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**[Review]**

# Harnessing allogeneic CD4$^+$ T cells to reinvigorate host endogenous antitumor immunity

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**Abstract**

Immune checkpoint blockade (ICB) therapies developed over the past decade have been among the most promising approaches for the treatment of patients with advanced cancers. However, the overall objective response rate of ICB therapy for various cancers remains insufficient. Hence, novel strategies are required to improve the efficacy of immunotherapy for advanced cancers. The graft-versus-tumor (GVT) effect, which reflects strong antitumor immunity, is known to occur after allogeneic hematopoietic stem cell transplantation (HSCT). The GVT effect is mainly caused by transplanted donor lymphocytes that recognize and react to distinct alloantigens on tumor cells. In contrast, transplanted allogeneic cells can, in some instances, induce endogenous antitumor immunity in recipients if the graft has been rejected. Because of this ability, allogeneic cells have also been used to induce endogenous antitumor immunity without HSCT, and their beneficial immune response is referred to as the "allogenic effect." Here, we review the usefulness of allogeneic cells, particularly allogeneic CD4$^+$ T cells, in cancer immunotherapy by highlighting their unique potential to induce host endogenous antitumor immunity.

## Introduction

Advanced cancer has a high mortality rate and is one of the most difficult diseases to cure. Immunotherapies developed over the past decade have been among the most promising approaches for the treatment of patients with advanced cancer[1]. Recent findings suggest that cancer cells shield themselves by expressing immune checkpoint molecules, such as PD-L1, which bind to the PD-1 receptors expressed on activated T cells, resulting in the loss of their capacity to attack cancers[2,3]. The blockade of these immune checkpoint molecules using either anti-PD-1 antibody (Ab) or anti-PD-L1 Ab can reinvigorate the host endogenous antitumor immunity[4,5]. Based on their clinical efficacy, these immune checkpoint inhibitors have been approved for many cancers, including melanoma, non-small cell lung cancer, and renal cell carcinoma[3-8]. However, the overall objective response rate of these therapies based on immune checkpoint blockade rarely exceeds 40% in various cancers[3,9,10]. On the other hand, blocking physiological immunosuppressive pathways such as PD-1/PD-L1 interactions often causes serious autoimmune-like adverse reactions[11,12]. Therefore, novel strategies are required to improve the efficacy of immunotherapy for advanced cancers.

Adoptive cell therapy is another innovative approach for treating advanced malignancies. Engineering human T cells with a chimeric antigen receptor (CAR) specific for the pan-B-cell CD19 antigen (CD19 CAR-T cells) has been associated with high response rates in patients with relapsed or refractory B-cell malignancies[13]. Many other types of CAR-T cell therapies have been developed to target various antigens presented on the surface of both hematological malignant cells and non-hematological solid tumors[14-16]. However, CAR-T cell therapies in solid tumors are not as effective as those in leukemia because of the paucity of preferable target antigens, as well as their inability to infil-

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trate and survive in the tumor microenvironment[17,18]. Furthermore, one of the challenges of these target-specific therapies is relapse due to emerging clones that have lost their target antigens[13,19]. Indeed, other target-specific immune therapies, such as bispecific antibodies and several monoclonal antibodies, have the same problem of relapse due to the loss of target antigens[20,21]. In this context, Kuhn *et al.* demonstrated that CD40 ligand-modified CAR-T cells not only elicited antitumor activity but also circumvented tumor immune escape by inducing endogenous antitumor immunity[22,23]. Li *et al.* also demonstrated that CAR-T cells secreting IL-36γ activate endogenous antigen-presenting cells (APCs) and T cells, which improves CAR-T cell-mediated antitumor responses[24]. These results suggest that endogenous antitumor immunity in concert with CAR-T cells may play a pivotal role in overcoming escaped clones and eliciting potent antitumor effects.

