

Advances in evidence-based cancer adoptive cell therapy

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Abstract: Adoptive cell therapy (ACT) has been developed in cancer treatment by transferring/infusing immune cells into cancer patients, which are able to recognize, target, and destroy tumor cells. Recently, sipuleucel-T and genetically-modified T cells expressing chimeric antigen receptors (CAR) show a great potential to control metastatic castration-resistant prostate cancer and hematologic malignancies in clinic. This review summarized some of the major evidence-based ACT and the challenges to improve cell quality and reduce the side effects in the field. This review also provided future research directions to make sure ACT widely available in clinic.

Keywords: Adoptive cell therapy (ACT); non-specific cell therapy; specific cell therapy; dendritic cell-based therapy

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Introduction

Cancer is a major global public health problem and the number of newly diagnosed cases continues to increase due to aging and growth of worldwide populations. Although advancement in early detection, prevention, and treatment options, cancer is still the second cause of human mortality. To date, most cancers are clinically diagnosed at the advanced stages of diseases, which result in curable surgery not an option, while chemotherapy and radiotherapy are not effective in cure of most cancer patients. During the past decade, development of modern medicine, such as target therapy has still relatively short-term benefits for selected patients (1).

In recent years, different studies demonstrated that enhancement of cytotoxicity to target the cellular immune system could help clinicians to fight human cancer (2). For instance, sipuleucel-T, the first therapeutic cancer vaccine approved by US FDA, is an autologous active cell immunotherapy to improve overall survival (OS) of

patients with metastatic castration-resistant prostate cancer in phase III clinical trials (3). Ipilimumab (4), a human monoclonal antibody that activates the immune system by cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), has increased the OS rate of patients with melanoma by 45.6% at 12 months, 33.2% at 18 months, and 23.5% at 24 months. Programmed cell death 1 (PD-1) is another negative co-stimulatory receptor expressed primarily on the surface of activated T cells (5,6) and anti-PD-1 antibodies (pembrolizumab and nivolumab) were able to not only increase OS of patients with metastatic melanoma (7,8), but also to reach better response rate and progression-free survival (PFS) of patients with advanced, previously treated squamous-cell NSCLC (9-11). Thus, immunotherapy shows encouraging in control of human cancers.

Adoptive cell therapy (ACT) is another potentially useful approach to treat human cancers (12) and previous studies showed that such a cancer immunotherapy can include non-specific cell therapy [lymphokine-activated killer (LAK) cells, cytokine-induced killer (CIK) cells and natural killer

cells (NK), specific cell therapy [cytotoxic T lymphocytes (CTLs), tumor-infiltrating lymphocytes (TILs), T cell therapy with modified T cell receptor (TCR) genes, T cell therapy with modified chimeric antigen receptors (CAR) genes] and so on. ACT is to stimulate body own immune system in order to trigger antitumor immune response, and eventually enable natural abilities to better recognize, target, and, finally, eliminate cancer cells from human body. Compared to other forms of cancer immunotherapy, ACT has multiple advantages, e.g., long-term benefit after short-term treatment and slight fewer adverse events. Although dendritic cells (DCs) and CTLs therapy have made a great progress, clinical applications are still somewhat limited. Recently, the genetic-modified T cells expressing specific TCRs or CARs are just now entering the clinical trials and the data have shown a great potential for high avidity to tumor-associated antigens and long-lasting anti-tumor responses, which encourages researchers to continuously study feasibility of this therapy in human cancers (13-15). Thus, our current review summarized the most recent advances and discussed and predicted the future research directions in this field.

Non-specific cell therapy

Non-specific cell therapy is an immunotherapy to non-specifically activate immune cells to induce their “non”-specific antitumor immune response to reject and destroy cancer cells. Based on different types of immune cells involved, non-specific cell therapy can be divided into LAK cell, CIK cell, and NK cell-mediated antitumor therapies. This non-specific cell therapy was used to treat different human cancers clinically (16-18).

