

Combination of anti-CD4 antibody treatment and donor lymphocyte infusion ameliorates graft-versus-host disease while preserving graft-versus-tumor effects in murine allogeneic hematopoietic stem cell transplantation

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Key words

Allogeneic hematopoietic stem cell transplantation, CD4, donor lymphocyte infusion, graft-versus-host disease, graft-versus-tumor

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Funding information

This work was supported by grants from the Japan Science and Technology Agency (15652196, 10104055), the Japanese Ministry of Education, Culture, Sports, Science and Technology (25460491, 25293113), the Ministry of Health, Labor and Welfare (13418429), and the Japan Agency for Medical Research and Development (15652908, 16768526).

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Received June 20, 2017; Revised July 25, 2017; Accepted July 27, 2017

Cancer Sci 108 (2017) 1967–1973

doi: 10.1111/cas.13346

Immune checkpoint therapies, such as those targeting cytotoxic T-lymphocyte-associated protein 4 or programmed cell death protein 1 inhibitory signaling in T cells, have emerged as standard options for certain types of cancer patients.⁽¹⁾ However, despite clear survival benefits in a subset of tumor patients, other groups of patients are refractory to these immune checkpoint modulations. Particularly, patients with a limited number of somatic mutations in the tumor are less sensitive to immune checkpoint therapy.⁽²⁾ T cells mainly recognize tumors through mutation-associated neoantigens that are presented as processed peptides in complexes with major histocompatibility complex (MHC) molecules. Thus, low mutation burden is associated with a low frequency of precursors to tumor-reactive T cells, and limits the size of antitumor

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is not only a well-established immunotherapy for hematologic malignancies, but is potentially useful for treating solid tumors refractory to available therapies. However, application of allo-HSCT to solid tumors is limited, despite the beneficial antitumor effects, by the risk of graft-versus-host disease (GVHD). CD4⁺ T cells have been implicated in several aspects of GVHD, and suppress antitumor CD8⁺ T-cell responses. In the present study, we investigated clinically applicable allo-HSCT protocols designed to maximize antitumor effects while reducing the risk of GVHD. We used a mouse model of allo-HSCT with s.c. tumors. We found that myeloablative conditioning was associated with better inhibition of tumor growth but with severe acute GVHD. Early treatment with anti-CD4 mAb substantially ameliorated GVHD while preserving antitumor effects, leading to improved survival in myeloablative allo-HSCT. Late treatment with anti-CD4 mAb also ameliorated GVHD to some extent. Donor lymphocyte infusion in GVHD mice treated with anti-CD4 mAb further suppressed tumor growth without exacerbating GVHD. Collectively, our results suggest that myeloablative allo-HSCT followed by anti-CD4 mAb treatment and donor lymphocyte infusion could be a potent and safe immunotherapy for patients with cancers refractory to available therapies.

T-cell responses, even in the absence of immunosuppressive signaling.

A possible way to increase the frequency of tumor-reactive T cells in cancer patients with low mutation burden is allogeneic hematopoietic stem cell transplantation (allo-HSCT), in which donor T cells in the graft recognize alloantigens on tumor cells and exert graft-versus-tumor (GVT) effects.^(3,4) In fact, allo-HSCT has been established as a curative immunotherapy for a variety of hematopoietic malignancies.⁽⁵⁾ However, application of allo-HSCT to solid tumors is limited due to the risk of graft-versus-host disease (GVHD), where donor T cells attack non-malignant cells and mediate severe immunotoxicity. As alloantigens can be mismatched MHC or minor histocompatibility antigens expressed on both tumor and

normal cells, segregation of GVT effects from GVHD remains as a major challenge limiting the application of allo-HSCT to solid tumors.

Despite the risk of GVHD, allo-HSCT has been tested for the treatment of patients with refractory solid tumors such as metastatic breast cancer and kidney cancer.^(6–10) In these clinical trials, patients who developed severe GVHD had a lower risk of relapse than patients without acute GVHD, despite a high risk of transplant-related mortality.⁽¹⁰⁾ These observations indicate that allo-HSCT would be an option for treating cancer patients with low mutation burden if we could establish a simple protocol to maximize the GVT effects while minimizing the risk of GVHD.

During acute GVHD, donor CD4⁺ T cells, and to a lesser extent CD8⁺ T cells, attack target organs such as the skin, liver, and intestine through the production of inflammatory cytokines.^(11,12) In addition, we previously reported that donor CD4⁺ T cells impair the hematopoietic niche in the bone marrow (BM) and severely suppress the production of T- and B-cell progenitors in mouse GVHD models.⁽¹³⁾ Early depletion of CD4⁺ T cells after allo-HSCT by administering an anti-CD4 mAb dramatically ameliorated systemic GVHD effects and, in addition, improved lymphocyte production in the BM. Notably, this simple treatment preserved graft-versus-leukemia (GVL) effects against i.v. injected mastocytoma, establishing a basic strategy for segregating GVL effects from the risk of GVHD. However, it remains elusive whether these findings are applicable to allo-HSCT for the treatment of solid tumors.

In the present study, we aimed to optimize allo-HSCT to maximize the GVT effects against solid tumors while reducing the risk of GVHD. We used a mouse model of major mismatched allo-HSCT, and investigated the effects of factors such as intensity of irradiation preconditioning, depletion of CD4⁺ T cells, and donor lymphocyte infusion (DLI).

