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High dose cyclophosphamide for GVHD prevention

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Abstract

Purpose of review—Administration of high-dose cyclophosphamide (Cy) after transplantation inhibits both graft rejection and graft-versus-host-disease (GvHD) in mouse models of allogeneic blood or marrow transplantation (alloBMT). This strategy has recently been adapted to human transplantation.

Recent findings—The safety and efficacy of high-dose post-transplantation Cy, when given in combination with tacrolimus and mycophenolate mofetil, was first demonstrated after nonmyeloablative conditioning and allografting using HLA-mismatched related donors. Further analysis shows that increasing HLA disparity does not worsen overall outcome. High-dose post-transplantation Cy was also found to be effective as sole prophylaxis of acute and chronic GvHD after HLA-matched alloBMT.

Summary—Taking advantage of the differential susceptibility of proliferating, alloreactive T cells over non-proliferating, non-alloreactive T cells to high dose Cy, and owing to the drug's stem cell sparing effects, this novel strategy provides a unique opportunity to optimize GvHD prophylaxis after HLA-matched alloBMT and increase the use of HLA-mismatched related donors. Safe and effective mismatched related alloBMT provides access to essentially everyone, such as patients with sickle cell anemia, in need of the procedure.

Keywords

graft-versus-host disease; allogeneic blood or marrow transplantation; posttransplantation cyclophosphamide; transplantation tolerance

Introduction

Despite advances in HLA typing, graft engineering, and supportive care, reducing the incidence and severity of GvHD remains a significant challenge to successful outcomes for patients undergoing alloBMT.¹ GvHD is caused by immune reactions of donor T cells against disparate host histocompatibility antigens.² Current GvHD prophylaxis regimens impair alloreactive T cell activation, proliferation, and interleukin-2 (IL-2) production and interfere with apoptosis of alloreactive T cells^{3,4} leading to global immunosuppression and delayed induction of transplantation tolerance.⁵ Among currently used immunosuppressants, only methotrexate (MTX) and cyclophosphamide (Cy) can induce the apoptosis of alloantigen-activated human T cells.³ Although active as a single agent for GvHD prevention, MTX is toxic and cannot be administered in the high doses required for alloreactive T cell apoptosis and tolerance induction.³ In contrast, Cy can be administered safely in the high doses that may be required to eliminate alloactivated T cells after allogeneic blood or marrow transplantation (alloBMT). We have shown that a single dose of

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post-transplantation Cy (200 mg/kg) augments the engraftment of major histocompatibility complex (MHC)-mismatched bone marrow in mice conditioned with the combination of pre-transplantation fludarabine and low dose (200 cGy) total body irradiation.⁶ In addition, post-transplantation Cy reduced the incidence and severity of GvHD across MHC barriers after myeloablative conditioning. These observations were not entirely novel since Cy administration was one of the first strategies shown to be effective in controlling acute GvHD in rodents.^{7,8} However, in an early clinical trial, intermittent low doses of post-transplantation Cy was inferior to cyclosporine in preventing GvHD after HLA-matched alloBMT.⁹ Thus, the drug may have been given at the wrong time or at a dose that was too low to be maximally effective at suppressing GvHD. The selection of low, intermittent doses of Cy was motivated by concerns that higher doses of the drug might be toxic to donor stem cells and would therefore impair engraftment. Our pre-clinical work as well as other data that have shown that high dose Cy is not toxic to primitive hematopoietic stem cells provided the rationale to test this approach in the clinical setting.^{10–12} Here, we describe the development and clinical use of high dose Cy as an approach for GvHD prophylaxis and review outcomes from several early phase clinical trials using this strategy in the HLA-matched and mismatched settings.