LAK cell-mediated non-specific cell therapy

Grimm and his colleagues first reported in 1982 (19) non-specific killer cells generated by culture of peripheral blood mononuclear cells (PBMC) with high dose of interleukin-2 (IL-2), which was named as LAK cells. LAK cells contain a mixture of T cells and NK cells, both of which are not restricted by the major histocompatibility complex (MHC) against a broad range of tumor cells *in vitro* (16,20).

Thereafter, Rosenberg and associates (16) showed effects of autologous LAK cells and IL-2 on patients with advanced cancers in whom standard therapy had failed. The objective tumor regression achieved in 11 out of 25 patients, i.e., a complete tumor regression (CR) occurred in 1 patient with

metastatic melanoma and partial responses (PRs) occurred in 9 patients with pulmonary or hepatic metastases from melanoma, colon cancer, or renal-cell cancer and in patients with primarily unresectable lung adenocarcinoma. In 1988, another study summarized a series of clinical trials using high-dose of IL-2 alone or in combination with LAK (17). Of 221 patients, 16 had a CR in patients with metastatic cancer and an additional 26 had a partial tumor regression (PR). Based on these studies, LAK cell therapy has been considered to be effective against metastatic melanoma, renal cell carcinoma, and other advanced solid tumors.

However, in another randomized clinical trial, the result suggested a trend toward improving survival when IL-2 was given together with LAK cells to melanoma patients, but not occur in patients with renal cell carcinoma (21). Moreover, a randomized phase III trial of IL-2 with or without LAK cells in treatment of patients with advanced renal cell carcinoma demonstrated that there was no difference in treatment response ($P=0.61$) and survival ($P=0.67$) between these two treatment arms and more patients on the LAK arm experienced pulmonary toxicity (22). In addition, several other studies showed the safety and efficacy of LAK cell therapy in patients with malignant gliomas (23-25). In one study, a median OS of 31 patients with glioblastoma multiforme (GBM) was 17.5 months versus 13.6 months in control group (23). Boiardi *et al.* reported that the focal injection of LAK cells and IL-2 in 9 recurrent GBM patients were well-tolerated and the response rate was 33%, although the median OS of patients didn't show significant improvement (26).

Thus, although LAK cell therapy seemed to effectively kill some tumor cells, high toxicity caused by high dose of IL-2 (such as vascular leakage and severe hypotension) (26,27) limited its clinical usage.

CIK cell-mediated non-specific cell therapy

CIK cells are obtained by isolated from PBMCs and then stimulated with a cocktail of interferon-gamma (IFN- γ), anti-CD3 monoclonal antibody, and IL-2 in a stepwise in a time-dependent *ex vivo* culturing process for approximately 2 weeks (28). CIK cells are a mixture of cells with non-MHC-restricted cytolytic activity, including $CD3^+CD56^-$ T cells and $CD3^+CD56^+NK$ -T cells, and a relatively minor population of $CD3^-CD56^+NK$ cells (29-31). Compared to standard IL-2 stimulated LAK cells, CIK cells have enhanced antitumor cytotoxic activity by over 70-fold (32). The lytic activity can be further enhanced by addition of

IL-1, IFN- γ , IL-7, IL-15, and other cytokines (33-36). To date, CIK cells have been evaluated as an adoptive cell immunotherapy for cancer patients in numerous clinical trials (summarized in *Table 1*). In the first phase I clinical study, autologous immunological effector cells were transfected with the IL-2 gene and to treat patients with metastatic renal cancer, colorectal cancer and lymphoma and data showed that 6 patients remained in disease progression, 3 showed stable disease (SD), and only 1 lymphoma patient had a complete response (CR) (37). PubMed search of the international registry on CIK cells (IRCC) (18) for "CIK cells clinical trials" found 11 such clinical trials in 2011 that contained 384 patients treated with autologous CIK cell immunotherapy, of which 24 patients had a CR, 27 patients had a PR, and 40 patients had a minor response. The total response rate (RR) was 23.7% (91/384), while 161 patients (41.9%) had a SD and 129 patients (33.6%) had a progressive disease (PD). Only 3 patients had tumor volume decreased. The side effects of CIK cell treatment were minimal and at final data analysis, which indicated that adjuvant immunotherapy with CIK cells could prevent tumor recurrence and improve quality of life and progression-free survival (PFS) rate in patients. A latest study published in *Gastroenterology* (62) showed the efficacy and safety of a multicenter, randomized, open-label, phase III trial using activated CIK cells as the adjuvant immunotherapy that included 230 hepatocellular carcinoma patients after surgical resection, radiofrequency ablation, or percutaneous ethanol injection in South Korea. The median time of recurrence-free survival (RFS) was 44.0 months in the immunotherapy group *vs.* 30.0 months in the control group. However, patients in the immunotherapy group had higher proportion of adverse events than in the control group, although the proportion of serious adverse events did not differ significantly between these two groups of patients.