Materials and Methods

Mice and tumor cell lines. Male C57BL/6 (B6; H-2^b) and (C57BL/6 × DBA/2) F₁ (BDF₁; H-2^{b×d}) mice were purchased from Japan SLC (Hamamatsu, Japan). Mice used for experiments were 6–8 weeks old at the time of HSCT. All mice were housed in a barrier system and all animal experiments were carried out in accordance with institutional guidelines with the approval of the Animal Care and Use Committee of the University of Tokyo (Tokyo, Japan). Colon-26 (H-2^d) adenocarcinoma cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging, and Cancer, Tohoku University (Sendai, Japan).

Allogeneic hematopoietic stem cell transplantation, anti-CD4 mAb treatment, and DLI. Allogeneic hematopoietic stem cell transplantation was carried out using B6 donors and BDF₁ recipients as described previously.^(13,14) In brief, BM cells were prepared from the femurs and tibias of B6 donor mice, and T cell depletion was carried out with anti-Thy1.2 mAb using the autoMACS system (Miltenyi Biotec, Tokyo, Japan). Splenocytes were prepared from donor mice, and unfractionated T cells were negatively enriched by autoMACS with antibodies against CD11b, B220, Ter-119, and NK1.1. Antibodies against CD8 or CD4 were added to the antibody cocktail for unfractionated T cells to prepare CD4⁺ or CD8⁺ T cells, respectively (T cells, CD3⁺ >92%; CD4⁺, >95%; and CD8⁺, >95%). BDF₁ recipient mice received myeloablative (9 Gy, split into two equal doses given 3 h apart) or non-myeloablative (4 Gy) X-ray total body irradiation on the day before allo-

HSCT, and were i.v. injected with 5×10^6 T cell-depleted BM (TCD BM) cells with or without 5×10^6 whole T cells. For the depletion of CD4⁺ cells *in vivo*, mice were injected i.p. with 200 µg anti-CD4 mAb (clone GK1.5;^(15,16) BioXcell, West Lebanon, NH, USA) at day 3, 6, or 17 after allo-HSCT. Donor lymphocyte infusion was carried out by i.v. injection of 5×10^6 whole T cells, CD4⁺ T cells, or CD8⁺ T cells on day 12 after allo-HSCT. Administration of anti-CD4 mAb-depleted CD4⁺ T cells for at least 1 week after treatment.⁽¹³⁾ Each group of experiments consisted of 10 mice except where otherwise specified.

Tumor inoculation. Recipient mice were s.c. inoculated with 2×10^5 colon-26 cells per mouse at 1 day after irradiation and 5 h before allo-HSCT. Tumor volume was evaluated by measuring the major axis and minor axis of the tumor, and the volume calculated by the following formula: tumor volume (mm³) = (major axis in mm) × (minor axis in mm)² × 0.5236.

Systemic assessment of GVHD. The severity of GVHD was assessed with a clinical GVHD scoring system, as described by Cooke *et al.*⁽¹⁷⁾ Recipients were individually scored two or three times per week for five clinical parameters (weight loss, posture, activity, fur texture, and skin integrity) on a scale from 0 to 2. A clinical GVHD score was generated by summation of the five criteria scores, generating a total ranging from 0–10. Survival was monitored daily.

Statistics. Statistical analyses were carried out using Stat-Mate IV software (ATMS, Tokyo, Japan) or GraphPad Prism version 6 (GraphPad Software, Inc., La Jolla, CA, USA). The data are presented as mean ± SE. For comparisons between groups in the *in vivo* study, we used one-way ANOVA with Dunnett's post-hoc tests. A probability value of $P < 0.05$ was considered significant.

Results

Combining myeloablative irradiation and anti-CD4 mAb treatment ameliorates GVHD with moderate effects on GVT. We first investigated the influence of irradiation preconditioning on GVHD and GVT using a [B6→BDF₁] major mismatched allo-HSCT model (Fig. 1a). BDF₁ mice (H-2^{b × d}) received myeloablative (9 Gy) or non-myeloablative (4 Gy) X-ray total body irradiation on the day before allo-HSCT. The recipients were s.c. inoculated with colon-26 tumor cells (H-2^d) 5 h before allo-HSCT, and then transplanted with B6 (H-2^b) mice-derived TCD BM alone (BMT) or together with unfractionated T cells (GVHD). Among the BMT mice that received TCD BM alone, tumor growth was slower in mice in the myeloablative group that received the higher radiation dose than in mice that received the lower dose (Fig. 1b, c). There was no obvious difference between the GVHD scores of BMT mice in the myeloablative and non-myeloablative groups (Fig. 1d). In contrast, in GVHD mice that received TCD BM together with unfractionated T cells, the GVHD score in the myeloablative group progressively increased from day 9 onward, whereas the score in the non-myeloablative group decreased after day 14 (Fig. 1d). Tumor growth in GVHD mice was slower in the myeloablative group than in the non-myeloablative group (Fig. 1b, c). In GVHD mice treated with anti-CD4 mAb on day 3, the GVHD scores decreased to levels comparable to those in the BMT group by day 14, irrespective of irradiation preconditioning (Fig. 1d). Although the tumor growth was accelerated in GVHD mice receiving anti-CD4 mAb treatment compared to those in the untreated GVHD mice, tumor growth