Clinical translation of high-dose posttransplantation Cy: Initial studies

Crossing the HLA barrier in alloBMT is one of the most formidable challenges in clinical transplantation immunology. Historically, partially HLA-mismatched, or HLA-haploidentical, alloBMT has been associated with high rates of graft failure, severe GvHD, and non-relapse mortality.¹³ Increasing HLA mismatch between donor and recipient has been associated with worse event-free survival, especially when donors are mismatched by two or more HLA antigens.¹⁴ Two independent Phase I/II clinical trials evaluated the safety and efficacy of high-dose, posttransplantation Cy, given in conjunction with mycophenolate mofetil (MMF) and tacrolimus after nonmyeloablative conditioning and partially HLA-mismatched bone marrow transplantation (mini-haploBMT).^{15,16} All patients were treated initially as outpatients and received conditioning modified slightly from the regimen of Storb et al¹⁷: fludarabine, low-dose Cy, and 200 cGy total body irradiation; after transplantation of T-cell replete donor bone marrow (on what is designated day 0), the patients received 50 mg/kg of Cy on day 3 (28 patients) or on days 3 and 4 (40 patients).¹⁵ Administration of tacrolimus (for 6 months) and MMF (for 35 days) was not initiated until the day following completion of posttransplantation Cy to avoid blocking Cy-induced tolerance.¹⁸ The median times to neutrophil and platelet recovery were 15 and 24 days, respectively. Graft failure occurred in 9 of 66 (13%) evaluable patients, and was fatal in one. The cumulative incidences of acute grade II–IV and grade III–IV GvHD were 34 and 6%, respectively. The cumulative incidence of non-relapse mortality at 1 year was 15%. There was a trend towards a lower incidence of extensive chronic GvHD among recipients of two versus one dose of post-transplantation Cy (5% versus 25%, $p=0.05$), the only difference in outcomes between these two groups of patients. Serious infections were relatively infrequent: there were no cases of CMV disease and only five cases of invasive fungal infection, two of which were fatal. It also appears that this strategy may allow the preservation of fertility in young females.¹⁹ These encouraging outcomes in patients with advanced hematologic malignancies served as a rationale for further studies in patients with hematologic malignancies and in those with life-threatening nonmalignant hematologic diseases.

Extended studies of nonmyeloablative, HLA-haploidentical BMT (“mini-haploBMT”) with posttransplantation Cy

We recently reported on the outcomes of 185 hematologic malignancies patients treated with mini-haploBMT and high-dose, posttransplantation Cy.^{20**} Most patients (median age 50, range 1–71) had advanced disease and 49 (26%) had failed autologous BMT. Diagnoses were acute leukemia or lymphoblastic lymphoma (58), non-Hodgkin lymphoma (42), Hodgkin lymphoma (25), myelodysplastic syndrome (MDS) (22), chronic lymphocytic leukemia (CLL) (15), chronic myeloid leukemia (CML) (11), multiple myeloma (9), and chronic myelomonocytic leukemia (CMML) (3). Molecular typing was at an allele level for HLA-A, -B, -Cw, and -DRB1 and at an allele group level for -DQB1. Patients and their donors were heavily mismatched, with 159 pairs mismatched for 3 or 4 HLA antigens (HLA-A, -B, -Cw, or -DRB1, not including HLA-DQB1) in any direction. Post-transplant GvHD prophylaxis consisted of Cy (50 mg/kg IV) either once (on day 3; n = 48) or twice (on days 3, 4; n = 137), MMF, and tacrolimus as already described. Nonengraftment attributed to primary graft failure or to residual bone marrow malignancy occurred in 29 of 177 evaluable patients (16%). Only three of these patients died in aplasia without evidence of disease persistence or recurrence. The cumulative incidences of NRM and relapse or progression at one year after transplantation were 15% and 50%, respectively. The cumulative incidence of grade II–IV acute GvHD by day 200 after transplantation was 31%, and of chronic GvHD by 1 year after transplantation was 15%. With a median of 20 months follow-up after BMT (range 2–71 months), the actuarial event-free survival (EFS) at one year was 35%. Figure 1 shows the EFS of 144 patients transplanted before March, 2009 according to their primary diagnosis. The EFS curves appear to achieve a plateau of 20–30% for diseases such as MDS and the acute leukemias (Figure 1A) versus 40–50% for diseases such as non-Hodgkin lymphoma (excluding mantle cell lymphoma) and Hodgkin lymphoma (HL; Figure 1B). The results of mini-haploBMT with post-transplantation Cy for HL are particularly encouraging since all but two of the patients had relapsed after a prior autologous BMT. In light of the generally poor outcomes of nonmyeloablative, HLA-matched BMT for HL,²¹ our results raise the possibility that T and/or natural killer cells reactive to HLA molecules may be uniquely effective in the treatment of multiply relapsed HL.