## Endogenous antitumor immunity

In the process of tumorigenesis, gene mutations accumulate in tumor cells, indicating that tumor cells have different features based on the mutated genes from the normal host cells, although they arise from the same host. These differences can be presented with major histocompatibility complex (MHC) molecules on tumor cells as targets for host immunity, which can evoke immunological tumor elimination. However, such endogenous antitumor immunoreaction does not usually occur in an established tumor because clinically evident tumors have already escaped host immunity through multiple mechanisms[25]. Tumor cells may downregulate the expression of classical MHC molecules, express immune checkpoint molecules, and produce immunosuppressive cytokines, all of which develop an immunosuppressive tumor microenvironment[25]. A major challenge has been the development of approaches to overcome this mechanism of tumor escape in tumor-bearing hosts.

One of the goals of cancer treatment is the elimination of cancer cells and the long-lasting suppression of cancer recurrence. In this context, acquisition of endogenous antitumor immunity may represent a desirable strategy. To induce effective antitumor immunity, Chen and Mellman suggested the importance of the cancer immunity cycle, which consists of the following seven steps : release of cancer cell antigens, cancer antigen presentation by APCs, priming and activation of T cells, trafficking and infiltration of activated T cells into the tumor, antigen recognition of cancer cells by T cells, and killing of cancer cells[26]. In the last step, cancer cell antigens are released again, which revolves the subsequent cycles to further enhance the antitumor responses[26].

Cancer vaccines are a representative strategy for inducing endogenous T cell responses, which potentially activate host immunity against tumor evasion[27]. To date, two major types of therapeutic cancer vaccines have been developed : cancer-associated peptide vaccination and peptide-pulsed dendritic cell (DC) vaccination. In general, DC vaccination is thought to be more efficient than peptide vaccination because DCs possess the potent ability to elicit strong T cell immune responses. DCs used for vaccination are usually derived from the peripheral blood monocytes or bone marrow cells of tumor-bearing hosts[28,29]. After pulsing with cancer-associated peptides, activated autologous DCs were administered to the host to induce endogenous immune responses. Although encouraging results from preclinical studies have been reported, most of the recent clinical studies of both peptide- and DC-based vaccination have shown limited efficacy with respect to the induction of objective responses[1,27,30-32]. Thus, antitumor vaccination using an autologous immune system may remain insufficient to revolve the host cancer immunity cycle and induce potent endogenous antitumor immunity.

## Allogeneic antitumor immunity after hematopoietic stem cell transplantation

Allogeneic immunity is preferentially discussed in the context of hematopoietic stem cell transplantation (HSCT) and organ transplantation. Owing to differences in major and/or minor histocompatibility complex molecules, transplanted donor cells and the host immune system recognize host tissues and transplanted cells/organs, respectively, as enemies, except in the case of transplantation between identical twins[33,34]. Therefore, immunosuppressants must be administered to the recipient to avoid fatal graft-versus-host disease (GVHD) and/or graft rejection. On the other hand, the graft-versus-tumor (GVT) effect is a beneficial anti-tumor effect in which hematological malignant cells are attacked by infused donor cells after allogeneic HSCT[33-36]. The GVT effect is mainly mediated by donor T cells that recognize and react to multiple alloantigens on tumor cells[33]. Many studies have shown durable remission in patients with refractory hematological

malignancies after allogeneic HSCT because of potential GVT effects[35,37-39]. The GVT effects have also been reported in refractory solid tumors, including renal cell carcinoma, breast cancer, colorectal cancer, and some childhood cancers[40-47]. Among them, tumor regression was often associated with withdrawal of immunosuppression, chimerism conversion, and/or occurrences of chronic GVHD, all of which suggested that the antitumor effects were elicited by transplanted donor immunity[44,45]. Although the objective response rates due to the GVT effects for patients with solid tumors are not as high as those for patients with hematological malignancies, accumulating knowledge from cases that achieved long-lasting remission may provide us with some clues to control the potential GVT effects on solid tumors.