in the myeloablative group was slower than that of mice in the non-myeloablative group (Fig. 1b, c). In the myeloablative group, GVHD mice receiving anti-CD4 mAb treatment showed better overall survival than that of mice in the BMT group, which died from tumors from day 29, or for those of untreated GVHD mice, which died by GVHD from day 11 (Fig. 1e). All nine GVHD mice that received myeloablative preconditioning and anti-CD4 mAb treatment (Fig. 1f, solid circles) had tumor size and GVHD scores that were less than the average values for all groups (Fig. 1f, dotted lines), indicating a benefit from anti-CD4 mAb treatment. These results suggest that irradiation-induced damage to host stroma contributes both to tumor

growth inhibition and to the severity of acute GVHD, and that a combination of myeloablative allo-HSCT and early anti-CD4 mAb treatment could provide benefit in terms of GVHD control and antitumor effects.

Timing effects of anti-CD4 mAb treatment on GVHD and GVT. We next investigated the timing effects of anti-CD4 mAb treatment in GVHD mice receiving myeloablative conditioning to maximize the antitumor effects while inhibiting GVHD. Anti-CD4 mAb was given to GVHD mice receiving myeloablative conditioning on day 3, 6, or 17. In the GVHD mice receiving anti-CD4 mAb on day 3 or 6, the GVHD score decreased to a level comparable to that of mice in the BMT

