Posttransplant Administration of Cyclophosphamide and Donor Lymphocyte Infusion Induces Potent Antitumor Immunity to Solid Tumor

Masatoshi Eto,1 Yoriyuki Kamiryo,1,3 Ario Takeuchi,1,3 Masahiko Harano,1 Katsunori Tatsugami,1 Mamoru Harada,2 Keiijo Kiyoshima,1,2 Masumitsu Hamaguchi,1 Takanori Teshima,4 Masazumi Tsuneyoshi,2 Yasunobu Yoshikai,3 and Seiji Naito1

Abstract

Purpose: Nonmyeloablative allogeneic stem cell transplantation (SCT) has been increasingly used for the treatment of hematologic and solid malignancies, and mature donor T cells are considered to be the main effectors of the graft-versus-tumor (GVT) activity. However, the association between degree of donor chimerism and intensity of GVT effects has not been fully elucidated. We recently proposed a unique nonmyeloablative cell therapy using posttransplant cyclophosphamide and donor lymphocyte infusion, by which a significant antitumor effect against murine renal cell carcinoma, RENCA, was induced, although the level of mixed chimerism was relatively low. In this study, we attempted to clarify a role of chimerism for in vivo antitumor effects on GVT effects in radiation-associated nonmyeloablative SCT.

Experimental Design: We assessed antitumor effects on RENCA tumors and the degree of donor chimerism after several doses of irradiation followed by allogeneic SCT and compared the results with those of cyclophosphamide-based cell therapy.

Results: Allogeneic SCT following sublethal irradiation (6 Gy) induced almost complete donor chimerism, whereas cyclophosphamide-based cell therapy produced low levels of donor chimerism. Nonetheless, GVT activity was much more potent in cyclophosphamide-based cell therapy than irradiation-conditioned SCT. Furthermore, cyclophosphamide-conditioned SCT induced more potent immune reconstitution with less severe graft-versus-host disease than irradiation-conditioned SCT.

Conclusions: Our results indicate that a high level of chimerism is not essential for the in vivo antitumor effect of nonmyeloablative allogeneic cell therapy against solid tumor and that the recovery of peripheral lymphocytes after the initial immunosuppression might be a critical event for the elicitation of in vivo antitumor effects of that treatment modality.

Allogeneic hematopoietic stem cell transplantation (SCT) is a curative treatment for hematologic malignancies and bone marrow (BM) failure syndrome. However, high incidence of treatment-related toxicity and mortality associated with intensive pretransplant conditioning limits wider application of this treatment strategy (1–4). Recently, nonmyeloablative conditioning was introduced to reduce treatment-related toxicity (5–14). Since the first report by Childs et al. (5), nonmyeloablative allogeneic hematopoietic SCT has been applied for the treatment of solid cancers. In allogeneic hematopoietic SCT, mature donor T cells are considered to be the main effectors of the graft-versus-tumor activity of this type of therapy (4, 15–20). Therefore, a high level of donor T-cell chimerism has been suggested to be a desirable situation for its elicitation of in vivo antitumor effect.

We have reported a series of studies about the cyclophosphamide-induced tolerance system that comprises an i.v. injection of 1 × 10⁶ allogeneic spleen cells and 2 × 10⁷ BM cells followed by an i.p. injection of 200 mg/kg cyclophosphamide (21, 22). In this system, cyclophosphamide destroys both donor-reactive T cells of host origin and host-reactive T cells of donor origin, resulting in a stable mixed chimerism and an immunologic tolerance (21, 22). We recently modified this tolerance-inducing protocol for the treatment of cancer and proposed a cyclophosphamide-using nonmyeloablative cell therapy, in which donor lymphocyte infusion was carried out 1 day after the cyclophosphamide treatment (23). The cyclophosphamide-using cell therapy can induce a significant...
antitumor effect against murine renal cell carcinoma (RCC) RENCA, although the level of chimerism is relatively low.

In the case of hematologic malignancies, it has been assumed that a complete donor chimerism is necessary for complete destruction of residual leukemia cells. However, it has not been well determined whether a high level of chimerism is really required for the \textit{in vivo} elicitation of antitumor effect of nonmyeloablative allogeneic cell therapy in the treatment of solid malignancies. In this study, we attempted to elucidate necessity of chimerism for \textit{in vivo} antitumor effect in radiation-associated nonmyeloablative cell therapy.