In China, there have been numbers of such clinical trials using CIK cell immunotherapy of human cancers. For example, Liu *et al.* (36) reported 148 patients with metastatic renal clear cell carcinoma randomized to autologous CIK cell immunotherapy (arm 1, n=74) or IL-2 treatment combination with IFN- α -2a (arm 2, n=74). The 3-year PFS and OS in arm 1 were 18% and 61%, respectively, as compared to 12% and 23%, respectively, in arm 2. The median PFS and OS in arm 1 were significantly longer than those in arm 2. Pan *et al.* (58) reported 90 patients with post-mastectomy triple-negative breast cancer in a retrospective study and 45 patients received chemotherapy

alone or with sequential radiotherapy and 45 patients received chemotherapy with/without radiotherapy and sequential CIK infusion. The 1-, 2-, 3-, and 4-year disease-free survival (DFS) rates in the CIK group were 97.7%, 90.1%, 83.4%, and 75.2%, respectively, *vs.* 88.9%, 64.4%, 62.1%, and 56.4%, respectively, in the control group. Also the 1-, 2-, 3-, and 4-year OS rates were significantly higher in treatment group (100.0%, 100.0%, 96.7%, and 92.4%, respectively, *vs.* 95.6%, 88.6%, 76.3%, and 72.7%, respectively, in the control group). In subgroup analyses, CIK adjuvant therapy increased DFS rate of patients with pathologic grade III disease and significantly increased the OS rate of patients with N1, N2, N3, IIB, or III TNM disease. These data indicate that adjuvant CIK treatment combined with chemotherapy was an effective therapeutic strategy.

Thus, overall, these trials certainly demonstrated that CIK cells therapy was feasible and safe in patients with hepatocellular carcinoma, renal cell carcinoma, non-small cell lung cancer, gastric cancer, and other solid tumors. In most cancer patients, adjuvant CIK cell therapy combination with conventional treatment had better clinical outcomes than standard therapy alone.

NK cell-mediated non-specific cell therapy

NK cells were first identified and characterized by Herberman *et al.* (63) and Kiessling *et al.* (64) in 1975 as a unique subset of lymphocytes that are larger in size than regular T and B lymphocytes and contain distinctive cytoplasmic granules. In human beings, NK cells are defined by expression of the surface marker CD56 and lack of the T cell markers, such as CD3 or TCR (65) and account for approximately 5% to 15% of human peripheral blood lymphocytes (66). NK cells are innate lymphocytes with the capacity to target foreign, damaged, malignant, and virally infected cells without prior immunization or MHC restriction. The cytotoxic granules released by NK cells up on targeting cells are largely composed of perforin and granzyme. Beyond their cytotoxicity, NK activation also leads to release of cytokines IFN- γ , TNF- α , G-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3 and others (67,68).

As NK cells are found primarily in blood, NK cell therapy has been most successful in hematopoietic malignancies (69-73). Currently, NK cells can be used to treat patients with refractory leukemia before conventional hematopoietic cell transplantation (HCT) for induction

