

The Future of Stem Cell Transplantation in Autoimmune Disease

Susumu Ikehara

© Humana Press Inc. 2009

Abstract We have previously found that conventional allogeneic bone marrow transplantation (allo BMT) can be used to treat various spontaneously developed autoimmune diseases in mice. In addition, we have found that autoimmune diseases can be transferred into the normal mice by conventional BMT from autoimmune-prone mice. Based on these findings, we have proposed that autoimmune diseases are “stem cell disorders.” To apply allo BMT to humans, we extensively carried out BMT to clarify which cells are essential for successful BMT, and finally found that three types of cells are essential for successful allogeneic BMT: (1) hemopoietic stem cells (HSCs), (2) natural suppressor cells, and (3) stromal cells (including mesenchymal stem cells, MSCs). We have very recently found that MSCs play a crucial role in preventing graft failure, since there is a major histocompatibility complex restriction between HSCs and MSCs. To recruit donor-derived MSCs, we have found that the injection of whole bone marrow cells into the bone marrow cavity (intra-bone marrow-BMT, IBM-BMT) is the best strategy for the treatment of various otherwise intractable diseases, including autoimmune diseases. In this review article, we provide evidence that IBM-BMT heralds a revolution in the field of transplantation and regeneration medicine.

Keywords Bone marrow transplantation (BMT) · Autoimmune disease · Intra-bone marrow (IBM)-BMT · Hemopoietic stem cell (HSC) · Mesenchymal stem cell (MSC)

S. Ikehara (✉)
First Department of Pathology, Kansai Medical University,
10-15 Fumizono-cho,
Moriguchi City, Osaka 570-85506, Japan
e-mail: ikehara@takii.kmu.ac.jp

Introduction

In 1985, we found that allogeneic (but not syngeneic or autologous) bone marrow transplantation (BMT) could be used to treat autoimmune diseases in autoimmune-prone mice [1, 2]. Since then, we have confirmed our findings using a variety of autoimmune-prone mice [3–5].

Conversely, we have succeeded in inducing autoimmune diseases in normal mice by transplanting T-cell-depleted bone marrow cells (BMCs) or partially purified hemopoietic stem cells (HSCs) from autoimmune-prone mice to normal mice [6, 7]. Based on these findings, we proposed that autoimmune diseases were “stem cell disorders (SCDs)” [6–8].

Our findings have also been confirmed in humans: patients with autoimmune diseases were cured after allogeneic BMT, while autoimmune diseases were found to be transferred to recipients of BMT from donors who were suffering from autoimmune diseases [9].

In this article, we show that various otherwise intractable diseases (including autoimmune diseases) can be cured by our novel BMT method.

Strategies for recruitment of donor stromal cells

Using radiosensitive and chimeric-resistant MRL/lpr mice, we have found that the recruitment of donor stromal cells is essential for successful allogeneic BMT. We found that three methods are effective in replacing recipient stromal cells with donor-derived stromal cells: (1) conventional intravenous BMT (IV-BMT) plus bone grafts [10, 11]; (2) BMT from the portal vein (PV-BMT) [12], since tolerance can be easily induced when the antigen is portal venously (PV) injected into the liver; and finally (3) intra-bone

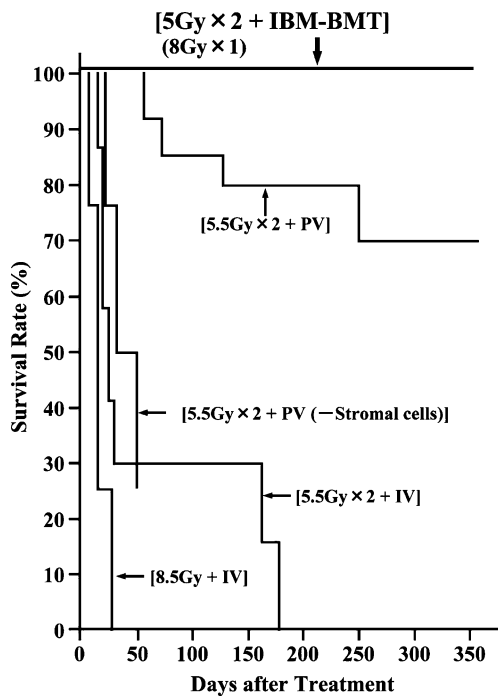


Fig. 1 Treatment of autoimmune diseases in MRL/lpr mice by IBM-BMT ($5\text{ Gy} \times 2$). IBM-BMT can be used to treat autoimmune diseases in MRL/lpr mice even when the radiation dose is reduced to $5\text{ Gy} \times 2$