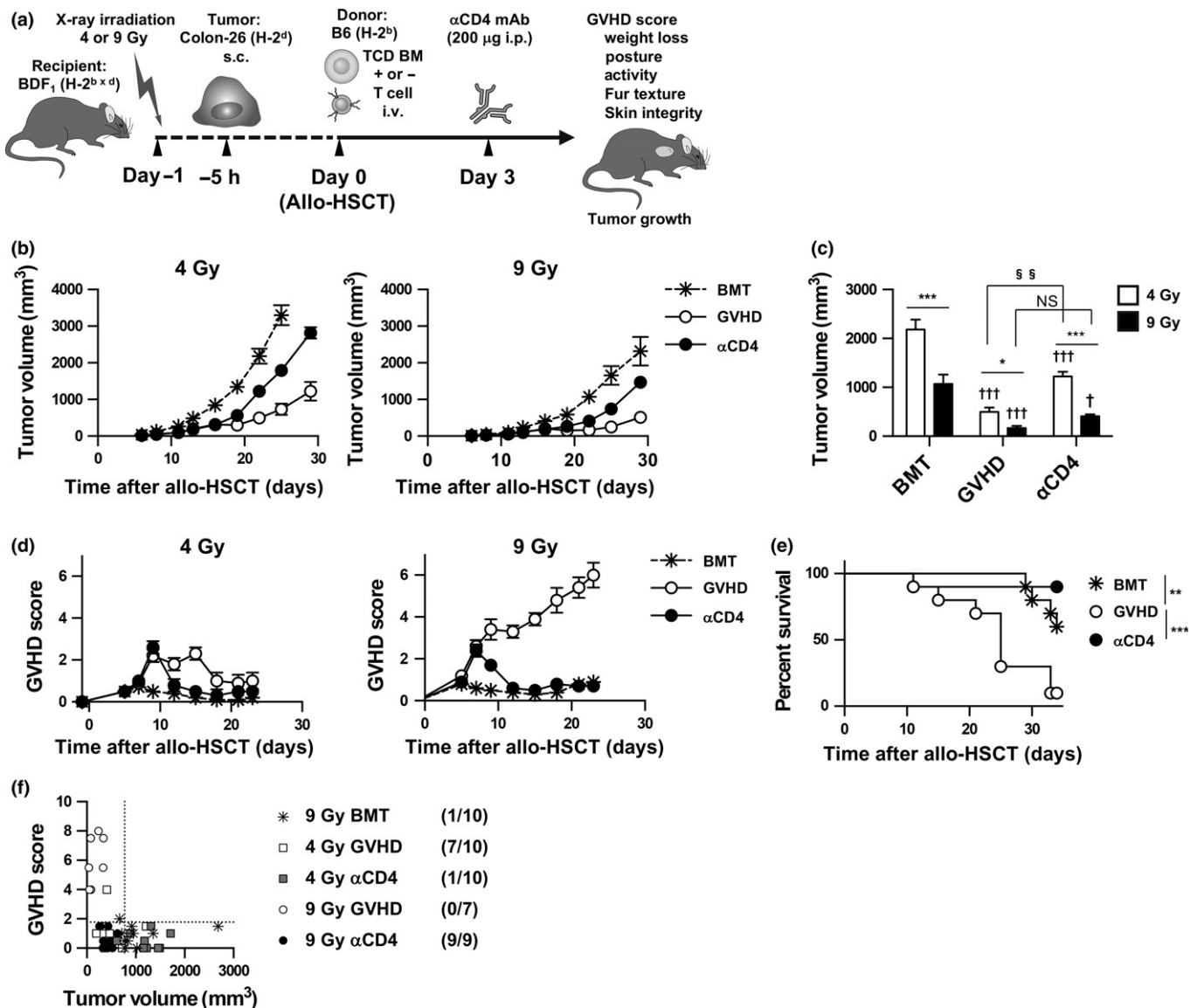


Fig. 1. Myeloablative allogeneic hematopoietic stem cell transplantation (allo-HSCT) in combination with anti-CD4 mAb treatment confers moderate graft-versus-tumor effects without graft-versus-host disease (GVHD). (a) Schema of the graft-versus-tumor model. BDF₁ mice (H-2^b × d) were irradiated with myeloablative (9 Gy) or non-myeloablative (4 Gy) X-ray irradiation on day -1, s.c. inoculated with colon-26 (H-2^d) cells 5 h before allo-HSCT, and transplanted with T cell-depleted bone marrow (TCD BM) alone (BMT) or with TCD BM plus T cells (GVHD) from B6 mice (H-2^b) on day 0. An anti-CD4 mAb was i.p. injected on day 3 after allo-HSCT (αCD4). (b) Tumor growth curves and (c) tumor volume at day 22 after allo-HSCT. **P* < 0.05; ****P* < 0.001 (4 Gy vs. 9 Gy). †*P* < 0.05; †††*P* < 0.001 (vs. BMT); §§*P* < 0.01 (vs. GVHD). NS, not significant. (d, e) GVHD scores (d) and survival (e). (f) Scatter plot of GVHD score versus tumor volume at day 24 after allo-HSCT. Dotted lines indicate average values across all groups; numbers to the right of the graph indicate the number of mice from each group that had less-than-average GVHD scores and tumor volumes (mice appearing in the lower left-hand corner of the plot), and the total number in each group. ***P* < 0.01; ****P* < 0.001. Data represent mean ± SEM (a–d; *n* = 10) from one of two independent experiments.

group at day 15 onward (Fig. 2a, b), but tumor growth was accelerated compared to untreated GVHD mice or GVHD mice treated with anti-CD4 mAb on day 17 (Fig. 2c). In GVHD mice treated with anti-CD4 mAb on day 17, tumor growth was comparable to those in untreated GVHD mice (Fig. 2c). Whereas the GVHD score decreased markedly after anti-CD4 mAb treatment on day 17, the score remained high compared to that of BMT control or early treatment groups (Fig. 2a, b), and some mice died before treatment. Half of the GVHD mice that received anti-CD4 mAb treatment on day 3 or 6 had less-than-average tumor size and GVHD scores (Fig. 2d). These results suggest that late antibody treatment is associated with better antitumor effects and moderate therapeutic effects against GVHD.

Combination of anti-CD4 mAb treatment and DLI augments GVT effects. Finally, we examined whether DLI would rescue reduction of GVT effects after anti-CD4 mAb treatment without exacerbating GVHD. We tried to determine whether CD4⁺ or CD8⁺ T cells were more suitable for DLI. The GVHD mice treated with anti-CD4 mAb on day 3 received an equal number of unfractionated T cells (containing both CD4⁺ and CD8⁺ T cells), CD8⁺ T cells, or CD4⁺ T cells on day 12 (Fig. 3a). The GVHD score of the DLI group was very similar to that of the BMT group, irrespective of the donor T cell population (Fig. 3b, c). Three out of five GVHD mice that had a GVHD score ≥ 3.0 at day 10 showed an increased GVHD score after DLI of unfractionated T cells, whereas the other mice with mild GVHD in the same group had a decreased GVHD score after DLI (Fig. 3d). Strikingly, DLI of unfractionated T cells showed strong tumor growth inhibition that was significantly higher than that of anti-CD4 mAb-treated GVHD mice, with a

tumor volume comparable to that of GVHD mice untreated with anti-CD4 mAb (Fig. 3e). However, DLI of CD8⁺ T cells augmented antitumor effects moderately in comparison with the group that did not receive DLI, but DLI of CD4⁺ T cells did not (Fig. 3e). Six of nine GVHD mice that received anti-CD4 mAb and DLI of unfractionated T cells had less-than-average tumor size and GVHD scores (Fig. 3f). These results suggest that a combination of anti-CD4 mAb treatment and DLI, particularly in recipients with mild GVHD, is an attractive option to maximize GVT effects without risk of GVHD, and both CD4⁺ and CD8⁺ T cells contribute to the antitumor effects obtained by the DLI.

Discussion

Recent advances in immune checkpoint therapy provide a way to inhibit peripheral tolerance and to augment T-cell responses against tumors with neoantigens. However, these approaches are not effective for tumors without neoantigens as a result of central tolerance, the mechanism by which self-reactive T cells are eliminated during development. Allogeneic hematopoietic stem cell transplantation is the only way to break self-tolerance by transferring a T-cell population that did not undergo thymic selection in the recipient. Using clinically relevant experimental models of allo-HSCT and DLI, we showed that myeloablative preconditioning combined with early anti-CD4 mAb treatment and DLI elicits potent GVT effects and a sustained remission of GVHD symptoms.