**Materials and Methods**

**Animals.** Female BALB/c (H-2\(^{a}\)) recipient mice and female DBA/2 (H-2\(^{b}\)) donor mice were obtained from Japan Charles River at 8 wk of age. All mice were kept in a specific pathogen-free condition and then used for experiments at 10 wk of age. All animal protocols were approved by the University Committee on the Use and Care of Animals at Kyushu University.

**Tumors.** RENCA is a murine carcinogen-induced RCC of BALB/c origin and is maintained \textit{in vitro} in a complete medium. RPMI 1640 (Life Technologies) supplemented with 10% heat-inactivated FCS (HyClone), \(5 \times 10^{-5}\) mol/L 2-mercaptoethanol, 20 mmol/L HEPES, 30 \(\mu\)g/mL gentamicin (Schering Corp.), and 0.2% sodium bicarbonate was used as the complete medium.

**Radiation-induced cancer treatment protocol.** To evaluate the \textit{in vivo} antitumor activity, BALB/c mice were injected s.c. with \(2 \times 10^5\) RENCA cells. Seven days after the tumor inoculation, these mice were irradiated with several doses of irradiation (3, 5, 6, 7, and 8.5 Gy). On the next day (day 0), 1.0 mL of RPMI 1640 containing a set quantity of a mixture of \(2 \times 10^7\) BM cells and \(1 \times 10^7\) lymph node (LN) cells originated from donor DBA/2 mice was injected i.v. into the tail vein of BALB/c mice.

**Cyclophosphamide-induced cancer treatment protocol.** As previously reported (23), 7 d after tumor inoculation, 1.0 mL of RPMI 1640 containing a set quantity of a mixture of \(1 \times 10^8\) spleen cells and \(2 \times 10^7\) BM cells originated from donor DBA/2 mice was injected i.v. into the tail vein of BALB/c mice (on day 0). Cyclophosphamide (Endoxan, Shionogi) dissolved in PBS (20 mg/mL) was injected i.p. at a dose of 200 mg/kg on day 2. Donor DBA/2 lymphocytes (\(1 \times 10^7\)) were injected i.v. to BALB/c mice on day 3.

**Fig. 1.** Levels of mixed chimerism in the BALB/c mice treated with radiation followed by the transfer of DBA/2 BM and LN cells. BALB/c mice were irradiated with the indicated doses of radiation. On the next day (on day 0), these mice were transferred with BM and LN cells from allogeneic DBA/2 (Ly1\(^{+}\)) mice. On day 15, chimerism in the peripheral blood was assessed by a flow cytometric analysis. In some experiments, BALB/c mice were treated with DBA/2 spleen and BM cells on day 0, cyclophosphamide on day 2, and DBA/2 LN cells on day 3, as shown in our previous study (23). Chimerism in the peripheral blood was also assessed by a flow cytometric analysis on day 15. Each panel shows the representative findings of three separate experiments. The other two experiments showed similar results. DLI, donor lymphocyte infusion.

**Fig. 2.** Tumor growth in the BALB/c mice irradiated at a dose of 6 or 3 Gy followed by the transfer of allogeneic BM and LN cells. After establishing s.c. RENCA tumor (7 d after tumor inoculation), the BALB/c mice were irradiated at a dose of 6 Gy (A) or 3 Gy (B) followed by the transfer of BM and LN cells from either syngeneic BALB/c or allogeneic DBA/2 mice. Each group consisted of five mice. Tumor growth was inspected every 3 or 4 d. Points, mean; bars, SD. The representative findings among three separate experiments are shown here. The other two experiments showed similar results. *, \(P < 0.05\); **, \(P < 0.01\); ***, not specific between the two groups.
Flow cytometric analysis. The expression of the lymphocyte derived from BALB/c or DBA/2 mice was analyzed by two-color flow cytometry using a FACScan cytometer (Becton Dickinson). Phycoerythrin-conjugated anti-mouse CD5 (Ly-1) monoclonal antibody (PharMingen International) and FITC-conjugated mouse anti-mouse CD5.1 (Ly-1.1) monoclonal antibody (PharMingen International) were used for the analysis of the lymphocyte origin from either BALB/c or DBA/2 mice. In some experiments, FITC-conjugated anti-foxp3, phycoerythrin-conjugated anti-CD25, and peridinin chlorophyll protein–conjugated anti-CD4 (e-Bioscience) were used for the analysis of regulatory T cells. The labeled cells were analyzed by FACScan and fluorescence histograms were accumulated on a logarithmic scale.