marrow (IBM)-BMT [13]. IBM-BMT was ultimately found to be best, since it allows us to use a mild conditioning regimen ($5\text{ Gy} \times 2$), as shown in Fig. 1. We therefore used IBM-BMT instead of the conventional IV-BMT in subsequent experiments.

IBM-BMT for organ transplantation

Since we have previously found that the combination of organ allografts and conventional IV-BMT from the same donors prevents the rejection of organ allografts [14], we attempted to apply IBM-BMT to organ allografts. IBM-BMT was the most effective strategy, since the radiation dose could be reduced to $4.0\text{ Gy} \times 2$ in skin allografts [15]. In addition, we found that IBM-BMT is applicable to allografts of other organs and tissues in rats, such as pancreas islets [16], legs [17], lungs [18], and heart [19].

IBM-BMT for regeneration therapy

As it was apparent that donor stromal cells could be effectively recruited by “IBM-BMT”, we next attempted to treat osteoporosis in SAMP6 mice; the SAMP6 mouse (a substrain of senescence-accelerated mice) spontaneously develops osteoporosis early in life and is therefore a useful model for examining the mechanisms underlying osteoporosis. After IBM-BMT, the hematolymphoid system was completely reconstituted with donor-type cells. Thus-treated SAMP6 mice (8 months after IBM-BMT) showed marked increases in trabecular bone even at 20 months of age (Fig. 2) and the bone mineral density remained similar to that of normal B6 mice. Bone marrow stromal cells in “IBM-BMT”-treated SAMP6 mice were replaced by donor stromal cells [20, 21]. Thus, we succeeded in curing osteoporosis in SAMP6 mice by IBM-BMT, which can recruit both donor-derived HSCs and MSCs.

Fig. 2 Treatment of osteoporosis in SAMP6 mice by IBM-BMT from normal B6 mice

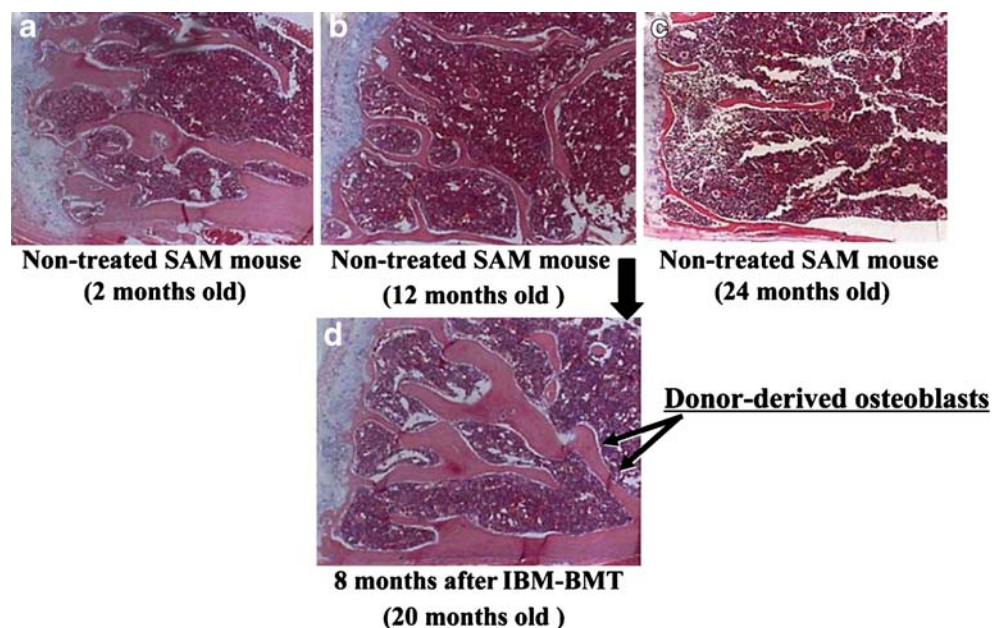
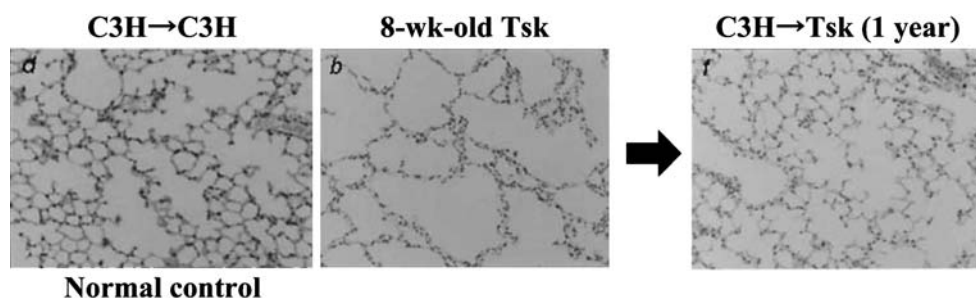


