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NONMYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION: TRANSPLANTATION FOR THE 21ST CENTURY

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1. ABSTRACT

Conventional approaches to allogeneic stem cell transplantation have used toxic high-dose conditioning therapy to achieve allogeneic engraftment and control of underlying disease. For engraftment purposes, preclinical studies and clinical observations have shown that conditioning regimens can be markedly reduced in intensity, resulting in reduced treatment toxicities. Preclinical canine studies demonstrated that the use of potent pre- and postgrafting immunosuppression allows for reduction in conditioning regimens while facilitating development of stable mixed chimerism. If attenuated conditioning regimens can be successfully translated to human stem cell transplantation, an improved safety profile will allow potentially curative treatment to a more representative patient profile not currently offered such therapy. Mixed chimerism could prove curative of disease phenotype of various nonmalignant disturbances of the hematopoietic and immune systems. For patients with hematopoietic malignancy, spontaneous conversion to full donor hematopoeisis after stem cell transplant may prove curative by virtue of graft versus host reactions directed against the malignancy, however infusion of additional donor lymphocytes may be needed to treat persistent disease.

2 INTRODUCTION

Modern hematopoietic stem cell transplantation (HSCT) can be traced back to 1949 with the observation by Jacobson *et al*. (1) that mice were protected from marrow lethal effects of ionizing total body irradiation (TBI) by shielding their spleens with lead. Subsequent studies showed that transplantable HSC was responsible for the radioprotection. A treatment schema evolved from these studies for patients with marrow-based diseases such as leukemias (2). In this simple schema, patients receive maximum doses of systemic chemoradiation therapy to eradicate the underlying malignant disease. The intensity of the therapy is only limited by toxicities to solid organs, such as lung, heart, liver and gut. The infused allogeneic HSCs are used to restore or "rescue" hematopoiesis, which is destroyed along with the underlying disease.

This treatment schema continues to be the basis for most current HSCT. Two observations have been used to question the validity of this transplant schema. The first observation is that many malignant diseases cannot be completely sterilized by high-dose chemoradiation, even after intensification of the pre-transplant therapy to a point where organ toxicities occur (2, 3). The second observation is that a substantial number of cures can be attributed to immunological anti-tumor reactions brought about by lymphocytes of the allografts (4-7). Furthermore, donor lymphocyte infusions (DLI) have been used to produce complete remissions in patients whose disease has relapsed after HSCT (8, 9). These two observations have led to a radical rethinking of how HSCT might be done in the future by attempting to minimize associated toxicities and taking advantage of a better understanding of how to influence both host and donor immune functions. The emphasis has shifted from trying to eradicate malignant cells through toxic therapy towards using the allogeneic donors' immune cells to eliminate the malignant cells. If this new schema proves effective, it would allow extending HSCT to include those patients who are too old or medically infirm to qualify for current allotransplants.

This new transplant approach which avoids serious marrow damage and associated pancytopenia is founded on the simple experimental fact that, in the setting of major histocompatibility complex (MHC) identity, the effector cells that mediate both host-versus-graft (HVG) reactions and graft-versus-host disease (GVHD) are T lymphocytes. These findings have opened up the

possibility of designing post-transplant immunosuppression regimens which would serve a dual purpose. The first is to reduce the risk of GVHD and the second to prevent HVG reactions. The latter would reduce the need for intensive and potentially organ-toxic pre-transplant conditioning therapy. This hypothesis was tested in a preclinical canine model in which nonmyelotoxic post-transplant immunosuppression was substituted for the otherwise necessary intensive cytotoxic pre-transplant conditioning regimen (10). In the transplant schema that evolved from these studies, pre-transplant immunosuppression is used for the exclusive purpose to reduce host immune responses, while the post-transplant immunosuppression aims to suppress both donor and residual host immune cells. Theoretically, with the discontinuation of post-transplant immunosuppression, mutual graft-host tolerance will be established which manifests itself as stable mixture of donor and host hematopoietic cells or mixed donor/host hematopoietic chimerism.

The regimen developed in the canine model uses a low and non-myeloablative dose of 200 cGy TBI before and a combination of the de novo purine synthesis inhibitor mycophenolate mofetil (MMF) and the T-cell activation blocker cyclosporine (CSP) for 4 and 5 weeks, respectively, after HSCT (10). In another set of experiments, stable mixed chimerism was also achieved in dogs given pre-transplant limited field irradiation targeted only to cervical, thoracic, and upper abdominal lymph nodes (11). In these dogs, mixed hematopoietic chimerism was established as early as 6 weeks after transplant even in non-irradiated marrow and lymph node sites. This finding argues that the "creation of marrow space" by cytotoxic agents is not needed for stable allogeneic engraftment, and that grafts created marrow space by immunologic means. This raises the possibility that pre-transplant irradiation can be replaced by non-toxic T-cell immunosuppression. The early results of further studies with an antibody to the T-cell receptor $\alpha\beta$ (12) and with T-cell costimulation blockade by CTLA4Ig (13) are encouraging and enables the lowering of the pre-transplant TBI dose needed for establishment of mixed chimerism in this model from 200 cGy to 100 cGy.