Measurement of tumor growth in vivo. After s.c. tumor inoculation, tumor growth was inspected every 3 or 4 d by measuring the largest perpendicular diameters with a caliper, which thus was recorded as the tumor area (mm$^2$).

Histopathologic examination. The RENCA tumors were removed and fixed in 10% buffered formalin, embedded in paraffin, and cut into 5-μm slices. The sections were then stained with H&E. The slides were coded without any reference to the prior treatment status and then tumor-infiltrating lymphocytes were examined pathologically by a single pathologist (K.K.).

Statistics. The statistical significance of the data was determined using the unpaired two-tailed Student's $t$ test. In some experiments, Kaplan-Meier survival curves were created, and log-rank test was used for the comparison of survival curves. A $P$ value of $<0.05$ was considered to be statistically significant.

**Results**

Effect of the levels of chimerism on tumor rejection. We first examined the levels of radiation-induced mixed chimerism in the periphery of the treated BALB/c mice (Fig. 1). BALB/c mice were irradiated with several doses of irradiation (3, 5, 7, and 8.5 Gy). On the next day (on day 0), these mice were transferred i.v. with a mixture of 2.0×10$^7$ BM cells and 1×10$^7$ LN cells originated from donor Ly1.1+ DBA/2 mice. On day 15 after transplantation, the levels of donor chimerism were examined in the peripheral blood. In the mice irradiated with 7 or 8.5 Gy, almost T lymphocytes in the peripheral blood cells were derived from donor DBA/2 mice. The percentage of donor-derived lymphocytes in the peripheral blood decreased as the dose of irradiation decreased. In the mice irradiated with 3 Gy, donor-derived cells were very few in the host peripheral blood. In the BALB/c mice that had been treated with DBA/2 spleen and BM cells on day 0, cyclophosphamide on day 2, and DBA/2 LN cells on day 3, although mixed chimerism was clearly detected, the degree of mixed chimerism on day 15 was obviously lower than that in the BALB/c mice treated with 5 Gy irradiation and donor cells (Fig. 1).

Next, we determined whether the percentage of mixed chimerism could have any influence on the in vivo antitumor effect. Considering the clinical situation, we started the cancer treatment after establishing the s.c. injected RENCA tumors.
Graft-versus-host disease–related death of the BALB/c mice treated with 6 Gy irradiation followed by the transfer of DBA/2 BM and LN cells. We next examined the survival of the BALB/c mice treated with 6 Gy irradiation followed by the transfer of DBA/2 BM and LN cells. To discriminate graft-versus-host disease (GVHD)-induced or tumor-related death, we compared the survival of tumor-bearing BALB/c mice treated with 6 Gy irradiation followed by the transfer of BM and LN cells derived from either syngeneic BALB/c or allogeneic DBA/2 mice. As shown in Fig. 3A, 80% of tumor-bearing BALB/c recipients of allogeneic bone marrow transplantation (BMT) following 6 Gy irradiation died by day 55. These dead mice exhibited GVHD-associated symptoms, including weight loss (Fig. 3C), hunching, and splenomegaly (data not shown). Similarly, 100% of tumor-bearing syngeneic mice died (Fig. 3B). However, these mice were not accompanied by GVHD-associated symptoms (data not shown). Instead, these mice had huge tumors and maybe died of tumor-related death. The weight loss in tumor-bearing BALB/c recipients of allogeneic BMT following 6 Gy irradiation was severer than that in tumor-bearing BALB/c recipients of syngeneic BMT following 6 Gy irradiation (Fig. 3C), although the RENCA tumor growth rate was significantly suppressed by the allogeneic transfer (Fig. 2A). On the other hand, all tumor-free BALB/c recipients of syngeneic BMT following 6 Gy irradiation survived beyond day 55, but 60% of tumor-free allogeneic animals died by day 30 (Fig. 3A) with weight loss and clinical signs of GVHD (data not shown). Overall, these results indicate that tumor-bearing BALB/c mice treated with 6 Gy irradiation followed by the transfer of allogeneic BM and LN cells died mainly due to GVHD.