In recent years, Guo *et al.* demonstrated that granulocyte colony-stimulating factor-mobilized human leukocyte antigen (HLA)-mismatched donor peripheral blood stem cell infusion after regular chemotherapy, which is referred to as "microtransplantation," improved the outcomes of patients with acute myeloid leukemia (AML)[48,49]. Notably, pentamer analysis revealed a significant increase in WT1$^+$CD8$^+$ T cells (donor and/or host origin) in 33 of 39 patients, and the number of infused CD3$^+$ T cells was correlated with the therapeutic effects[49]. The underlying mechanism of their anti-leukemic activity remains incompletely understood, but it has been noted that microtransplantation provides an effective and safe strategy for patients with AML[48-51].

In addition, Rubio *et al.* reported an ingenious approach in a mouse model of leukemia in which mandatory or spontaneous loss of donor chimerism after HSCT was associated with improved leukemia-free survival[52]. They demonstrated that host-derived interferon-γ was critical for mediating anti-leukemic effects[52]. Along with this preclinical study, some clinical cases with advanced hematologic malignancies who achieved complete remission after non-myeloablative HSCT, even though the transplanted graft was rejected, have been reported[53,54]. These preclinical and clinical observations suggest that host-versus-graft immunity in graft rejection also elicits host-versus-tumor effects[52,54,55]. Collectively, allogeneic cells that can cause a strong immunoreaction in the recipient may be useful for inducing not only donor-derived but also host-derived antitumor immunity.

## Allogeneic immunotherapy to induce endogenous antitumor immunity

In the 1960s, before establishing the current style of HSCT, Alexander *et al.* proposed the concept of inducing host endogenous antitumor immunity by adoptive transfer of heterologous cells without utilizing HSCT[56]. The induced antitumor immunity prolonged the survival of the tumor-bearing host even though the transferred heterologous cells were rejected[56]. Other researchers have applied the same idea to induce host antitumor immunity in preclinical models in which lymphocytes with/without pre-immunization isolated from the spleen or lymph nodes were used as allogeneic cell sources[57-59]. The favorable immune response elicited by infused heterologous/allogeneic cells is referred to as the "allogenic effect"[60]. Then, several clinical studies have been conducted on patients with intractable cancers, with or without low-dose irradiation, cytokine therapy, or low to regular doses of chemotherapy prior to allogeneic cell infusion[61-63]. As a result, an objective response was sometimes observed in patients with hematologic malignancies and renal cell carcinoma[62]. However, such a preferable response is rarely observed in other refractory solid tumors, and the achievement of stable disease or a transient effect is usually the best response in many studies[61,63]. Furthermore, a shortage of information about the sustainability of responses due to allogeneic effects leaves the long-term efficacy of these strategies undetermined.

## Therapeutic antitumor vaccinations with CD4$^+$ T lymphocytes containing allogenic cells

More recently, studies have started using separated populations of allogeneic cells for the induction of host endogenous antitumor immunity, in which some of them used CD4$^+$ T lymphocytes containing allogeneic cells (Table 1). Symons *et al.* conducted CD8$^+$ T cell-depleted allogeneic donor lymphocyte infusion after the administration of cyclophosphamide. As a result, activated host immunity prolonged survival by a median of 10 and 7 days in mouse models of hematologic malignancy and solid tumors, respectively[64]. Their antitumor effect requires the presence of donor CD4$^+$ T cells, host CD8$^+$ T cells, and expression of alloantigens in normal host tissues[64]. Su *et al.* performed multiple injections of mitomycin C (MMC)-inactivated MHC-fully mismatched allogeneic leukocytes, which in-