Myeloablative preconditioning by irradiation is associated with increased risk of GVHD, delayed immune reconstitution, and overall treatment-related mortality, but is also associated

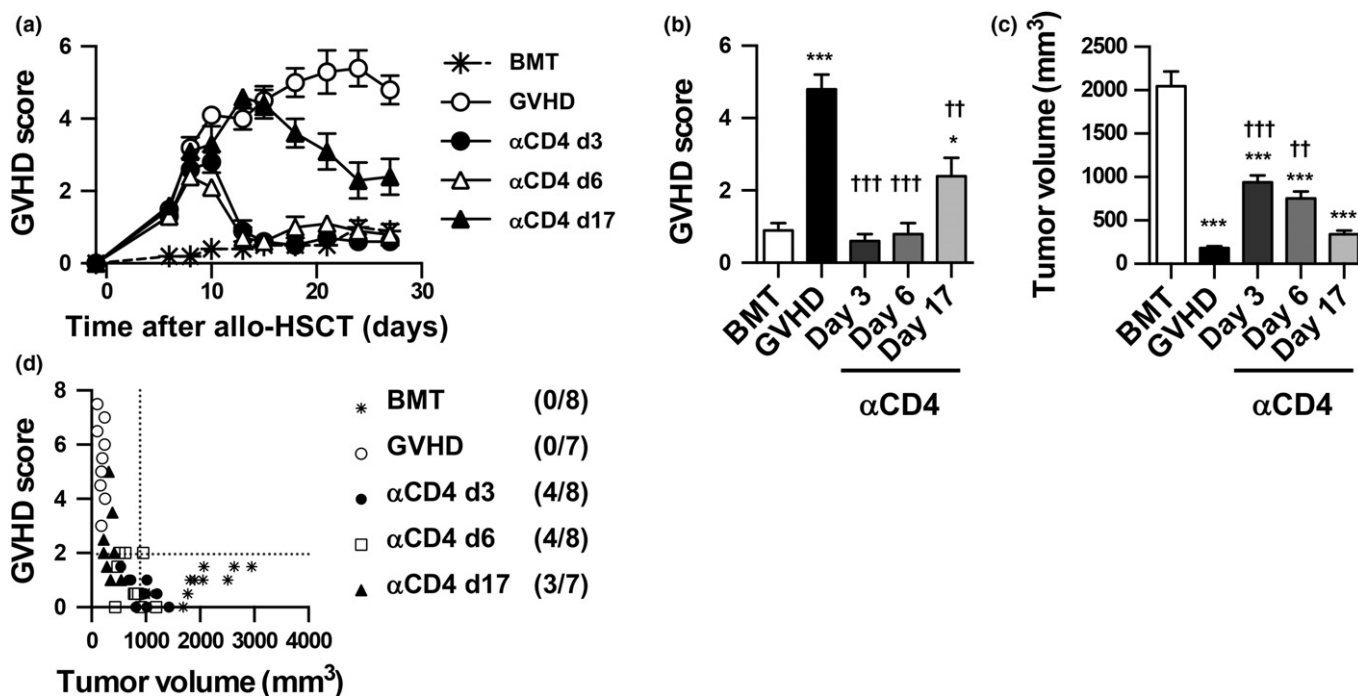


Fig. 2. Timing effects of anti-CD4 mAb treatment on graft-versus-tumor and graft-versus-host disease (GVHD) in murine allogeneic hematopoietic stem cell transplantation (allo-HSCT). BDF₁ mice receiving myeloablative X-ray irradiation were s.c. inoculated with colon-26 cells and transplanted with T cell-depleted bone marrow (TCD) BM alone (BMT) or with TCD BM plus T cells (GVHD) on day 0. GVHD mice were untreated or treated with anti-CD4 mAb on day (d) 3, 6, or 17. (a) Kinetics of GVHD score. (b,c) GVHD score (b) and tumor volume (c) at day 27 after allo-HSCT. (d) Scatter plots of GVHD score versus tumor volume at day 23 after allo-HSCT. Dotted lines indicate average values across all groups. The numbers to the right of the graph indicate the number of individuals plotted in the lower left box and the total number in each group. * $P < 0.05$; *** $P < 0.001$ (vs. BMT). †† $P < 0.01$; ††† $P < 0.001$ (vs. GVHD). Data represent mean \pm SEM ($n = 10$) from one of two independent experiments.