Fig. 3 Amelioration of emphysema in [C3H→Tsk] mice by IBM-BMT



Since IBM-BMT appeared to be a powerful strategy in regeneration therapy, we next used tight-skin (Tsk) mice (an animal model for emphysema) to examine whether emphysema could be cured by IBM-BMT.

IBM-BMT was carried out from C3H mice into Tsk mice (8–10 weeks old) that had already shown emphysema. Eight months after the transplantation, the lungs of all the Tsk mice treated with IBM-BMT [C3H→Tsk] showed structures similar to those of normal mice, whereas the [Tsk→Tsk] mice showed emphysema, as seen in age-matched Tsk mice (Fig. 3). Next, we attempted to transfer emphysema from Tsk mice to C3H mice by IBM-BMT. Six months after IBM-BMT, the [Tsk→C3H] mice showed emphysema [22]. These results strongly suggested that emphysema in Tsk mice originates from defects in the stem cells (probably MSCs and/or HSCs) in the bone marrow [22].

IBM-BMT + donor lymphocyte infusion for treatment of malignant tumors

It is well known that the graft-versus-leukemia reaction can cure patients of a variety of hematological malignancies [23, 24]. Recently, it has been reported that graft-versus-tumor (GvT) effects can induce partial (complete in some)

remission of metastatic solid tumors such as breast cancer [14, 25, 26] and renal cell carcinoma [27–33]. Based on these findings, donor lymphocyte infusion (DLI) has recently been used for the treatment of malignant solid tumors even in humans. However, it is very difficult to completely eradicate the tumors, since extensive DLI induces graft-versus-host disease (GvHD). We therefore attempted to establish a new method for the treatment of malignant tumors, this method consisting of intra-bone marrow-IBM-BMT plus DLI, since we have recently found that IBM-BMT can allow a reduction in radiation doses as a conditioning regimen and prevent GvHD in mice [13, 34]. Using the Meth-A cell line (BALB/c-derived fibrosarcoma), we found that IBM-BMT plus the injection of CD4⁺ T-cell-depleted (but not CD8⁺ T cell depleted) spleen cells (as DLI) can prevent GvHD while suppressing tumor growth (Fig. 4). In addition, we have found that IBM-BMT plus extensive DLI (three times every 2 weeks) leads to the complete rejection of the tumor, although the success rate (three out of 50) is not high so far [34].

In addition, we have examined whether this strategy (IBM-BMT plus DLI) is applicable to other tumors in other animals. We have obtained similar results in another system (colon cancer, ACL-15 in rats) [35]. We are now establishing more efficient strategies to eradicate malignant tumors.