Table 1. Therapeutic vaccination with $CD4^+$ T lymphocytes containing allogenic cells in mouse models of cancer

| Study group | Tumor cell | Particularity of allogenic cells | Times (routes) of Administration | Required concomittant therapy | Reference |
|---|---|---|---|---|---|
| SKCCC at JH | A20/RENCA | $CD8^+$ T cell-depleted lymphocytes | 1 time (i.v.) | CY | Symons *et al.* 2008 |
| PUMC and CAMS | B16F10 | MMC inactivated MHC-fully mismatched leukocytes | 2 times (i.v.) | Non | Su *et al.* 2008 |
| | TC-1 | Tumor antigen primed and inactivated MHC-haploidentical T lymphocytes | 3 times (i.v.) | Non | Shi *et al.* 2014 |
| | TC-1 | MMC inactivated MHC-fully mismatched leukocytes | 3 times (i.t.) | CY+ MMC inactivated TC-1 vaccine | Tang *et al.* 2017 |
| HHUMC | BCL1 | CD3/CD28 cross-linked memory Th1 cells | 1-3 times (i.v.) | Non | Har-Noy *et al.* 2008 |
| | | | 3 times (i.d.) | BCL1 lysate vaccine | Har-Noy *et al.* 2009 |
| | | | 2 times (i.v. and i.t.) | Cryoablation of the tumor | Har-Noy *et al.* 2009 |
| UA and HHUMC | 12B1 | Effector/memory $CD4^+$Th1 cells | 3 times (i.f.) | 12B1- derived CRCL vaccine | Janikashvili *et al.* 2011 |
| FMU | B16F1 | $CD4^+$T cells activated by the host derived antigen presenting cells | 1 time (i.t.) | Non | Mochizuki *et al.* 2021 |

SKCCC at JH : Sidny Kimmel Complehensive Cancer Center at Johns Hopkins, PUMC : Pekin Union Medical College, CAMS : Chinese Academy of Medical Science, HHUMC : Hadassha-Hebrew University Medical Center, UA : University of Alizona, FMU : Fukushima Medical University, i.v. : intravenous, MMC : mytomycin C, 1.t. : intratumoral, Th1 : T helper 1, i.d. : intradermal, i.f. : intrafootpad, CRCL : chaperone-rich cell lysate

duced both innate and adaptive immune responses in B16F10 tumor-bearing mice[65]. The same group reported that multiple injections of tumor-antigen-primed inactivated haploidentical lymphocytes into mice with TC-1 lung cancer induced host tumor-specific cytotoxic T lymphocytes (CTLs), in which tumor growth was delayed with prolonged overall survival[66]. According to their latest study, the sequential administration of cyclophosphamide, MMC-inactivated MHC-fully mismatched allogeneic leukocytes, and cancer cell vaccinations were required to achieve the best antitumor efficacy, which inhibited tumor growth and extended survival with a mean survival advantage of 8.8 days[67].

With activated $CD4^+$ T cells, Har-Noy *et al.* used T-helper 1 (Th1) memory cells generated by the *ex vivo* activation of allogeneic $CD4^+$ T cells with anti-CD3/anti-CD28 stimulation. Prior to use, Th1 memory cells were further activated by anti-CD3/anti-CD28-coated nanobeads, which were then administered with the attached beads (named CD3/CD28 cross-linked Th1 memory cells)[68,69]. A single infusion of CD3/CD28 cross-linked Th1 memory cells switched the cytokine environment of tumor-bearing mice from Th2-dominant to Th1-dominant, which was capable of enhancing host endogenous antitumor immunity, with 31% of the mice cured[68]. In their combined method of CD3/CD28 cross-linked Th1 memory cells with tumor lysate vaccination, the mean survival time of mice with BCL1 leukemia was prolonged by 14.5 days in comparison to control mice, although no mice were cured[69]. They also demonstrated that cryoimmunotherapy, which consisted of intratumoral/intravenous administration of allogeneic CD3/CD28 cross-linked Th1 memory cells with cryoablation of the solid tumor, resulted in 40% of mice surviving for more than 90 days[69]. A subsequent study showed that approximately 40% of mice with leukemia survived for up to 40 days when allogeneic effector/memory $CD4^+$ Th1 cells were combined with chaperone-rich cell lysate vaccination[70]. Based on these preclinical studies, a phase IIb clinical trial is currently being conducted in patients with metastatic colorectal cancer (NCT04444622).