Fig. 4. Percentage of donor cells in the BALB/c mice treated with radiation followed by the transfer of allogeneic BM and LN cells, and the absolute cell number of the mesenteric LN 15 d after the treatment. A, BALB/c mice were treated with either 3 Gy (3G) or 6 Gy (6G) of irradiation. On the next day (day 0), these mice were transferred with BM and LN cells from either syngeneic BALB/c or allogeneic DBA/2 mice. In some experiments, BALB/c mice were treated with DBA/2 spleen and BM cells on day 0, cyclophosphamide on day 2, and DBA/2 LN cells on day 3 (CP), as shown in our previous study (23). Thereafter, the positive percentage of donor DBA/2 (Ly1.1+) cells in the BALB/c mice given allogeneic cells was sequentially examined by flow cytometry. Columns, mean; bars, SD. Eight mice were used in each experiment. The absolute cell number in the mesenteric LNs was counted. Columns, mean; bars, SD. Untreated BALB/c mice were used as a control. Each panel shows the representative findings of three separate experiments. The other two experiments showed similar results. *, P < 0.01, compared with 6G or 3G; **, P < 0.01, compared with 6G (allo. and syn.) and 3G (allo. and syn.) groups, but not specific compared with the control group.

About the dose of irradiation, we selected 6 Gy because it is nonlethal but is expected to induce high levels of mixed chimerism based on the results of Fig. 1. We also selected 3 Gy as a control of low levels of mixed chimerism. Figure 2 shows the RENCA tumor growth in the BALB/c mice treated with 6 or 3 Gy of irradiation followed by the adoptive transfer of BM and LN cells from either allogeneic BALB/c or DBA/2 mice. In the 6 Gy–irradiated mice, the RENCA tumor growth rate was significantly suppressed by the allogeneic transfer compared with the syngeneic transfer 20 days after the tumor inoculation (Fig. 2A). However, no mice with the allogeneic transfer could reject the tumor (data not shown). In the 3 Gy–irradiated mice, no significant difference in the RENCA tumor growth rate was observed among the three groups (Fig. 2B). No mice in the irradiation group were able to reject s.c. established RENCA, irrespective of the dose of irradiation. On the other hand, 40% of RENCA tumors disappeared in the BALB/c mice treated with the cyclophosphamide-using cell therapy, as previously reported (23).

No antitumor effect of the BALB/c mice treated with 3 Gy irradiation followed by the transfer of DBA/2 BM and LN cells. We next compared the survival of the tumor-bearing BALB/c mice treated with 3 Gy irradiation followed by the transfer of BM and LN cells from either syngeneic BALB/c or allogeneic DBA/2 mice. As shown in Fig. 3D, almost half of tumor-bearing BALB/c mice treated with 3 Gy irradiation followed by the transfer of allogeneic or syngeneic BM and LN cells died by day 30, and no difference was detected between the two groups. Irradiation (3 Gy) was considered to induce a slight prolongation of survival in both allogeneic and syngeneic transfer groups compared with the untreated group (Fig. 3D). These results indicated that, in the 3 Gy–irradiated mice, no antitumor effect was induced by the transfer of allogeneic BM and LN cells.

Level of mixed chimerism in the BALB/c mice treated with 6 or 3 Gy of irradiation followed by the transfer of BM and LN cells from DBA/2 mice. The level of donor chimerism was sequentially evaluated in the BALB/c recipients by a flow cytometric analysis of the donor (Ly1.1+) T cells in the peripheral blood (Fig. 4A). Almost complete donor chimerism was detected in the allogeneic BALB/c mice treated with 6 Gy
irradiation 8 days after transplantation and thereafter. In contrast, donor-derived cells were rarely detected in the allogeneic mice conditioned with 3 Gy irradiation. **Impaired immune reconstitution after irradiation-conditioned allogeneic BMT.** We next investigated the mechanisms of the impaired antitumor effects in the BALB/c recipients of allogeneic BMT following 6 or 3 Gy of irradiation. We counted the absolute cell number in the mesenteric LNs after the treatment with irradiation (Fig. 4B). The absolute cell number of the mesenteric LN cells on day 15 after the treatment with 6 or 3 Gy of irradiation followed by the transfer of allogeneic or syngeneic BM and LN cells was significantly small compared with that of control mice. However, there was no difference in the absolute cell number of the mesenteric LN cells between the control mice and the mice treated with the cyclophosphamide-using cell therapy 15 days earlier.