Table 1 Published clinical studies using CIK cell immunotherapy

Year	Cancer type	# of patients	Therapy	Clinical response
1999 (37)	Metastatic renal cancer, colorectal cancer and lymphoma	10	Autologous CIK cells transfected with IL-2 gene	CR [1]; SD [3]
2002 (38)	Patients with advanced malignant tumor	63	Autologous CIK	PR + MR: 44.46%
2005 (39)	Hodgkin disease and non-Hodgkin lymphoma	9	Autologous CIK cells	PR: 2; SD: 2
2006 (40)	Advanced gastric cancer	57	Autologous CIK cells with chemotherapy	2-year life-span was prolonged
2007 (41)	Acute and chronic myelogenous leukemia and Hodgkin disease	11	Autologous CIK	CR: 1; PR: 2; PD: 6
2008 (42)	Hepatocellular carcinomas	85	Autologous CIK	1-year and 18-month recurrence rates: 8.9% and 15.6% vs. 30.0% and 40.0%
2008 (43)	Hepatocellular carcinoma	127	Autologous CIK	Disease-free survival rates were significantly higher than in the control group
2008 (44)	Non-small cell lung cancer	59	Chemotherapy plus CIK cells vs. chemotherapy alone	ORR: 44.8% vs. 43.3%; DCR: 89.7% vs. 65.5%; time to progression: 6.65 vs. 4.67 mos; median survival time: 15 vs. 11 mos; PFS and OS were significantly longer
2009 (45)	Advanced lymphomas; metastatic kidney carcinoma; hepatocellular carcinoma	12	Autologous CIK	CR:3; PR:1; SD:1
2010 (46)	Hepatocellular carcinoma	146	TACE combination with autologous CIK vs. TACE	6-month, 1-year, and 2-year PFS rates: 72.2%, 40.4%, 25.3% vs. 34.8%, 7.7%, 2.6%; 6-month, 1-year, and 2-year OS rates: 90.3%, 71.9%, 62.4% vs. 74.6%, 42.8%, 18.8%
2010 (47)	B-cell malignant lymphoma	9	Autologous CIK + IL-2	CR:8; PR:1
2011 (48)	Advanced solid malignancies	40	CIK combined with second-line chemotherapy vs. second-line chemotherapy	ORR: 30% vs. 15%; DCR: 80% vs. 70%; PFS and OS were significantly longer
2012 (49)	Large B-cell lymphoma	9	Autologous CIK	CR: 9
2012 (36)	Metastatic renal carcinoma	148	Autologous CIK vs. IL-2 combination with IFN- α -2a	3-year PFS and OS: 18% and 61%; vs. 12% and 23%; mPFS and OS were significantly longer
2012 (50)	Metastatic nasopharyngeal carcinoma	60	GC + CIK vs. CIK	CR: 3 vs. 0; PR: 18 vs. 14; SD: 2 vs. 3; PD: 7 vs. 13; ORR: 70% vs. 46.7%
2012 (51)	Lung cancer	87	Chemotherapy combination with autologous CIK vs. chemotherapy alone	PFS and OS were significantly longer
2012 (52)	Locally advanced gastric cancer	151	Autologous CIK vs. no	PFS and OS were significantly longer
2012 (53)	Hematological malignancies	20	Autologous CIK	CR: 11; PR: 7; SD: 2

Table 1 (continued)

Table 1 (continued)