The pre-clinical studies have resulted in the development of a schema for allogeneic HSCT for human patients with both nonmalignant and malignant hematological diseases (14, 15). The underlying hypothesis of the schema is that creation of marrow space by cytotoxic agents is not necessary for transplant success, and grafts can be established by immunosuppressive drugs which suppress HVG reactions. As a first step we tested the hypothesis that post-transplant control of HVG and GVH reactions allows development of mutual graft-host tolerance in patients with deficiency in T-cell function. In this study using post-transplant CSP/MMF alone without pre-transplant TBI, the development of mixed donor/host chimerism in hematopoietic lineages other than lymphocytic has provided proof of principle that creation of marrow space by cytotoxic agents is not needed for stable allogeneic engraftment (16). Theoretically, generation of stable multi-lineage mixed chimerism will be sufficient to ameliorate other non-malignant genetic disorders, as suggested by the association of mixed chimerism with improved disease symptoms following conventional HSCT (17). However, full donor chimerism may be required for certain genetic disorders as exemplified in a dog model of hereditary hemolytic anemia as a result of pyruvate kinase deficiency where mixed chimerism did not prevent progressive cirrhosis and death because of continued host erythrocyte hemolysis (18). The use of a non-myeloablative regimen to achieve stable mixed chimerism has the potential advantage of reducing both short- and long-term toxicities associated with conventional transplant regimens. Among five patients with genetic disorders who have received non-myeloablative HSCT thus far, four had pre-transplant conditions that would have greatly increased the risk for mortality following conventional HSCT. Mixed donor/host chimerism was achieved in all patients initially, without significant morbidity attributed to the non-myeloablative regimen. Non-fatal graft rejection occurred in one patient, and one died from complications associated with chronic GVHD. These early results support using a non-myeloablative regimen as the first HSCT in patients with non-malignant disorders, particularly those with risk factors for poor outcome after conventional HSCT.

In patients with malignant hematological diseases, we hypothesized that mixed chimerism would serve as a platform for subsequent adoptive anti-tumor immunotherapy using donor lymphocytes. This concept has been successfully explored in patients with leukemia, lymphoma and multiple myeloma who were ineligible for conventional transplants because of age or medical infirmity (19). The transplant regimen employed TBI, 200 cGy, given as a single fraction at a rate of 7 cGy/min on day 0, CSP at 6.25 mg/kg b.i.d. p.o. on days -1 to 35, and MMF at 15 mg/kg b.i.d. p.o. on days 0 to 27. All patients were infused on day 0 with G-CSF mobilized HSC collected from the peripheral blood of HLA-identical sibling donors within hours of TBI. Forty-four patients have received this nonmyeloblative treatment at the Fred Hutchinson Cancer Research Center in Seattle and at two collaborating institutions, Stanford University and the University of Leipzig, Germany. The diagnoses of patients transplanted include acute myeloid leukemia (n=10), multiple myeloma (n=8), chronic myelocytic leukemia (n=9), Hodgkin's disease (n=4), chronic lymphocytic leukemia (n=8), acute lymphoblastic leukemia (n=1), non-Hodgkin's lymphoma (n=3) and myelodysplastic syndrome (n=1) (20). The procedure has been well tolerated, and in the majority of patients, took place in the outpatient setting. Patients experienced no mucositis, diarrhea or alopecia, had minimal nausea and vomiting, and typically, very mild myelosuppression. In fact, patients who had normal peripheral blood cell counts at transplant usually did not require platelet or red blood cell transfusions. Initial engraftment was seen in all patients and was manifested by either mixed or full chimerism present through at least 2 months. Graft rejections were seen in 19% of all patients and usually occurred between 2 and 5 months after transplant. These events were non-fatal with recovery of autologous hematopoiesis. Fifty-eight percent

of patients with sustained engraftment developed grade II, or at most grade III, acute GVHD after MMF was discontinued, but this complication was readily managed with the administration of corticosteroids. Donor lymphocyte infusions were given to 17 patients 65 days or more after transplant who showed mixed chimerism and no active acute GVHD. The reason for administering donor lymphocyte infusions included evidence of rejection (9/17) and disease progression (8/17). Significant GVHD (> grade III) developed in 3 patients and neutropenia in two patients following donor lymphocyte infusions. Projected survival at 1.5 years after transplant was 76%. Transplant-related mortality during the first year was only 6.5%. Death occurred in 11% of patients from relapse of malignancy or persistent disease (in 5% of cases, death occurred after second conventional transplant).