We next did histopathologic analysis on the tumor-infiltrating lymphocytes in the RENCA-bearing BALB/c mice treated with 3 or 6 Gy of irradiation followed by the transfer of allogeneic BM and LN cells (Fig. 5). Consequently, in the 6 Gy–treated mice, only a slight infiltrate of lymphocytes was observed in tumor tissues, in spite of a high level of chimerism (Fig. 5A and D). In the 3 Gy–treated mice, only a few tumor-infiltrating lymphocytes were also detected, probably due to the failure in the acceptance of donor cells (Fig. 5B and D). In the BALB/c mice treated with cyclophosphamide-using cell therapy, however, a diffuse infiltration of mononuclear cells was observed around the tumor cells (Fig. 5C and D), supporting our previous report (23).

**Decreases of regulatory T cells in the mice treated with the cyclophosphamide-using cell therapy.** We finally evaluated the changes in the subsets of regulatory T cells in the spleen cells of the BALB/c mice treated with the cyclophosphamide-using cell therapy because cyclophosphamide has been recently reported to decrease regulatory T cells, resulting in the enhancement of antitumor immunity (24–26). As shown in Fig. 6A and B, CD4^+CD25^+Foxp3^+^ regulatory T cells obviously decreased 4 days after the cyclophosphamide treatment in the spleen cells of the mice treated with the cyclophosphamide-using cell therapy compared with the control mice.

**Discussion**

In nonmyeloablative allogeneic hemopoietic cell transplantation for the treatment of malignancy, it is widely believed that
the graft-versus-tumor activity is associated with complete chimerism (1, 11, 27, 28) because mature donor T cells are considered to be the main effectors of the graft-versus-tumor activity. However, some reports suggest that host-derived cells participate in in vivo antitumor effect (29). In addition, we recently proposed a unique nonmyeloablative cell therapy using cyclophosphamide, by which a significant antitumor effect against s.c. established RENCA was induced at a low level using cyclophosphamide, by which a significant antitumor effect was modest. This result suggests that allogeneic derived anti-host T cells caused lethal GVHD, but in vivo antitumor effect was modest. This result suggests that allogeneic T-cell responses, which could cause lethal GVHD, were not sufficient to eradicate solid cancers. This scenario represents clinical nonmyeloablative SCT against solid cancers; cure of solid cancers is rare with nonmyeloablative SCT that often induces severe GVHD. As shown in this study, irradiation destroyed host immune system (Fig. 4B). In contrast, our cyclophosphamide-using cell therapy protocol is not associated with such long-lasting immunologic incompetency because of the preservation of host immune system (Fig. 4B). Although the level of chimerism is low, the induced GVHD reaction facilitates to evoke antitumor immune responses because antitumor effects were not induced without donor lymphocyte infusion in the cyclophosphamide-using cell therapy (23). In addition, most of the recovered peripheral LN cells were

due to excessive immunosuppression was too strong to recover peripheral LN cells of the recipients after the cyclophosphamide-using cell therapy (21, 22). In addition, the recovery of peripheral LN cells of the recipients after the cyclophosphamide-using cell therapy was much quicker than that after the radiation-induced system even with 3 Gy irradiation (Fig. 4B). Furthermore, regulatory T cells obviously decreased 4 days after the cyclophosphamide was much quicker than that after the radiation-induced system even with 3 Gy irradiation (Fig. 4B). Furthermore, regulatory T cells obviously decreased 4 days after the cyclophosphamide treatment, CD4+CD25+foxp3+ regulatory T cells were examined in the spleen cells of the recipients (4 days after CP) in A and B). A. representative fluorescence-activated cell sorting data. B. columns, mean percentage of regulatory T cells; bars, SD. The analysis of regulatory T cells in untreated BALB/c mice was used as a control (Control in A and B). Each panel shows the representative findings of three separate experiments. The other two experiments showed similar results. *, P < 0.01, compared with control group. 