Although many of the abovementioned strategies require additional treatments such as chemotherapy, cancer vaccination, and/or cryoablation concomitantly with allogeneic cell infusions, these studies suggest that $CD4^+$ T lymphocytes containing allogeneic cells have the unique potential to activate host endogenous antitumor immunity.

## A novel antitumor immunotherapy using alloantigen-activated $CD4^+$ T cells

We recently developed a novel strategy using allogeneic $CD4^+$ T cells to reinvigorate host endogenous antitumor immunity in a mouse model of melanoma[71]. In a mixed lymphocyte culture, MHC class II-mismatched allogeneic $CD4^+$ T cells were activated by host strain-derived DCs (Fig. 1). A single intratumoral injection of alloantigen-activated $CD4^+$ (named AAA-$CD4^+$) T cells causes Th1 inflammation in the tumor. The inflammation accelerates the infiltration of host professional APCs, host $CD4^+$ T cells, and host $CD8^+$ T cells into

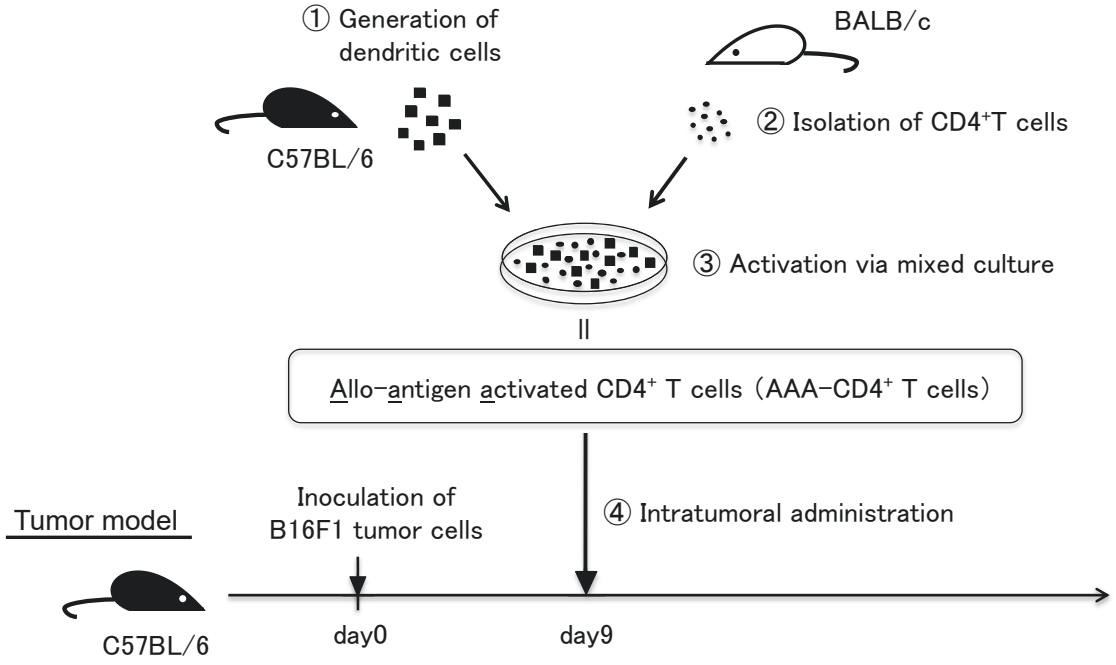


Fig. 1. Tumor mouse model : Host C57BL/6 mice were subcutaneously injected with B16F1 tumor cells on day zero. ① : Dendritic cells (DCs) were generated from c-kit-positive cells in the bone marrow of other C57BL/6 mice. ②-③ : $CD4^+$ T cells isolated from the spleens of BALB/c mice were activated in mixed-lymphocyte cultures with C57BL/6 mouse-derived DCs. ④ : Nine days after B16F1 inoculation, alloantigen-activated $CD4^+$ T cells (AAA-$CD4^+$ T cells) were directly injected into the tumor. The tumor size and changes in the physical condition of the animals were monitored every two-three days.

the tumor, which licenses the host endogenous CTLs to eliminate preestablished melanoma. The induced CTLs persisted long-term *in vivo* as memory CTLs to protect animals from tumor rechallenge. Notably, injected allogeneic $CD4^+$ T cells diminished *in vivo* soon after tumor regression and failed to cause any immune-related complications or other unexpected adverse reactions[71].