In each of the patient groups, complete remissions of the underlying malignancy have been observed. These included molecular remissions in three patients with chronic myelogenous leukemia and two with chronic lymphocytic leukemia, cytogenetic and *in situ* FISH remissions in two additional patients with chronic myelogenous leukemia, and remissions in patients with acute lymphoblastic leukemia, acute myelogenous leukemia, multiple myeloma, and Hodgkin's disease (21). These encouraging results have prompted us to employ this treatment schema in other clinical settings, including patients with HLA-matched unrelated HSC donors (22).

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4. REERENCES

1. Jacobson LO, Marks EK, Robson MJ, Gaston EO, Zirkle RE : Effect of spleen protection on mortality following x-irradiation. *J Lab Clin Med* 34, 1538-1543 (1949)

2. Thomas ED, Storb R, Clift RA, Fefer A, Johnson L, Neiman PE, Lerner KG, Glucksberg H, Buckner CD: Bone-marrow transplantation. *N Engl J Med* 292, 832-843, 895-902 (1975)

3. Burchenal JH, Oettgen HF, Holmberg EAD, Hemphill SC, Reppert JA: Effect of total body irradiation on the transplantability of mouse leukemias. *Cancer Res* 20, 425 (1960)

4. Barnes DWH, Loutit JF: Treatment of murine leukaemia with x-rays and homologous bone marrow: II. *Br J Haematol* 3, 241-252 (1957)

5. Mathe G, Amiel JL, Schwarzenberg L, Catton A, Schneider M: Adoptive immunotherapy of acute leukemia: Experimental and clinical results. *Cancer Res* 25, 1525-1531 (1965)

6. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, Storb R: Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med* 300, 1068-1073 (1979)

7. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED and the Seattle Marrow Transplant Team: Antileukemic effect of chronic graft-versus-host disease. Contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med* 304, 1529-1533 (1981)

8. Kolb HJ, Mittermüller J, Clemm C, Holler G, Ledderose G, Brehm G, Heim M, Wilmanns W: Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 76, 2462-2465 (1990)

9. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, Ljungman P, Ferrant A, Verdonck L, Niederwieser D, van Rhee F, Mittermüeller J, De Witte T, Holler E, Ansari H: Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. *Blood* 86, 2041-2050 (1995)

10. Storb R, Yu C, Wagner JL, Deeg HJ, Nash RA, Kiem H-P, Leisenring W, Shulman H: Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. *Blood* 89, 3048-3054 (1997)

11. Storb R, Yu C, Barnett T, Wagner JL, Deeg HJ, Nash RA, Kiem H-P, McSweeney P, Seidel K, Georges G, Zaucha JM: Stable mixed hematopoietic chimerism in dog leukocyte antigen-identical littermate dogs given lymph node irradiation before and pharmacologic immunosuppression after marrow transplantation. *Blood* 94, 1131-1136 (1999)

12. Barsoukov AA, Moore PF, Storb R, Santos EB, Sandmaier BM: The use of an anti-TCRαβ monoclonal antibody to control host-versus-graft reactions in canine marrow allograft recipients conditioned with low dose total body irradiation. *Transplantation* 67, 1329-1335 (1999)

13. Storb R, Yu C, Zaucha JM, Deeg HJ, Georges G, Kiem H-P, Nash RA, McSweeney PA, Wagner JL: Stable mixed hematopoietic chimerism in dogs given donor antigen, CTLA4Ig, and 100 cGy total body irradiation before and pharmacologic immunosuppression after marrow transplant. *Blood* 94, 2523-2529 (1999)

14. Storb R, Yu C, McSweeney P: Mixed chimerism after transplantation of allogeneic hematopoietic cells. In: Thomas ED, Blume KG, Forman SJ (eds). *Hematopoietic Cell Transplantation*, 2nd Edition. Blackwell Science: Boston 287-295 (1999)

15. Storb R: Nonmyeloablative preparative regimens: experimental data and clinical practice. In: Perry MC (ed). *ASCO Education Book* 241-249 (1999)

16. Woolfrey AE, Nash RA, Frangoul HA, McSweeney PA, Sanders JE, Ochs HD, Storb R: Non-myeloablative transplant regimen used for induction of multi-lineage allogeneic hematopoietic mixed donor-host chimerism in patients with T-cell immunodeficiency. *Blood* 92(Suppl 1) 520a (abstract) (1998)

17. Walters MC, Storb R, Patience M, Leisenring W, Taylor T, Sanders JE, Buchanan GE, Rogers ZR, Dinndorf P, Davies SC, Roberts IAG, Dickerhoff R, Yeager AM, Hsu L, Kurtzberg J, Ohene-Frempong K, Bunin N, Bernaudin F, Wong W-Y, Scott JP, Margolis D, Vichinsky E, Wall DA, Wayne AS, Pegelow C, Redding-Lallinger R, Wiley J, Klemperer M, Mentzer WC, Smith FO, Sullivan KM: Impact of bone marrow transplantation for symptomatic sickle cell disease: an interim report. *Blood* 95, 1918-1924 (2000)

