioning, but at much lower doses.[37,49]

The intensity of regimens increases with the doses of chemotherapy. Similar doses of fludarabine plus intermediate doses of one or two alkylating agents or low dose total body irradiation would be more powerful in host cytoreduction. Lim et al defined the intermediate doses of alkylating agents as oral busulfan (8–10 mg/kg), intravenous melphalan (80–140 mg/m^2), intravenous cyclophosphamide (600–1200 mg/m^2), or intravenous thiotepa (5–10 mg/kg).[48] The doses employed by different clinical centers may be quite different. For example, the combination of fludarabine and melphalan varies from fludarabine 100–150 mg/m^2 and melphalan 140–180 mg/m^2 to fludarabine 90–120 mg/m^2 and melphalan 90–140 mg/m^2.[49,53,54] In the regimen consisting of fludarabine and cyclophosphamide, the dose of cyclophosphamide in the research of Anderlini et al is 2.5–3 times the dose used by Lim et al.[48,54]

New drugs have been developed and added to the conditioning regimens. Treosulfan has been used as a substitute for busulfan in frail patients, because the side effects and toxicity are supposedly less severe. Treosulfan-based conditioning regimens have shown a favorable safety profile with fast

and sustained engraftment.[18,19,55] Recently, Nemecek et al have reported that a conditioning regimen consisting of treosulfan and fludarabine is well tolerated and yields encouraging survival rates and disease control with minimal nonrelapse mortality in patients with high-risk hematological malignancies.[39] Clofarabine is a second-generation purine nucleoside analog that combines the properties of fludarabine and cladribine. It is one of the most effective single agents against leukemic blast.[56] The combination of clofarabine with the reduced-intensity conditioning regimen showed good antileukemic efficacy, even in patients with high-risk acute myeloid leukemia or myelodysplastic syndrome.[57] Tyrosine kinase inhibitors can lead to cytogenetic remissions in patients with chronic myeloid leukemia and have been used before reduced-intensity or nonmyeloablative conditioning regimens. Warlick et al reported that allogeneic reduced-intensity conditioning BMT for older patients with chronic myeloid leukemia can control relapse with acceptable toxicity.[58]

Novel reduced-intensity and nonmyeloablative conditioning regimens
Total lymphoid irradiation
Efforts have been made clinically to reduce toxicity through using total lymphoid irradiation rather than total body irradiation to protect critical organs. Total lymphoid irradiation was initially used with the combination of conventional myeloablative regimens to increase immunosuppression and engraftment further.[59] Research in animals showed that total lymphoid irradiation increased the proportion of regulatory natural killer T cells. These natural killer T cells prevented acute graft versus host disease by inhibiting the proliferation and cytokine secretion of conventional T cells without affecting graft versus tumor activity.[60,61] Lowsky et al took advantage of the immune system's regulatory natural killer T cells and evaluated the total lymphoid irradiation-based reduced-intensity conditioning regimen in patients with lymphoid malignancies or acute leukemia undergoing allogeneic BMT. Eight Gy total lymphoid irradiation was delivered with fractions using fields to encompass the thymus, spleen, and lymph nodes. The results showed that 95% of patients scored as grade 0 according to standard scores for graft versus host disease, and the incidence of severe acute graft versus host disease was only 3%. The reduced-intensity conditioning regimen containing total lymphoid irradiation did not adversely affect the graft versus tumor effects of the allogeneic graft.[62]

Monoclonal antibodies
In 1994, Storb et al reported a conditioning regimen for patients with aplastic anemia using a high-dose combination of cyclophosphamide and monoclonal antibody to CD3 (antithymocyte globulin).[63] Subsequently, reduced-intensity conditioning regimens that consisted of antithymocyte globulin and alkylating agents were applied to both nonmalignant and malignant hematological disorders.[15,64] Promising outcomes were confirmed after regimens that contained antithymocyte globulin by the low incidence of acute graft versus host disease, although chronic graft versus host disease remained a major problem.[65,66] Therefore the use of other antibodies was explored, a representative being alemtuzumab (monoclonal antibody to CD52, marketed as Campath®, Genzyme Corporation, Cambridge, MA). Alemtuzumab has since been proven to be effective and safe in the reduced-intensity conditioning regimens by several goups.[67–69] Recently, a minimal-intensity conditioning regimen using alemtuzumab with fludarabine and cyclophosphamide has been developed by Marsh et al.[70] The results show that the regimen consisting of fludarabine, cyclophosphamide and alemtuzumab was associated with not only durable engraftment but also a much lower risk of chronic graft versus host disease compared with the conventional regimen containing antithymocyte globulin.[70] A retrospective study concluded that reduced-intensity conditioning consisting of fludarabine, melphalan, and alemtuzumab significantly improved survival rates over a myeloablative conditioning regimen consisting of busulfan, cyclophosphamide, and antithymocyte globulin plus or minus etoposide.[71] The anti-CD20 monoclonal antibody, rituximab, has also been used in both myeloablative and reduced-intensity conditioning regimens.[72] For patients with lymphoma who experienced disease recurrence following autologous BMT, allogeneic BMT prepared with a nonmyeloablative conditioning regimen consisting of fludarabine, cyclophosphamide and rituximab was suggested to be an effective option.[73]