The major challenge in HLA-haploidentical BMT is not finding a donor, but rather selecting among multiple potential donors. Historically, the extent of HLA mismatch between donor and recipient has been the paramount consideration in donor selection, because increasing degrees of HLA mismatch have been associated with worse overall outcomes.^{13,22} The low incidence of severe GvHD¹⁵ and NRM prompted us to examine the relationship between HLA mismatch and outcome of mini-haploBMT with posttransplantation Cy. Notably, increasing degrees of HLA mismatch at either class I or class II loci had no significant effect on the cumulative incidence of acute or chronic GvHD or on NRM. Moreover, the presence of a HLA-DRB1 antigen mismatch in the graft-versus-host direction was associated with a significantly lower cumulative incidence of relapse (Figure 2A; p = 0.04) and improved EFS (Figure 2B; p = 0.009), whereas DQB1 antigen and class II allele mismatch status had no effect. Additionally, the presence of two or more class I allele mismatches (composite of A, B, and Cw) in either direction was associated with a significantly lower cumulative incidence of relapse (Figure 2C; p = 0.045 for GVH direction, p = 0.01 for HVG direction) and improved EFS (Figure 2D; p = 0.07 for GVH direction, p = 0.001 for HVG direction). Thus, when nonmyeloablative, HLA-haploidentical BMT incorporates high-dose post-transplantation Cy, increasing HLA disparity between donor and recipient was not detrimental to overall outcome and appeared to be beneficial. While the results of this retrospective analysis need to be validated prospectively, the results raise the possibility that

post-transplantation Cy dissociates the graft-versus-leukemia GvL effect from clinically significant GvHD, and that donors for alloBMT could be selected based upon genetic factors other than HLA.

The application of BMT to treat non-malignant disorders affecting the lymphohematopoietic system, especially sickle cell anemia, is limited by the lack of suitable donors.²³ Nonmyeloablative, HLA-haploidentical BMT with post-transplant CY is a promising approach for patients with life-threatening nonmalignant hematologic disease who lack an HLA-matched sibling donor. We treated three patients with thrombotic PNH, one of whom also had sickle cell disease, with a nonmyeloablative, HLA-haploidentical BMT with post-transplant Cy.²⁴ Rapid engraftment without GvHD occurred in two of the patients, including the patient with sickle cell disease. Both patients are disease free with full donor chimerism and require no immunosuppressive therapy, with follow-up of 1 and 4 years, respectively.

High-dose Cy as sole GvHD prophylaxis after HLA-matched alloHSCT

There is no consensus regarding the most effective and least toxic approach for GvHD prevention after HLA-matched alloBMT.²⁵ The most commonly used regimens for GvHD prophylaxis consist of a calcineurin inhibitor (CNI; cyclosporine or tacrolimus) in combination with either methotrexate, MMF, or sirolimus.^{26–30} However, despite the use of pharmacological GvHD prophylaxis, acute GvHD still occurs in 35–55% of BMT recipients from HLA-matched siblings, and more frequently in recipients of unrelated donor BMT.^{28,30–33} As importantly, the incidence of chronic GvHD is 50% or higher with most commonly used prophylaxis regimens.^{26–30,34,35} Patients that do not develop chronic GvHD can be withdrawn from immune suppression within 6–9 months of transplantation. These patients are considered to have developed early donor/recipient tolerance. Patients that develop acute GvHD require secondary treatment and the majority of them remain on multiagent immunosuppression beyond the first 12 months after transplantation.³⁶ The median duration of immunosuppressive treatment in patients that develop chronic GvHD is 23 months with approximately 15% of them requiring treatment beyond 7 years.³⁷ Thus, it can be argued that the frequency and duration of use of secondary immunosuppression should be considered when judging the efficacy of any GvHD prophylaxis regimen.³⁸ A GvHD prophylaxis strategy that promotes tolerance induction may obviate the need for secondary immunosuppressive treatment. By avoiding the use of CNIs, such an approach may also improve immune reconstitution and optimize anti-tumor immunity.^{39,40} It has been proposed that CNI by disrupting the thymic architecture⁴¹ and by blocking the induction of transplantation tolerance,⁵ could also play a role in the development of chronic GvHD.^{42,43} Thus, their omission may decrease the incidence of chronic GvHD.