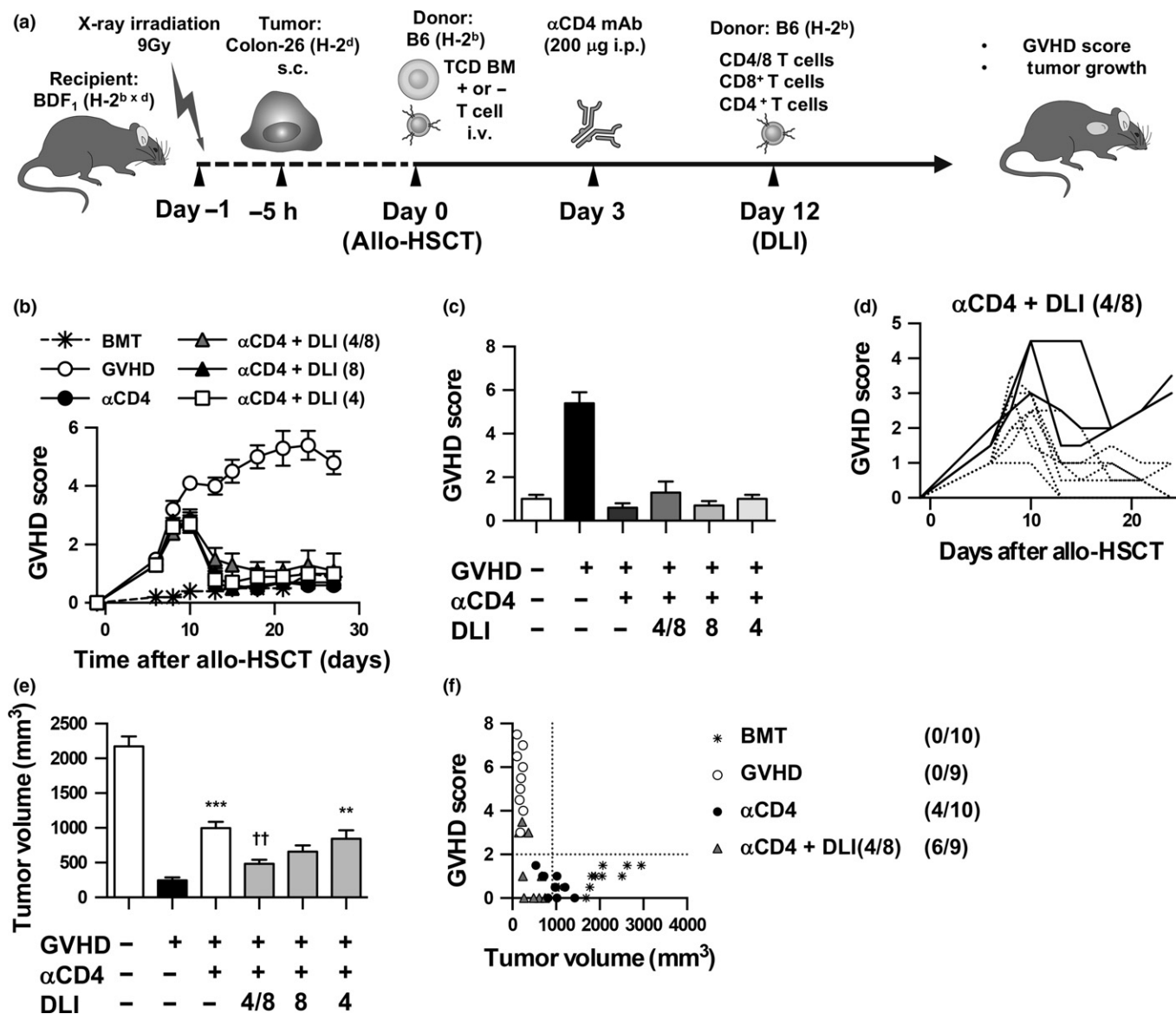


Fig. 3. Combination of anti-CD4 mAb treatment and donor lymphocyte infusion (DLI) enhances graft-versus-tumor effects without exacerbating graft-versus-host disease (GVHD) in murine allogeneic hematopoietic stem cell transplantation (allo-HSCT). (a) Schema of DLI model. BDF₁ mice receiving myeloablative X-ray irradiation were s.c. inoculated with colon-26 cells and transplanted with T cell-depleted bone marrow (TCD BM) alone (BMT) or with TCD BM plus T cells (GVHD) on day 0. GVHD mice were untreated or treated with anti-CD4 mAb on day 3. On day 12, DLI group mice received i.v. injection of B6-derived unfractionated T cells (CD4/8 T cells [“4/8” in subsequent panels]), CD8⁺ T cells (“8”), or CD4⁺ T cells (“4”). (b) Kinetics of GVHD score. (c) GVHD score at day 23 after allo-HSCT. (d) Kinetics of GVHD score for individual GVHD mice that were treated with anti-CD4 mAb and received DLI with unfractionated (CD4/8) T cells. (e) Tumor volume at day 23. (f) Scatter plots of GVHD score versus tumor volume at day 24 after allo-HSCT. Dotted lines indicate average values. Numbers to the right indicate the number of individuals plotted in the lower left box over the total group number. **P < 0.01; ***P < 0.001 (vs. GVHD⁺/αCD4⁻/DLI⁻); ††P < 0.01 (vs. GVHD⁺/αCD4⁺/DLI⁻). Data represent mean ± SEM (n = 10), from one of two independent experiments.

with reduced risk of rejection and leukemia relapse.^(18,19) Similar risks and benefits of myeloablative preconditioning were observed in our allo-HSCT model; however, early anti-CD4 mAb treatment almost completely ameliorated GVHD, while leaving considerable antitumor effects intact. These results suggest that the adverse effects of irradiation preconditioning were, at least in part, associated with the CD4⁺ T-cell-mediated immunotoxicity, and CD8⁺ T cells alone could mediate GVT effects. Our results are inconsistent with a report showing a major role of donor CD4⁺ T cells in GVT effects against bladder tumor.⁽²⁰⁾ The difference might be due to the differences in preconditioning. We used irradiation

myeloablative preconditioning before allo-HSCT, which permanently and completely reconstitutes all hematopoietic cells as donor type, but the previous study used non-myeloablative preconditioning with cyclophosphamide, which transiently and partially establishes chimerism in T cells, a very different setting from clinical allo-HSCT. Interestingly, myeloablative preconditioning 1 day before tumor inoculation, even in the absence of allogeneic T-cell responses, suppressed tumor growth more strongly than non-myeloablative preconditioning. These results suggest that better antitumor effects following myeloablative preconditioning are not only mediated by direct damage to tumor cells, but also mediated by damage to the

tumor stroma that is essential for tumor growth, and includes endothelial cells and fibroblasts. As the requirement for stroma in tumor growth may vary depending on the tumor type and affected organs, the benefits of myeloablative conditioning need to be further investigated in clinical settings.