Radioimmunotherapy usage
Antibodies conjugated with radionuclides, known as radioimmunotherapy, have been used for the treatment of cancer both in animal experiments and clinically.[74,75] By way of radioimmunotherapy, radiotherapy could be directly delivered to the surface of the targeted cells in continuous low-dose rate irradiation without increasing the toxicity, thereby sparing normal tissue. Therefore, radioimmunotherapy has been used in conditioning regimens to reduce the tumor burden while allowing for long-term disease control through graft versus

tumor effects in both myeloablative and reduced-intensity conditioning regimens.[76] Clinically, the radiolabeled anti-CD20 antibody (yttrium-90 ibritumomab tiuxetan) had been administered prior to reduced-intensity conditioning regimens for patients with advanced lymphoma and refractory disease or relapse after a previous autologous BMT. The treatment is associated with favorable outcomes, including no additional toxicity, enhanced cytoreduction, acceptable graft versus host disease, and absence of relapse.[77,78] Pagel et al have combined iodine-131 labeled anti-CD45 antibody with a standard reduced-intensity conditioning regimen for the treatment of older high-risk patients with acute myeloid leukemia or myelodysplastic syndrome. The results showed that CD45-targeted radiotherapy could be safely combined with a

reduced-intensity conditioning regimen to yield encouraging overall survival.[79] The frequently used reduced-intensity and nonmyeloablative conditioning regimens are summarized in Table 3.

Conclusion

BMT remains a potentially dangerous procedure due to the many possible complications. The myeloablative conditioning regimens depending on high doses of radiotherapy and chemotherapy induce intense toxicity and have a high nonrelapse mortality rate; therefore, the conventional myeloablative conditioning regimens have been modified with the goal of reducing toxicity while maintaining or improving efficacy. Reduced-intensity and nonmyeloablative conditioning regimens have proven to be less toxic, making them suitable for older patients and those with comorbidities. These novel regimens are also associated with a lower rate of nonrelapse mortality and incidence of severe acute graft versus host disease. Host immunity is not completely destroyed in reduced-intensity and nonmyeloablative conditioning regimens and provides partial protection from infections. Reduced-intensity and nonmyeloablative conditioning regimens are appealing alternatives to myeloablative conditioning regimens and make BMT more acceptable by directly or indirectly ameliorating the complications. Efforts to improve effects and outcomes further continue to be explored by researchers, with potentially promising results.[80]

Acknowledgments

Table 3 Summary of frequently used reduced-intensity and nonmyeloablative conditioning regimens

Regimen	Radiotherapy and chemotherapy	Total dose	Reference
TBI	TBI	2–3 Gy	11,42–44, 51
FluTBI	Fludarabine	iv: 90 mg/m²	11,38,
	TBI	2–3 Gy	42–44,50, 51
FAI	Fludarabine	iv: 120 mg/m²	49
	Cytarabine	iv: 4 g/m²	
	Idarubicin	iv: 36 mg/m²	
BuFlu	Busulfan	iv: 3.2 mg/kg	37
	Fludarabine	iv: 120 mg/m²	
FluCy	Fludarabine	iv: 125 mg/m²	54
	Cyclophosphamide	iv: 3 g/m²	
FluCyATG	Fludarabine	iv: 125 mg/m²	54
	Cyclophosphamide	iv: 3 g/m²	
	ATG	iv: 60 mg/kg	
FluMel	Fludarabine	iv: 90–150 mg/m²	49,53,54
	Melphalan	iv: 90–180 mg/m²	
TreoFlu	Treosulfan	iv: 30–42 g/m²	39
	Fludarabine	iv: 150 mg/m²	
TLIATG	TLI	8 Gy	62
	ATG	iv: 7.5 mg/kg	
TreoFluATG	Treosulfan	iv: 30–42 g/m²	18,39
	Fludarabine	iv: 150 mg/m²	
	ATG	iv: 30–90 mg/m²	
BuFluATG	Busulfan	iv: 12.8 mg/kg	66
	Fludarabine	iv: 250 mg/m²	
	ATG	iv: 4.5 mg/kg	
FCC	Fludarabine	iv: 120 mg/m²	70
	Cyclophosphamide	iv: 1200 mg/m²	
	Alemtuzumab	iv or sc: 40–100 mg	
FluCyRit	Fludarabine	iv: 90 mg/m²	73
	Cyclophosphamide	iv: 2250 mg/m²	
	Rituximab	iv: 1375 mg	

Abbreviations: TBI, total body irradiation; iv, intravenous; TLI, total lymphoid irradiation; ATG, antithymocyte globulin; sc, subcutaneous.

Disclosure

The authors declare no competing financial interests in this work.

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