Fig. 4 Prevention of GvHD and suppression of tumor growth by IBM-BMT + DLI (CD4⁺)

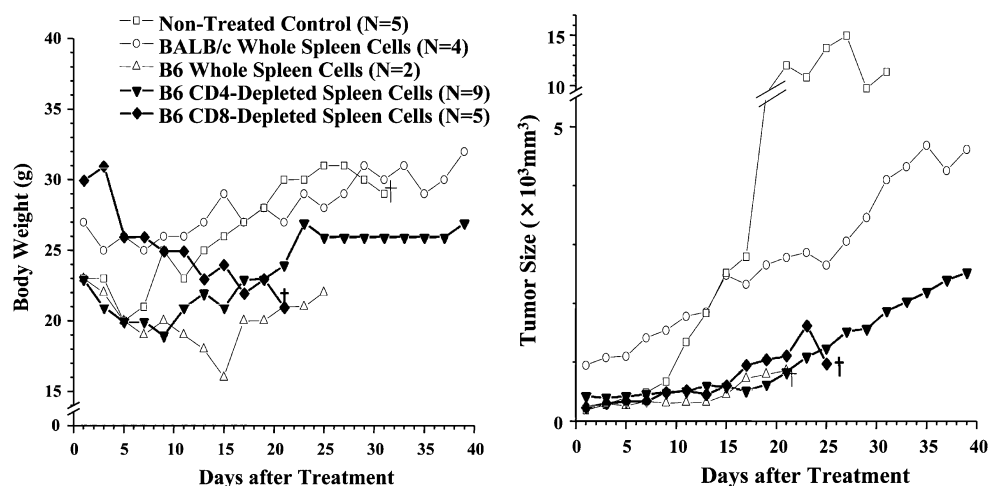
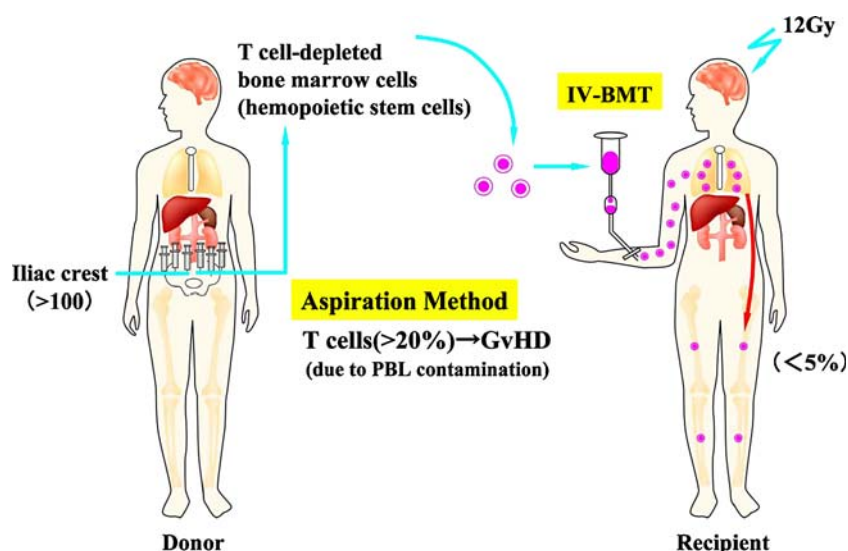


Fig. 5 Conventional BMT method for allogeneic BMT. Conventional BMT is carried out using an aspiration method (AM), followed by the intravenous injection of BMCs (IV-BMT)