After allo-HSCT, one study showed that the majority of the donor T cells migrated to the secondary lymphoid tissues within a few days, where they were primed by host-type professional antigen-presenting cells (APCs) and differentiated into effector T cells.⁽²¹⁾ In the induction phase, CD4⁺ T cells may augment the induction of CD8⁺ T cells by providing activation signals to APCs or by secreting pro-inflammatory cytokines such as interleukin-2.⁽¹¹⁾ In the effector phase, in which effector T cells redistributed to the tumor or target organs of GVHD where they attacked tumors or non-malignant tissues, CD4⁺ T cells played a major role in inducing a cytokine storm.^(11,22) In our analysis of the effects of timing of anti-CD4 mAb treatment, we did not see significant differences in the GVHD and GVT effects between treatment on day 3 or day 6. These results suggest that the presence of CD4⁺ T cells before day 3 of allo-HSCT is sufficient to maximize the GVT effects mediated by CD8⁺ T cells. Our data showing that giving anti-CD4 mAb treatment to mice with severe GVHD on day 17 partially ameliorated GVHD indicated that CD4⁺ T cells persistently contributed to GVHD, and anti-CD4 mAb therapy would be an option to treat patients with severe GVHD. Although strong GVT effects were observed in the mice treated late with anti-CD4 mAb, late administration intended to obtain strong GVT effects may not be recommended because of the relatively high risk of GVHD.

Donor lymphocyte infusion is a well-established treatment for leukemia relapse after allo-HSCT.^(23–25) Some clinical studies showed that DLI of CD4⁺ T cells reduced the risk of GVHD while preserving GVL effects in leukemia patients.^(23,26) As most of the leukemia cells lack expression of MHC class II markers, GVL effects of CD4⁺ DLI have been considered to be mediated by reactivation of pre-existing tumor-reactive CD8⁺ T cells by infusion of CD4⁺ T cells. Unexpectedly, CD4⁺ DLI did not augment GVT effects in the GVHD recipients receiving early anti-CD4 mAb treatment in our allo-HSCT model. This may be due, in part, to the influence of residual anti-CD4 mAb in the recipients.⁽¹⁶⁾ Even

allowing for the possible influence of residual anti-CD4 mAb, DLI with unfractionated T cells⁽¹⁶⁾ exerted more potent GVT effects than CD8⁺ DLI, suggesting that infused CD4⁺ T cells may contribute to GVT effects when co-infused with CD8⁺ T cells. As to the risk of DLI-associated GVHD, recipients with relatively severe GVHD should be excluded from receiving unfractionated DLI. Importantly, antigen recognition of graft-derived CD8⁺ T cells and DLI-derived CD8⁺ T cells may differ, as the former population is primed by host-type APCs but the latter population is primed by repopulated donor-type APCs. Because clonal diversity of tumor-reactive CD8⁺ T cells is an important factor for obtaining optimal antitumor effects, a combination of early anti-CD4 mAb treatment and unfractionated DLI would be an option to treat solid tumors.

In summary, we investigated the optimal strategy for adapting allo-HSCT for the treatment of solid tumors. Using a major mismatched mouse allo-HSCT model, we found that a combination of myeloablative preconditioning, early anti-CD4 mAb treatment, and DLI with unfractionated T cells could be a potent and safe immunotherapy that is theoretically effective against cancers lacking neoantigens. Of course, there are additional factors that may affect the risk of GVHD and the effects of GVT in clinical settings, such as the age of the patient, histocompatibility of the donor and recipient, and the type of tumor. The validity of our findings should be carefully investigated in future clinical studies. To this end, we are in the process of developing a humanized anti-CD4 mAb with potent antibody-dependent cell-mediated cytotoxicity.

Acknowledgments

The authors thank S. Fujita and K. Hachiga for invaluable assistance and support throughout this project.

Disclosure Statement

Satoshi Ueha, Yusuke Shono, Satoru Ito, and Kouji Matsushima own stock in IDAC Theranostics, Inc. Shoji Yokochi, Yoshiro Ishiwata, and Satoru Ito are employees of IDAC Theranostics, Inc. Shiro Shibayama is an employee of Ono Pharmaceutical Company. The other authors have no conflict of interest.