Based on the results with high-dose Cy as GvHD prophylaxis in mismatched alloBMT, we studied it as sole prophylaxis of GvHD after myeloablative HLA matched related or unrelated donor BMT.⁴⁴ One hundred and seventeen consecutive hematologic malignancies patients were treated on this phase I/II clinical trial; 78 patients received HLA-matched related and 39 received HLA-matched unrelated donor allografts. Transplantation conditioning comprised oral or intravenous busulfan from days -7 to -4 (target AUC 800–1400) and Cy 50 mg/kg on days -3 and -2, followed by an infusion of donor marrow obtained in a targeted collection of 4×10^8 nucleated cells/kg. No growth factors were administered. The median patient age was 50 years with 14 patients being 60 years or older. The most common diagnosis was acute myeloid leukemia (AML; n=58, or 50%), and 68/117 patients (58%) were not in remission at the time of transplantation. Sustained engraftment of donor cells occurred in 114 patients (98%), with the median time to neutrophil recovery of 23 days for recipients of related donor and 25 days for recipients of

unrelated donor allografts. High-dose post-transplantation Cy was well-tolerated; the most common toxicities were transient mild renal dysfunction or elevations of serum liver enzymes, while hepatic veno-occlusive disease developed in 10 patients (9%) and was fatal in two. The cumulative incidences of grades II–IV GvHD by day 200 after transplantation were 42% and 46% among recipients of related versus unrelated donor grafts, respectively. The incidence of grades III–IV GvHD for all patients was 10%. At 2 years after transplantation, the cumulative incidences of chronic GvHD for recipients of related versus unrelated donor grafts were 9% and 11%, respectively. In a multivariable analysis, the only variable associated with an increased risk of developing moderate-severe acute GvHD was a male recipient of a female donor graft ($p=.05$).

These initial results were recently updated in a follow-up report on 139 consecutive patients (79 related and 60 unrelated) treated on this study.⁴⁵ With a median follow-up of 26 months, the cumulative incidences of acute grades II–IV and chronic GvHD for all patients were 45% and 10%, respectively. Only 3 patients have died with refractory GvHD. We also retrospectively analyzed the use of secondary systemic immunosuppressants (SSI) in this expanded cohort. Overall, the cumulative incidence of SSI use was 45%. The median time to initiation of SSI was 42 days (range, 19–142 days) and the median duration of SSI use was 152 days (range, 13–981 days). At 6 months and 1 year after transplantation, 63% and 85% of patients were off all immunosuppressive therapy, respectively. These results extend our previous observations that post-transplantation Cy is effective single agent prophylaxis of acute GvHD with a low rate of grade III–IV GvHD and more than half of the patients never requiring additional SSI. The limited use of SSI may be responsible for the low infection rate seen in these patients.

The overall survival (OS) and event-free survival (EFS) for all patients at 1 year after transplantation were 63% and 48%, respectively, and at 2 years after transplantation were 55% and 39%, respectively, suggesting that use of high-dose Cy for GvHD prophylaxis retains the GvL effect in these high-risk patients.⁴⁴ Consistent with other recent studies, EFS and OS did not differ according to the donor type. The cumulative incidence of relapse for patients transplanted in remission was 26% at two years after transplantation. Among patients with MDS or AML in remission at the time of transplantation, the 1- and 2- year EFS were 61% and 52%, respectively. The cumulative incidence of relapse for patients with AML/MDS that were in CR at the time of allografting was 28% at 2 years. AML/MDS patients who were not in CR at the time of transplantation had a worse EFS than patients in CR, but the difference was not statistically significant ($p=0.26$), while the presence of circulating blasts in patients with active disease was associated with significantly poorer outcome compared to patients in CR ($P=0.01$, Figure 3). In conclusion, high-dose post-transplantation Cy is effective as sole prophylaxis of GvHD after HLA-matched alloHSCT, and does not appear to eliminate the allogeneic GvL effect in patients with advanced hematologic malignancies.