Conventional BMT versus novel BMT

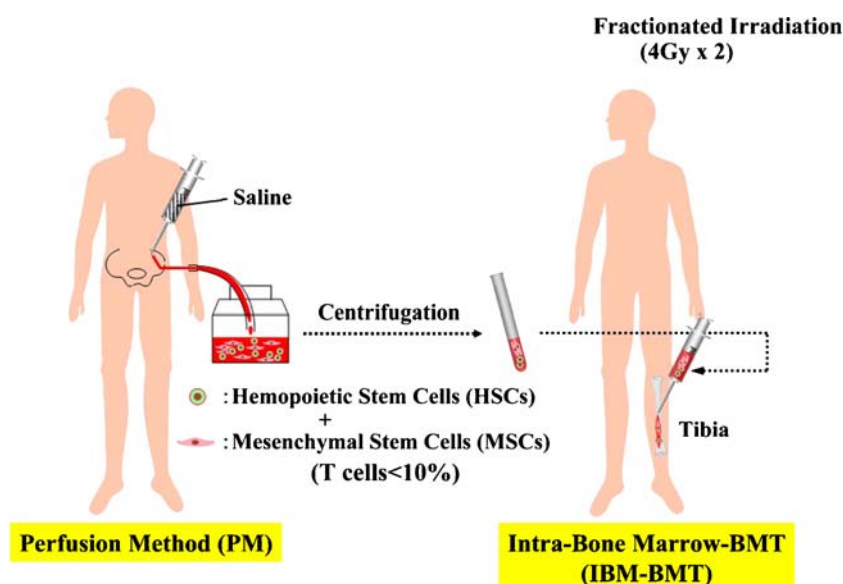
Conventional BMT is carried out as follows: bone marrow needles are inserted into the iliac bones more than 100 times, and the BMCs are collected by the aspiration method (AM; Fig. 5). Therefore, contamination with peripheral blood (particularly T cells) is inevitable. When thus-collected cells are intravenously injected, most cells become trapped in the lung and only a few cells migrate into the bone marrow (Fig. 5).

To apply our new BMT methods to humans, we established, using cynomolgus monkeys, a “perfusion method (PM)”, which minimizes the contamination of BMCs with T cells. As shown in Fig. 6, two needles are

inserted into a long bone such as the humerus, femur, or tibia. The end of the extension tube is connected to a needle. The other end is placed in a syringe containing 0.5 ml heparin. The second needle is connected to a syringe containing 30 ml of saline, and the saline is then pushed gently from the syringe into the medullary cavity to flush out the bone marrow. The saline containing the BM fluid is then collected (Fig. 6).

There is significantly less contamination with T cells when using the PM (<10%) than with the conventional AM (>20%). Therefore, T cell depletion is unnecessary with the PM, and whole BMCs can be used. However, in the case of the conventional AM, T cell depletion is necessary, and the loss of some important cells such as MSCs during the

Fig. 6 New BMT method for allogeneic BMT. New BMT method is carried out using a perfusion method (PM), followed by IBM-BMT



process of T cell depletion is inevitable. Furthermore, the number and progenitor activities of the cells harvested using the PM are greater than when using the conventional AM [36, 37].

Future directions

We have also found that the PM is applicable to the iliac bones as well as the long bones not only in monkeys but also in humans.

As described here, the new BMT method (PM + IBM-BMT) can be used to treat various otherwise intractable diseases, including (1) autoimmune diseases, (2) age-associated diseases (osteoporosis, emphysema, etc.), (3) diseases curable by organ transplantation, and (4) malignant tumors (including solid tumors). The PM can efficiently be used to collect whole BMCs (including HSCs and MSCs) without them being contaminated with T cells, and no GvHD therefore develops. IBM-BMT can efficiently transfer donor whole BMCs (both HSCs and MSCs) into recipients and this method can therefore be used to quickly replace not only HSCs but also MSCs with donor-derived cells.

From the findings to date, it is conceivable that all the body's cells originate in the bone marrow, and that all diseases might therefore originate from defects in the bone marrow. One paper already suggests that gastric cancer originates from bone-marrow-derived cells [38].

We believe that the development of our BMT method heralds a revolution in the field of transplantation (BMT and organ transplantation) and regeneration therapy.

Acknowledgements I thank Mr. Hilary Eastwick-Field and Ms. K. Ando for their help in the preparation of the manuscript. These studies were mainly supported by the 21st Century Center of Excellence (COE) program of the Ministry of Education, Culture, Sports, Science, and Technology. Supported also by a grant from Haiteku Research Center of the Ministry of Education, Health, and Labor Sciences Research Grants; a grant from the Science Frontier program of the Ministry of Education, Culture, Sports, Science, and Technology; and a grant from the Department of Transplantation for Regeneration Therapy (sponsored by Otsuka Pharmaceutical Company, Ltd.), a grant from Molecular Medical Science Institute, Otsuka Pharmaceutical Co., Ltd.; and a grant from Japan Immunoresearch Laboratories Co., Ltd. (JIMRO).