Year	Cancer type	# of patients	Therapy	Clinical response
2014 (54)	Hepatocellular carcinoma	132	Autologous CIK plus standard treatment vs. standard treatment only	1-year OS: 74.2% vs. 50.0%; 2-year OS: 53.0% vs. 30.3%; 1-year OS: 42.4% vs. 24.2%
2013 (55)	Renal cell carcinoma	20	Autologous CIK vs. no	PFS: 32.2 vs. 21.6 mos
2014 (56)	Pancreatic cancer	20	Autologous CIK	Disease control rate: 25%; PFS: 11 weeks; OS: 26.6 weeks
2014 (57)	Colorectal cancer	60	Chemotherapy combination with autologous CIK vs. chemotherapy alone	mPFS: 25.8 vs. 12.0 mos; mOS: 41.3 vs. 30.8 mos
2014 (58)	Triple-negative breast cancer	90	Chemotherapy with/without radiotherapy and sequential CIK infusion vs. chemotherapy alone or with sequential radiotherapy	1-, 2-, 3-, and 4-year DFS: 97.7%, 90.1%, 83.4%, and 75.2% vs. 88.9%, 64.4%, 62.1%, and 56.4%; 1-, 2-, 3-, and 4-year OS: 100.0%, 100.0%, 96.7%, and 92.4% vs. 95.6%, 88.6%, 76.3%, and 72.7%
2015 (59)	Lung cancer	120	Chemotherapy with autologous CIK vs. chemotherapy alone	3-, 5-year PFS: 74% and 62% vs. 44.7% and 26.8%; mPFS and mOS: 24 and 72 mos vs. 14 and 44 mos
2015 (60)	Hepatocellular carcinoma	1,031	Hepatectomy with CIK vs. hepatectomy alone	mPFS and mOS: 16 and 41 mos vs. 12 and 28 mos
2015 (61)	Metastatic nasopharyngeal carcinoma	222	GC + CIK vs. GC (gemcitabine + cisplatin)	1-, 2-, and 3-year PFS: 76.0%, 32.1% and 23.8% vs. 70.0%, 24.5% and 17.0%; 1-, 2-, and 3-year OS: 90.2%, 65.2% and 25.9% vs. 85.5%, 47.3% and 19.1%
2015 (62)	Hepatocellular carcinoma	230	CIK vs. no	Median time of recurrence-free survival: 44.0 vs. 30.0 mos

CIK, cytokine-induced killer; IL-2, interleukin-2.

of remission, after HCT as consolidation, or replace of HCT. A number of studies have shown encouraging results of NK adoptive infusion in patients with acute myeloid leukemia (AML) (74-77). At the same time, NK cells have also been assessed in many non-hematopoietic forms of cancer. Clinical trials showed transfusion of autologous NK cells was safe with no negative side effects on patients with metastatic colorectal cancer, non-small cell lung cancer, metastatic melanoma or renal cell carcinoma, although there was no significant clinical response observed (78). Thus, due to the limited clinical response associated with autologous NK cell therapy, adoptive transfusion of allogeneic NK cells was explored as an alternative.

Furthermore, allogeneic NK cells are thought to be of non-cross-resistant mechanisms and minimal overlapping toxicities for cancer therapy. Safety and efficacy of allogeneic NK cell transfusions were established in patients

with metastatic melanoma, renal cell carcinoma, refractory Hodgkin's disease, and refractory AML (74,79-81). In a study of allogeneic NK cell therapy of AML patients, complete remissions were observed in 26% of patients and nearly all patients showed an expansion in NK cells after IL-2 therapy (74). In a NKAML pilot study (82), children with high risk of AML who achieved first complete remission after conventional chemotherapy received infusion of haploidentical NK cells. Non-hematologic toxicity was limited with no graft-versus-host disease (GVHD). The 2-year event-free survival was 100%. In another study, 5 of 19 adults with advanced AML achieved a complete hematological response.

NK-92 cells, a pure allogeneic activated NK cell line (83), have been successfully used as effector cells for cancer therapy. Arai's group (83) first used NK-92 cells in patients with advanced renal cell carcinoma or melanoma and

showed the safety and efficacy of a large-scale NK-92 expansion and 2 patients' experienced transient minor decreases in tumor size. Although NK-92 therapy was reported to be safe, limited data were accumulated to date on the efficacy of this approach. In summary, NK cells could be promising cancer therapy approach, especially allogeneic NK cell infusion, which may become a new area of novel cell-based immunotherapy against human cancer.

Specific immunotherapy

Compared to non-specific cell therapy, specific immunotherapy is to specifically activate or modulate lymphocytes for target particular genes that are activated tumor cells (12); therefore, to destroy these tumor cells.

TIL-mediated specific immunotherapy

More than 100 years ago, it was noted that malignant tumors contain variable numbers of lymphocytes (84), which have come to be known as tumor infiltrating lymphocytes (TILs), which represent the local immune response directed against tumor growth and metastasis. TILs are composed of a mixture of lymphocytes with multiple phenotypic and functional properties, such as CD4⁺ and CD8⁺ lymphocytes. Several previous studies demonstrated that CD8⁺ TILs are generally associated with tumor regression, whereas the role of CD4⁺ TILs in cancer is controversial. Generally, both CD4⁺ and CD8⁺ TILs are necessary for effective tumor elimination (85,86). TILs grade was an independent predictor of sentinel lymph node status and survival of patients with cutaneous melanoma (87). Recently, TILs were also recognized to be associated with pathologic response to neoadjuvant therapy and DFS and OS after adjuvant chemotherapy of triple-negative and human epidermal growth factor receptor 2 (HER2)-positive breast cancers (88).