In terms of applying *ex vivo* activated allogeneic $CD4^+$ T cells that induce a Th1 environment and host antitumor immunity, the strategies of Har-Noy *et al.* and ours seem to be similar, but the cell products differ from each other. One of the most significant differences is the method of activation, and therefore, the reactivity of allogeneic $CD4^+$ T cells. They used a CD3/CD28 antibody to activate allogeneic $CD4^+$ T cells, in which nearly all allogeneic $CD4^+$ T cells, including both host antigen-reactive and host antigen-nonreactive fractions, were activated. As the estimated frequency of allogeneic $CD4^+$ T cells that are reactive to alloantigens is approximately 1 in 10-100 of all allogeneic $CD4^+$ T cells, only a limited fraction of their activated allogeneic $CD4^+$ T cells can be reactive to the alloantigens of the tumor-bearing host[72]. In contrast, many of our AAA-$CD4^+$ T cells were reactive to host alloantigens because they were activated by DCs derived from the same host strain. The mismatched MHC class II molecules between the tumor-bearing host and $CD4^+$ T cell donor are the most likely target antigens of AAA-$CD4^+$ T cells. Because MHC molecules are among the strongest antigens in transplantation immunology, AAA-$CD4^+$ T cells can cause robust graft-versus-host-directed inflammation in tumors after intratumoral administration. AAA-$CD4^+$ T cells may then be eliminated by host-versus-graft-directed inflammation, during which time the host endogenous antitumor immunity can be reinvigorated. Although the molecular mechanisms remain to be elucidated, our results suggest that alloantigen-activated $CD4^+$ T cells can be used to induce host antitumor CTLs in cancer immunotherapy.

## Future perspectives

The allogeneic immune response is a conserved immune reaction ; thus, the current concept of AAA-$CD4^+$ T cell therapy in animal models may be translated into human clinical trials. Since a large number of human DCs are induced from monocytes obtained by peripheral blood apheresis, human AAA-$CD4^+$ T cells can be generated by the activation of allogeneic $CD4^+$ T cells with monocyte-derived DCs from patients with cancer, which recognize and react

to the mismatched HLA class II antigens of patients[28]. Along with clinical development, we also aimed to understand the molecular mechanisms underlying the induction of strong endogenous antitumor immunity using our strategy. Because we found a significant accumulation of host-activated DCs and activated macrophages in the tumor after AAA-$CD4^+$ T cell injection, these professional APCs may play important roles in inducing current antitumor immunity[71]. However, their practical roles in our model have not been elucidated, although allogeneic inflammation can lead to their migration and activation in tumors. Intriguingly, it has been reported that many distinct types of macrophages exist in tumors, but their functions remain undefined[73-77]. For instance, some of them elicit antitumor immunity, whereas others are tumor supportive[73-77]. Furthermore, many other types of immune-related cells also exist in the tumor, such as tumor-infiltrating neutrophils, myeloid-derived suppressor cells, and cancer-associated fibroblasts, all of which may be involved in the induction of current endogenous antitumor immunity[78-82]. The discovery of a novel molecular mechanism through which endogenous antitumor immunity is reinvigorated by allogeneic cells could be further translated for the development of new therapeutic targets in next-generation cancer immunotherapy.

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## Conflict of interest disclosure

Our university received financial support from Nobelpharma Co., Ltd. to obtain a patent associated with AAA-$CD4^+$ T cell therapy.

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