18. Yu C, Nash R, Lothrop C, Zaucha J, Storb R : Severe canine hereditary hemolytic anemia treated by marrow grafts using nonmyeloablative immunosuppression. *Blood* 92(Suppl 1) 263a, #1078 (abstract) (1998)

19. McSweeney PA, Wagner JL, Maloney DG, Radich J, Shizuru J, Bensinger WI, Bryant E, Chauncey TR, Flowers MED, Kauffman M, Minor CS, Nash RA, Blume K, Storb R: Outpatient PBSC allografts using immunosuppression with low-dose TBI before, and cyclosporine (CSP) and mycophenolate mofetil (MMF) after transplant. *Blood* 92(Suppl 1) 519a, #2133 (abstract) (1998)

20. McSweeney P, Niederwieser D, Shizuru J, Molina A, Wagner J, Minor S, Radich J, Chauncey T, Hegenbart U, Maloney D, Nash R, Sandmaier B, Blume K, Storb R: Outpatient allografting with minimally myelosuppressive, immunosuppressive conditioning of low-dose TBI and postgrafting cyclosporine (CSP) and mycophenolate mofetil (MMF). *Blood* 94(Suppl. 1) 393a, #1742 (abstract) (1999)

21. McSweeney P, Niederwieser D, Shizuru J, Radich J, Molina A, Hegenbart U, Chauncey T, Sandmaier B, Wolff D, Blume K, Storb R: Molecular remissions after non-myeloablative allografting for chronic myelocytic leukemia (CML). *Blood* 94(Suppl. 1) 710a, #3135 (abstract) (1999)

22. Niederwieser D, Wolff D, Hegenbart U, Mantovani L, Ponisch W, Deininger M, McSweeney P, Edelmann J, Kiefel V, Blume K, Storb R: Hematopoietic stem cell transplants (HSCT) from HLA-matched and one-allele mismatched unrelated donors using a nonmyeloablative regimen. *Blood* 94(Suppl. 1) 561a, #2506 (abstract) (1999)

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Hematopoietic stem cell transplant (HSCT) is the treatment of choice for patients with severe forms of MPS I. Transplantation before the age of 2 years (before the onset of irreversible neurological decline), favorably alters the natural history of the disease. From: Handbook of Clinical Neurology, 2013.Â Hematopoietic stem cell transplantation (HSCT) has become an accepted therapeutic modality for the treatment of malignant and nonmalignant disorders. Tables 31.1 and 31.2 list the indications for allogeneic and autologous HSCT. Most allogeneic transplants performed in patients <20 years old are for acute leukemias (43%) or nonmalignant indications (35%). Autologous stem cell transplantation for progressive multiple sclerosis: update of the European Group for Blood and Marrow Transplantation autoimmune diseases working party database. Mult Scler Dec;12(6): Saiz A, Blanco Y, Berenguer J, GÃ³mez M, Carreras E, Arbizu T, Graus F. [Clinical outcome 6 years after autologous hematopoietic stem cell transplantation in multiple sclerosis.] Neurologia Jan Ni XS, Ouyang J, Zhu WH, Wang C, Chen B. Autologous hematopoietic stem cell transplantation for progressive multiple sclerosis: report of efficacy and safety at three yr of follow up in 21 patients.Â Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA Apr 11;297(14): 28 28.

Autologous peripheral hematopoietic stem cells transplantation (auto-HSCT) was for the first time performed at Hammersmith Hospital in London in 1981 to treat the patient in accelerated phase of CML. Although auto-HSCT does not play any role in the treatment of CML nowadays, indications for this valuable therapeutic method have evolved for many years. In acute leukemia auto-HSCT should be recommended only in the context of clinical studies.Â Allogeneic hematopoietic stem cell transplantation connected with application of high-dose chemo- and radiotherapy was first carried out by Thomas et al. in 1957 to treat leukemia patient in advanced stage [18].Â In the beginning of 21st century, the similar trend occurred also in allo-HSCT. Other (stem) cells for the treatment of multiple sclerosis. Conclusion. Acknowledgements.Â Autologous hematopoietic stem cell transplantation-related changes of the immune repertoire The restoration of immune tolerance following AHSCT is characterized by a profound renewal of the T-cell repertoire mainly due to the expansion of naive CD4Ã¾ T cells of recent thymic origin [12]. This study [12] suggests for the rst time that AHSCT results in the induction of a new immune system less prone to self-reactivity.Â Autologous nonmyeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II. study. Lancet Neurol 2009; 8:244â€“253.