The ability to shorten the duration of post-grafting immunosuppression after HLA-matched alloHSCT with high-dose, post-transplantation Cy was marked by prompt immune reconstitution and a low incidence of opportunistic infections. Among 54 patients studied consecutively, the median absolute lymphocyte counts on day 30 and 60 after transplantation were 440/ μ l and >700/ μ l, respectively. The median CD4⁺ T cell count on day 60 after transplantation was 119/ μ l. Levels of Foxp3 mRNA, the signature transcript of regulatory T cells, in the peripheral blood of patients who did not develop GvHD were significantly greater than those detected in patients who developed GvHD. These results correlated with significantly lower levels of CD4⁺CD25⁺Foxp3⁺ cells in the blood of patients who developed GvHD. A lack of GvHD also correlated with high levels of IL-1R α and TGF- β (mRNA transcripts), whereas the development of GvHD correlated with high

levels of IFN- γ , IP-10, TNF- α and IL-6.⁴⁶ No patients died of CMV or invasive fungal infection. Reactivation of CMV occurred in 24% of patients, and there were only two documented case of CMV disease. The rapid recovery of CMV-specific immunity correlated with the results of in vitro ELISPOT assays. The frequency of cells secreting interferon gamma in response to stimulation with pentadecapeptides of the immunodominant CMV protein, pp65, at day 30–60 after alloHSCT did not differ from pre-transplantation specimens from CMV-seropositive donor/recipient pairs. The absence of post-transplantation Epstein–Barr virus-associated lymphoproliferative disease is another indicator of the prompt immunologic recovery seen with post-transplantation Cy.

Conclusions

Preclinical results suggesting that high-dose Cy given after transplantation can prevent rejection and GvHD had been successfully incorporated in conditioning strategies using HLA-mismatched and –matched donors. High-dose, post-transplantation Cy facilitates partially HLA-mismatched HSCT without severe GvHD and can mitigate if not completely nullify the negative impact of HLA-disparity on transplantation outcome. High dose Cy is also effective as single agent, short course prophylaxis of GvHD after myeloablative conditioning and HLA-matched alloBMT. The first step in the ongoing development of post-transplantation Cy for GvHD prophylaxis is to determine whether these data can be replicated in multi-center trials. Nonetheless, the overall ability of high-dose post-transplant Cy to limit the use of post-transplant immunosuppression and thus allow improved immune reconstitution, makes an excellent platform for the addition of novel strategies to decrease relapse. Such strategies include vaccinating donors against tumor antigens, use of post-transplant vaccines, and novel post-transplantation adoptive immunotherapy with donor T and/or NK cells. By promoting bi-directional transplant tolerance, high dose post-transplantation Cy creates the possibility of using alloBMT to treat autoimmune diseases and non-malignant hematologic disorders, and to induce tolerance to solid organ transplants through induction of donor hematopoietic chimerism.

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Figure 1. Disease specific outcomes in patients that received nonmyeloablative, HLA-haploidentical BMT with posttransplantation Cy
 Event-free survival in patients with acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) (A). Event-free survival in patients with Hodgkin's, mantle and Non-Hodgkin's lymphoma (B).

Figure 2. The relationship between HLA mismatch and outcome of mini-haploBMT with posttransplantation Cy

Outcomes (relapse and non-relapse mortality) according to donor-recipient HLA-mismatch (A)(B). Event-free survival according to the number of antigen mismatches (C) (D).

Adapted with permission from: Greater HLA Disparity Is Associated with Reduced Risk of Relapse and Improved Event-Free Survival after Nonmyeloablative, HLA-Haploidentical BMT with Post-Transplantation High-Dose Cyclophosphamide; Yvette L. Kasamon, Mary S. Leffell, Jeanne Kowalski et al; Blood (ASH Annual Meeting Abstracts), Nov 2008; 112: 150.

Figure 3. Event-free survival in patients AML according to disease status pretransplant after myeloablative conditioning and post-transplantation Cy as sole GVHD prophylaxis
Abbreviations: Complete remission (CR), peripheral blasts (PB).