References

- Ikehara S, Good RA, Nakamura T et al (1985) Rationale for bone marrow transplantation in the treatment of autoimmune diseases. *Proc Natl Acad Sci* 82:2483–2487
- Ikehara S, Ohtsuki H, Good RA et al (1985) Prevention of type I diabetes in nonobese diabetic mice by allogeneic bone marrow transplantation. *Proc Natl Acad Sci* 82:7743–7747
- Oyaizu S, Yasumizuy R, Miyama-Inabam M et al (1988) (NZW x BXS_B)F₁ mouse; a new animal model of idiopathic thrombocytopenic purpura. *J Exp Med* 167:2017–2022
- Than SOE, Ishida H, Inaba M et al (1992) Bone marrow transplantation as a strategy for treatment of non-insulin-dependent diabetes mellitus in KK-Ay mice. *J Exp Med* 176:1233–1238
- Nishimura M, Toki J, Sugiura K et al (1994) Focal segmental glomerular sclerosis, a type of intractable chronic glomerulonephritis, is a stem cell disorder. *J Exp Med* 179:1053–1058
- Ikehara S, Kawamura M, Takao F et al (1990) Organ-specific and systemic autoimmune diseases originate from defects in hematopoietic stem cells. *Proc Natl Acad Sci* 87:8341–8344
- Kawamura M, Hisha H, Li Y et al (1997) Distinct qualitative differences between normal and abnormal hemopoietic stem cells in vivo and in vitro. *Stem Cells* 15:56–62
- Ikehara S (2003) A new concept of stem cell disorders and their new therapy. *J. Hematother Stem Cell Res* 12:643–653
- Marmont AM (1994) Immune ablation followed by allogeneic or autologous bone marrow transplantation: a new treatment for severe autoimmune diseases? *Stem Cells* 12:125–135
- Ikehara S, Inaba M, Ishida S et al. (1991) Rationale for transplantation of both allogeneic bone marrow and stromal cells in the treatment of autoimmune diseases. In: Champlin RE, Gale RP Eds. *New Strategies in Bone Marrow Transplantation UCLA Symposia on Molecular and Cellular Biology, New Series*. Wiley-Liss, New York 137:251–257
- Ishida T, Inaba M, Hisha H et al (1994) Requirement of donor-derived stromal cells in the bone marrow for successful allogeneic bone marrow transplantation: complete prevention of recurrence of autoimmune diseases in MRL/MP-lpr/lpr mice by transplantation of bone marrow plus bones (stromal cells) from the same donor. *J Immunol* 152:3119–3127
- Kushida T, Inaba M, Takeuchi K et al (2000) Treatment of intractable autoimmune diseases in MRL/lpr mice using a new strategy for allogeneic bone marrow transplantation. *Blood* 95:1862–1868
- Kushida T, Inaba M, Hisha H et al (2001) Intra-bone marrow injection of allogeneic bone marrow cells: a powerful new strategy for treatment of intractable autoimmune diseases in MRL/lpr mice. *Blood* 97:3292–3299
- Nakamura T, Good RA, Inoue S et al (2004) Successful liver allografts in mice by combination with allogeneic bone marrow transplantation. *Proc Natl Acad Sci U S A* 83:4529–4532
- Ikehara S (2008) A novel method of bone marrow transplantation (BMT) for intractable autoimmune diseases. *J Autoimmun* 30:108–115
- Ikebukuro K, Adachi Y, Suzuki Y et al (2006) Synergistic effects of induction in transplantation of allogeneic pancreatic islets. *Bone Marrow Transplant* 38:657–664
- Esumi T, Inaba M, Ichioka N et al (2003) Successful allogeneic leg transplantation in rats by combination of intra-bone marrow (IBM) injection of donor bone marrow cells. *Transplantation* 76:1543–1548
- Kaneda H, Adachi Y, Saiyo Y et al (2005) Long-term observation after simultaneous lung and intra-bone marrow-bone marrow transplantation. *J Heart Lung Transplant* 24:1415–1423
- Guo K, Inaba M, LI M et al (2008) Long-term donor-specific tolerance in rat cardiac allografts by intra-bone marrow injection of donor bone marrow cells. *Transplantation* 85:93–101
- Ichioka N, Inaba M, Kushida T et al (2002) Prevention of senile osteoporosis in SAMP6 mice by intra-bone marrow injection of allogeneic bone marrow cells. *Stem Cells* 20:542–551
- Takada K, Inaba M, Ichioka N et al (2006) Treatment of senile osteoporosis in SAMP6 mice by intra-bone marrow injection of allogeneic bone marrow cells. *Stem Cells* 24:399–405
- Adachi Y, Oyaizu H, Taketani S et al (2006) Treatment and transfer of emphysema by a new bone marrow transplantation method from normal mice to Tsk mice and vice versa. *Stem Cells* 24:2071–2077