Rosenberg's group first described the expansion of human TILs as immunotherapy in 1987 (89). TILs were successfully expanded from 24 of 25 consecutive human tumors, including 6 melanomas, 10 sarcomas, and 8 adenocarcinomas and used for immunotherapy of human cancers. In 1988, they further showed the adoptive transfusion of autologous TILs in treatment of patients with metastatic melanoma (90) and objective regression of tumor occurred in 9 of 15 patients (60%) who had not previously been treated with IL-2 and in 2 of 5 patients (40%) in whom previous failed with IL-2 therapy. In 1994 (91),

this group increased number of the patients to 86, and showed that treatment with TILs and IL-2 with or without cyclophosphamide could result in objective responses in approximately one third of patients with metastatic melanoma. These data illustrated the potential value of lymphocytes in treatment of melanoma.

In the past few years, an increasing number of TILs infusion in combination with high-dose IL-2 and non-myeloablative (NMA) lymphodepletion chemotherapy has been reported and metastatic melanoma patients after such therapy had clinical responses up to 50% (92-94). For instance, a clinical trial (94) of 93 refractory melanoma with NMA and with or without 2 to 12 Gy of total-body irradiation (TBI) showed 48%, 52%, and 72% of the overall response rates (ORR) and 13%, 20%, and 40% of the CR, respectively. Data from Besser *et al.* (95) showed 29% and 9.8 months of the ORR and median survival in control patients *vs.* 40% and 15.2 months, respectively, in stage IV melanoma patients after treated with TILs and a high-dose of IL-2 following NMA. Five patients achieved CR and 18 PR and the 3-year survival of responding patients was 78%. Thus, ACT using autologous TILs is considered to be the most effective approach to induce ORR in metastatic melanoma patients.

However, TIL therapy may not be effective on other cancer types and the major limitation is the difficulty to identify antigen-specific T cells in those cancers, although it is now being increase in developing modifications for treatment of other solid tumors, such as cervical, pancreatic, lung, and head and neck cancers (<http://www.clinicaltrials.gov/>). For example, in a phase I trial of patients with locoregionally advanced nasopharyngeal carcinoma using adoptively transferred TILs following concurrent chemoradiotherapy (96), 19 of 20 patients exhibited an objective antitumor response, while 18 patients displayed DFS longer than 12 months after TILs infusion. There were only mild adverse events (AEs) observed and 1 patient had Grade 3 neutropenia (1/23, 5%).

Although TIL therapy of different human cancers developed slowly, a continuing progress has been made during the past several decades. The main advantage of TILs is the ability to specifically recognize tumor antigens, which is unfortunately also the disadvantage since most solid tumors don't display such tumor antigens and those naturally occurring TILs fail to eliminate malignant cells. Thus, the main objectives of TIL-ACT are enhancing the immunogenicity and enlarging the numbers of activated tumor-specific T cells in tumor lesions.

Genetically engineered T cells-mediating specific immunotherapy

As described in the above, TILs have been shown to induce a durable tumor regression in melanoma patients. However, TILs therapy may not be effective in other types of cancers. Moreover, TILs therapy requires a surgical resection of tumor lesions from each patient to isolate and generate T cells with antitumor activity. Advances in genetically engineered T cells have overcome such obstacles by introducing tumor-antigen-targeting receptors into human peripheral blood T cells. During the past two decades, genetically engineered T cells expressed highly active T-cell receptors (TCRs) or CARs have translated from a laboratory technology to clinical evaluation.