Fig. 6. Changes of regulatory T cells in the BALB/c mice treated with the cyclophosphamide-using cell therapy. BALB/c mice were treated with DBA/2 spleen and BM cells on day 0 and cyclophosphamide on day 2. In this experiment, DBA/2 LN cells were not injected to investigate the direct effects of cyclophosphamide on recipient lymphocytes. Four days after the cyclophosphamide treatment, CD4+CD25+foxp3+ regulatory T cells in untreated BALB/c mice was used as a control (Control BALB/c). A. Data 002 17.0% 4 days after CP CD25 Data 003 5.6% foxp3 CD4 gated B. Data 002 17.0% 4 days after CP Data 003 5.6%

synergistic effects were not induced without donor lymphocyte infusion in the cyclophosphamide-using cell therapy (23). In addition, most of the recovered peripheral LN cells were

the graft-versus-tumor activity is associated with complete chimerism (1, 11, 27, 28) because mature donor T cells are considered to be the main effectors of the graft-versus-tumor activity. However, some reports suggest that host-derived cells participate in in vivo antitumor effect (29). In addition, we recently proposed a unique nonmyeloablative cell therapy using cyclophosphamide, by which a significant antitumor effect against s.c. established RENCA was induced at a low level of chimerism (23). These findings lead us to a question that a high level of chimerism is really necessary for in vivo antitumor effect of nonmyeloablative allogeneic cell therapy for the treatment of solid malignancies. Therefore, we undertook this study.

A notable finding of the present study is that a high level of chimerism does not necessarily associate with in vivo antitumor effect. Although a high level of chimerism could be induced by 6 Gy irradiation followed by the transfer of allogeneic BM and LN cells, tumor-bearing BALB/c mice died mainly due to GVHD. In addition, immune reconstitution was significantly impaired in such mice (Fig. 4B). It has been shown that an acute GVHD caused immunodeficiency (30, 31). However, this may not be the case in this study because immune reconstitution was similarly impaired in

synergistic effects were not induced without donor lymphocyte infusion in the cyclophosphamide-using cell therapy (23). In addition, most of the recovered peripheral LN cells were

the graft-versus-tumor activity is associated with complete chimerism (1, 11, 27, 28) because mature donor T cells are considered to be the main effectors of the graft-versus-tumor activity. However, some reports suggest that host-derived cells participate in in vivo antitumor effect (29). In addition, we recently proposed a unique nonmyeloablative cell therapy using cyclophosphamide, by which a significant antitumor effect against s.c. established RENCA was induced at a low level of chimerism (23). These findings lead us to a question that a high level of chimerism is really necessary for in vivo antitumor effect of nonmyeloablative allogeneic cell therapy for the treatment of solid malignancies. Therefore, we undertook this study.

A notable finding of the present study is that a high level of chimerism does not necessarily associate with in vivo antitumor effect. Although a high level of chimerism could be induced by 6 Gy irradiation followed by the transfer of allogeneic BM and LN cells, tumor-bearing BALB/c mice died mainly due to GVHD. In addition, immune reconstitution was significantly impaired in such mice (Fig. 4B). It has been shown that an acute GVHD caused immunodeficiency (30, 31). However, this may not be the case in this study because immune reconstitution was similarly impaired in

the graft-versus-tumor activity is associated with complete chimerism (1, 11, 27, 28) because mature donor T cells are considered to be the main effectors of the graft-versus-tumor activity. However, some reports suggest that host-derived cells participate in in vivo antitumor effect (29). In addition, we recently proposed a unique nonmyeloablative cell therapy using cyclophosphamide, by which a significant antitumor effect against s.c. established RENCA was induced at a low level of chimerism (23). These findings lead us to a question that a high level of chimerism is really necessary for in vivo antitumor effect of nonmyeloablative allogeneic cell therapy for the treatment of solid malignancies. Therefore, we undertook this study.