References

- 1 Khalil DN, Smith EL, Brentjens RJ, Wolchok JD. The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. *Nat Rev Clin Oncol* 2016; **13**: 273–90.
- 2 Le DT, Uram JN, Wang H *et al.* PD-1 blockade in tumors with mismatch-repair deficiency. *N Eng J Med* 2015; **372**: 2509–20.
- 3 Porter DL. Allogeneic immunotherapy to optimize the graft-versus-tumor effect: concepts and controversies. *Hematology Am Soc Hematol Educ Program* 2011; **2011**: 292–8.
- 4 Ringden O, Karlsson H, Olsson R, Omazic B, Uhlin M. The allogeneic graft-versus-cancer effect. *Br J Haematol* 2009; **147**: 614–33.
- 5 Jenq RR, van den Brink MR. Allogeneic haematopoietic stem cell transplantation: individualized stem cell and immune therapy of cancer. *Nat Rev Cancer* 2010; **10**: 213–21.
- 6 Barkholt L, Bregni M, Remberger M *et al.* Allogeneic haematopoietic stem cell transplantation for metastatic renal carcinoma in Europe. *Ann Oncol* 2006; **17**: 1134–40.
- 7 Takahashi Y, Harashima N, Kajigaya S *et al.* Regression of human kidney cancer following allogeneic stem cell transplantation is associated with recognition of an HERV-E antigen by T cells. *J Clin Invest* 2008; **118**: 1099–109.
- 8 Ljungman P, Bregni M, Brune M *et al.* Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe 2009. *Bone Marrow Transplant* 2010; **45**: 219–34.
- 9 Slavina S, Or R, Prighozina T *et al.* Immunotherapy of hematologic malignancies and metastatic solid tumors in experimental animals and man. *Bone Marrow Transplant* 2000; **25**(Suppl 2): S54–7.
- 10 Ueno NT, Rizzo JD, Demirek T *et al.* Allogeneic hematopoietic cell transplantation for metastatic breast cancer. *Bone Marrow Transplant* 2008; **41**: 537–45.
- 11 Coghill JM, Sarantopoulos S, Moran TP, Murphy WJ, Blazar BR, Serody JS. Effector CD4⁺ T cells, the cytokines they generate, and GVHD: something old and something new. *Blood* 2011; **117**: 3268–76.
- 12 Teshima T, Ordemann R, Reddy P *et al.* Acute graft-versus-host disease does not require alloantigen expression on host epithelium. *Nat Med* 2002; **8**: 575–81.
- 13 Shono Y, Ueha S, Wang Y *et al.* Bone marrow graft-versus-host disease: early destruction of hematopoietic niche after MHC-mismatched hematopoietic stem cell transplantation. *Blood* 2010; **115**: 5401–11.
- 14 Suenaga F, Ueha S, Abe J *et al.* Loss of lymph node fibroblastic reticular cells and high endothelial cells is associated with humoral immunodeficiency in mouse graft-versus-host disease. *J Immunol* 2015; **194**: 398–406.
- 15 Dialynas DP, Quan ZS, Wall KA *et al.* Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5: similarity of L3T4 to the human Leu-3/T4 molecule. *J Immunol* 1983; **131**: 2445–51.

- 16 Ueha S, Yokochi S, Ishiwata Y *et al.* Robust Antitumor Effects of Combined Anti-CD4-Depleting Antibody and Anti-PD-1/PD-L1 Immune Checkpoint Antibody Treatment in Mice. *Cancer Immunol Res* 2015; **3**: 631–40.
- 17 Cooke KR, Kobzik L, Martin TR *et al.* An experimental model of idiopathic pneumonia syndrome after bone marrow transplantation: I. The roles of minor H antigens and endotoxin. *Blood* 1996; **88**: 3230–9.
- 18 Abdul Wahid SF, Ismail NA, Mohd-Idris MR *et al.* Comparison of reduced-intensity and myeloablative conditioning regimens for allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia and acute lymphoblastic leukemia: a meta-analysis. *Stem Cells Dev* 2014; **23**: 2535–52.
- 19 Shabbir-Moosajee M, Lombardi L, Ciurea SO. An overview of conditioning regimens for haploidentical stem cell transplantation with post-transplantation cyclophosphamide. *Am J Hematol* 2015; **90**: 541–8.
- 20 Kamiryo Y, Eto M, Yamada H *et al.* Donor CD4 T cells are critical in allogeneic stem cell transplantation against murine solid tumor. *Can Res* 2009; **69**: 5151–8.
- 21 Beilhack A, Schulz S, Baker J *et al.* In vivo analyses of early events in acute graft-versus-host disease reveal sequential infiltration of T-cell subsets. *Blood* 2005; **106**: 1113–22.
- 22 Henden AS, Hill GR. Cytokines in graft-versus-host disease. *J Immunol* 2015; **194**: 4604–12.
- 23 Bachireddy P, Wu CJ. Understanding anti-leukemia responses to donor lymphocyte infusion. *Oncoimmunology* 2014; **3**: e28187.
- 24 Lu YF, Gavrilescu LC, Betancur M, Lazarides K, Klingemann H, Van Etten RA. Distinct graft-versus-leukemic stem cell effects of early or delayed donor leukocyte infusions in a mouse chronic myeloid leukemia model. *Blood* 2012; **119**: 273–84.
- 25 Martin PJ. Reversing CD8+ T-cell exhaustion with DLI. *Blood* 2014; **123**: 1289–90.
- 26 Soiffer RJ, Alyea EP, Hochberg E *et al.* Randomized trial of CD8+ T-cell depletion in the prevention of graft-versus-host disease associated with donor lymphocyte infusion. *Biol Blood Marrow Transplant* 2002; **8**: 625–32.