T cell immunotherapy with modified TCR genes

TCR is a molecule found on the surface of T lymphocytes and be responsible for recognizing antigens bound to MHC, which contains two different protein chains α and β . These TCR α and β chains can be isolated from T cells of the rare patients who responded to tumors (97-99). Using the expression vectors, retrovirus, or lentivirus, we can genetically engineer TCRs into T cells (100,101) and then introduce these genetically modified T cells back to cancer patients. Thus, this novel strategy can produce a large amount of antigen-specific T cells and target tumor cells that express the target tumor-associated antigens (TAAs) presented by MHC molecules and release Th1 cytokines, including IFN- γ , GM-CSF, and TNF- α (102); therefore, to eliminate tumor lesions in patients.

The first specific gene target-transferred TCR-clinical trial was reported in 1999 that utilized a melanoma-antigen specific TCR MART-1 to introduce genetically modified T cell immunotherapy of patients with metastatic melanoma (103). They first prepared autologous lymphocytes from peripheral blood of patients and then successfully encoded a TCR through the retrovirus carrying genetically modified TCR chains. The data showed that 2 out 15 (13%) patients had responded after infusion of autologous TCRs, which was low than predicted 50% of such an approach; however, this method has potential for cancer patients for whom TILs are not available. Afterwards, the same team (104) showed the data on a subsequent clinical trial using newly established MART-1 and gp100-specific TCR genes to modified T cells for treatment of patients with metastatic melanoma. Objective cancer regression rates were 30% (6 of 20) and 19% (3

of 16) in patients who received the MART-1 and gp100-modified TCR, respectively. Another promising TAA was carcinoembryonic antigen (CEA), which is frequently overexpressed in many human cancers, most notably in colorectal adenocarcinoma. One study reported (104) that infusion of T cells after modified to target CEA cDNA resulted in decrease in serum CEA levels by 74–99% in patients and 1 patient had an objective regression of cancer metastatic to the lung and liver after T cells infusion. Another clinical trial (105) enrolled patients with positive NY-ESO-1, which is expressed in 80% of patients with synovial cell sarcoma and in approximately 25% of patients with melanoma and common epithelial tumors, for treatment with autologous TCR-transduced T cells plus 720,000 IU/kg of IL-2. Objective clinical responses were observed in 4 of 6 patients with synovial cell sarcoma and 5 of 11 melanoma patients. Two of 11 melanoma patients demonstrated complete tumor regression for more than one year. Moreover, a synovial cell sarcoma patient showed a PR lasting 18 months. In 2013, Morgan *et al.* (106) reported another study of 9 patients using autologous anti-MAGE-A3 TCR-engineered T cells and 5 patients experienced clinical regression of their cancers including 2 on-going responders. Furthermore, other cancer antigens, such as LAGE-1, MAGE-A4, and SSX-2, have also been investigated as tumor target antigens for genetically modified T cell immunotherapy. Some of their anti-tumor activities against different tumor cell lines and tumor models have also shown promising (107,108), although there is no report thus far in clinical trials.

However, although clinical response rate of infusion of genetically modified T cells is promising and the data supported more clinical usage of this approach, there have also been a number of reported toxicities related to “on target” toxicity to normal tissues but “off tissue” autoimmune toxicities effects on patients. The common side effects included exhibition of destruction in the skin, eye, and earod patients after infusion MART-1 and gp100-transduced T cells, while patients may also develop a severe transient inflammatory colitis after infusion of CEA-targeted T cells (104) and 3 of 9 patients had severe neurological toxicity in MAGE-A3 clinical trial (106). Thus, further investigation is needed to reduce side effects of genetically modified T cell immunotherapy but maximally to maintain their antitumor activities in clinic.