A notable finding of the present study is that a high level of chimerism does not necessarily associate with in vivo antitumor effect. Although a high level of chimerism could be induced by 6 Gy irradiation followed by the transfer of allogeneic BM and LN cells, tumor-bearing BALB/c mice died mainly due to GVHD. In addition, immune reconstitution was significantly impaired in such mice (Fig. 4B). It has been shown that an acute GVHD caused immunodeficiency (30, 31). However, this may not be the case in this study because immune reconstitution was similarly impaired in synergistic effects were not induced without donor lymphocyte infusion in the cyclophosphamide-using cell therapy (23). In addition, most of the recovered peripheral LN cells were

the graft-versus-tumor activity is associated with complete chimerism (1, 11, 27, 28) because mature donor T cells are considered to be the main effectors of the graft-versus-tumor activity. However, some reports suggest that host-derived cells participate in in vivo antitumor effect (29). In addition, we recently proposed a unique nonmyeloablative cell therapy using cyclophosphamide, by which a significant antitumor effect against s.c. established RENCA was induced at a low level of chimerism (23). These findings lead us to a question that a high level of chimerism is really necessary for in vivo antitumor effect of nonmyeloablative allogeneic cell therapy for the treatment of solid malignancies. Therefore, we undertook this study.

A notable finding of the present study is that a high level of chimerism does not necessarily associate with in vivo antitumor effect. Although a high level of chimerism could be induced by 6 Gy irradiation followed by the transfer of allogeneic BM and LN cells, tumor-bearing BALB/c mice died mainly due to GVHD. In addition, immune reconstitution was significantly impaired in such mice (Fig. 4B). It has been shown that an acute GVHD caused immunodeficiency (30, 31). However, this may not be the case in this study because immune reconstitution was similarly impaired in
host-derived cells (Fig. 4A), and our previous study showed the existence of the RENCA-specific production of IFN-γ even after the disappearance of donor-derived cells in the cyclophosphamide-using cell therapy (23). Indeed, several reports indicate that antitumor T-cell responses of host cells are induced (32). Therefore, we suppose that the recovery of host-derived immune cells might be a critical event for the in vivo antitumor effect of the cyclophosphamide-using cell therapy against solid cancer.

Recently, significant advances in understanding the molecular mechanisms underlying RCC have led to the development of rationally designed therapies, which are now being clinically tested. To date, the vascular endothelial growth factor receptor pathway has been the most promising target, and two agents (BAY 43-9006 and SU11248) that inhibit not only vascular endothelial growth factor receptor but also other receptors, including the platelet-derived growth factor receptor, FMS-like tyrosine kinase 3, KIT, or Raf kinase, have been recently approved by Food and Drug Administration for advanced RCC (33, 34). Consequently, much attention is now being paid to such molecular targeting therapies. However, the underlying mechanisms for the antitumor effects are different between nonmyeloablative allogeneic SCT and molecular targeting therapy. Therefore, nonmyeloablative allogeneic SCT should be done especially for patients with advanced RCC who are resistant to molecular targeting therapies. In such patients with a poor performance status because of advanced metastatic tumors, the risk of GVHD should be particularly minimized in the process of nonmyeloablative allogeneic SCT. In this sense, the cyclophosphamide-using cell therapy may be a choice of treatment because the lower levels of mixed chimerism can reduce the risk of GVHD (35, 36). Recently, similar postransplantation of cyclophosphamide has been applied to clinical nonmyeloablative allogeneic SCT for hematologic malignancies (37, 38). We hope that the concept that a high level of chimerism may not be essential for the in vivo antitumor effect against solid cancer in our current study will help reduce the risk of GVHD during the course of nonmyeloablative allogeneic SCT for the treatment of patients with advanced RCC.

References
Posttransplant Administration of Cyclophosphamide and Donor Lymphocyte Infusion Induces Potent Antitumor Immunity to Solid Tumor

Masatoshi Eto, Yoriyuki Kamiryo, Ario Takeuchi, et al.

*Clin Cancer Res* 2008;14:2833-2840.

Updated version  Access the most recent version of this article at: [http://clincancerres.aacrjournals.org/content/14/9/2833](http://clincancerres.aacrjournals.org/content/14/9/2833)

Cited articles  This article cites 38 articles, 21 of which you can access for free at: [http://clincancerres.aacrjournals.org/content/14/9/2833.full#ref-list-1](http://clincancerres.aacrjournals.org/content/14/9/2833.full#ref-list-1)

Citing articles  This article has been cited by 1 HighWire-hosted articles. Access the articles at: [http://clincancerres.aacrjournals.org/content/14/9/2833.full#related-urls](http://clincancerres.aacrjournals.org/content/14/9/2833.full#related-urls)

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link [http://clincancerres.aacrjournals.org/content/14/9/2833](http://clincancerres.aacrjournals.org/content/14/9/2833). Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.