23. Thomas ED, Blume KG (1999) Historical markers in the development of allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 5:341–346
24. Weiden PL, Flournoy N, Thomas ED et al (1979) Antileukemic effect of graft-versus-host diseases in human recipients of allogeneic-marrow grafts. *N Engl J Med* 300:1068–1073
25. Ben-Yosef R, Or R, Nagker A et al (1996) Graft-versus-tumor and graft-versus-leukaemia effect in patient with concurrent breast cancer and acute myelocytic leukaemia. *Lancet* 348:1242–1243
26. Eibi B, Schwaigofer H, Nachbaur HD et al (1996) Evidence for a graft-versus-tumor effect in a patient treated with marrow ablative chemotherapy and allogeneic bone marrow transplantation for breast cancer. *Blood* 88:1501–1508
27. Ueno NT, Rondon G, Mirza NQ et al (1998) Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. *J Clin Oncol* 16:986–993
28. Childs RW, Clave E, Tisdale J et al (1999) Successful treatment of metastatic renal cell carcinoma with a nonmyeloablative allogeneic peripheral-blood progenitor-cell transplant: evidence for a graft-versus-tumor effect. *J Clin Oncol* 17:2044–2049
29. Childs R, Chernoff A, Contentin N et al (2000) Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med* 343:750–758
30. Appelbaum FR, Sandmaier B (2002) Sensitivity of renal cell cancer to nonmyeloablative allogeneic hematopoietic cell transplantation: unusual or unusually important? *J Clin Oncol* 20: 1965–1967
31. Bregni M, Doderio A, Peccatori J et al (2002) Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer. *Blood* 99:4234–4236
32. Hentschke P, Barkholt L, Uzunel M et al (2003) Low-intensity conditioning and hematopoietic stem cell transplantation in patients with renal and colon carcinoma. *Bone Marrow Transplant* 31:253–261
33. Rini BI, Zimmerman T, Stadler WM et al (2002) Allogeneic stem-cell transplantation of renal cell cancer after nonmyeloablative chemotherapy: feasibility, engraftment, and clinical results. *J Clin Oncol* 20:2017–2024
34. Suzuki Y, Adachi Y, Minamino K et al (2005) A new strategy for treatment of malignant tumor: intra-bone marrow-bone marrow transplantation plus CD4⁺ donor lymphocyte infusion. *Stem Cells* 23:365–370
35. Koike Y, Adachi Y, Suzuki Y et al (2007) Allogeneic intra-bone marrow-bone marrow transplantation plus donor lymphocyte infusion suppresses growth of colon cancer cells implanted in skin and liver of rats. *Stem Cells* 25:385–391
36. Inaba M, Adachi Y, Hisha H et al (2007) Extensive studies on perfusion method plus intra-bone marrow-bone marrow transplantation using cynomolgus monkeys. *Stem Cells* 25:2098–2103
37. Kushida T, Inaba M, Ikebukuro K et al (2002) Comparison of bone marrow cells harvested from various bones of cynomolgus monkeys of various ages by perfusion or aspiration methods: a preclinical study for human BMT. *Stem Cells* 20:155–162
38. Houghton J, Stoicov C, Nomura S et al (2004) Gastric cancer originating from bone marrow-derived cells. *Science* 306:1568–1571