T cell therapy with modified CAR genes

T cell targeting specificity can be altered after expressing a

single-chain CAR (109). The latter is composed of a specific antigen-binding motif derived from a monoclonal antibody that links V_H with V_L sequences to recognize a single chain fragment variable (scFv) region and signaling components derived from the ζ chain of the TCR/CD3 complex on co-stimulatory molecules T lymphocytes (110,111). To date, CAR research reached to four generations (112), i.e., the first-generation CAR contains a single signaling domain most commonly derived from the CD3 ζ chain of the CD3/TCR complex. The second-generation CAR incorporates an additional intracellular co-stimulatory endodomains (such as CD28, OX40, or 4-1BB) to the basic first-generation receptor configuration in order to improve T-cell effector function. The third generation CAR includes a combination of CD28, 4-1BB, and CD3 ζ signaling moieties. The fourth generation CAR or TRUCKS employ a vector or vectors to encode a CAR and also a CAR-responsive promoter (e.g., nuclear factor of activated T cells) to respond upon successful signaling of the CAR after the transgenic production of cytokines, such as IL-12. In preclinical models, T cells engrafted with the second and third generation CARs possessed greater effector functions and had potent non-cross-resistant clinical activity after infusion in three of three patients treated with advanced chronic lymphocytic leukemia (CLL) (112). Moreover, clinical protocols for CAR-T cells immunotherapy usually involve previously conditioned NMA and high dose of IL-2 therapy after CAR-T infusions, which facilitated the engraftment and persistence of CAR-T cells in tumor lesions. To date, previous studies demonstrated some successful pre-clinical models and phase I clinical trials in ovarian cancer (111), renal cell carcinoma (113,114), neuroblastoma (115,116), B-cell non-Hodgkin lymphoma (NHL), and mantle cell lymphoma (MCL) (117) with the first-generation CARs. The early phase clinical trials indicated this approach was feasible, but the ORR was mild and most patients did not have visibly or significantly clinical benefit. However, this CAR-modified T cell immunotherapy did show great response rate in NHL and MCL patients using genetically modified CD20 autologous T cell electroporation. Of the 7 treated patients, 2 had a CR, 1 achieved a PR, and 4 had SD (117).

Thus far, both second- and the third-generation CARs are in progress in the clinical trials and the third generation CAR-T cells showed to be more effective, although there is no study reporting the comparison to the effectiveness of the second generation CARs. Most clinical trials focused on CD19 or CD20 antigen in hematologic malignancies, such as NHL and lymphocytic leukemia (*Table 2*).

Indeed, the CAR-T cells have been successful in treating hematological malignancies, but their application for solid tumors has been greatly hampered. Clinical studies with CAR-T cells targeting HER2, CEA, VEGF-R2, EGFR, or GD2 in solid tumors (111,115,116,131-133) have demonstrated the feasibility, but the clinical effectiveness was generally disappointed.

In addition, different clinical trials indicated that CAR-T cell-based therapy was associated with noticeable side effects. To date, there were 2 death cases reported (CAR T cells targeted ERBB2 in 1 patient with widely metastatic colon cancer and another 1 with bulky chronic lymphocytic leukemia by targeting CD19). Significant toxicity such as hepatic toxicity (113), fatal pulmonary dysfunction (113), systemic inflammatory response syndrome (SIRS) or even “cytokine storm” (109,124) has been also reported in patients. These obvious toxicities were usually associated with the lack of discrimination between tumor and normal cells by CAR-T cells. However, the technology will continue to improve, the side effects will be overcome, and future directions will likely include combination therapies.

DC-based immunotherapy

DCs are antigen-presenting cells in the mammalian immune system and display an extraordinary capacity to stimulate antigen-specific cytolytic and memory T-cell responses to antigens by processing antigen material and presenting it on the cell surface to T-lymphocytes (134). Immature DCs are particularly efficient in uptake of tumor derived material, while mature DCs can activate tumor-reactive CD8⁺ CTLs and CD4⁺ T cells (135,136). Moreover, DCs also can induce NK cell cytotoxicity and the latter essentially contribute to eliminating tumor cells (137-139). DCs can also directly mediate tumor-directed cytotoxicity (140,141). Due to the numerous antitumor effects, DCs evolved as promising candidates in cancer immunotherapy (142). For example, DCs can be used for antitumor vaccination through various means, including tumor lysates, tumor antigen-derived peptides, synthetic MHC class I—restricted peptides, and whole protein. The first DC vaccine was used in 4 B-cell lymphoma patients using autologous antigen-pulsed DCs and the data were published in 1996 (143). In this pilot study, all patients developed measurable antitumor cellular immune responses. Since then, several DC vaccine clinical trials in patients with prostate cancer (144,145), melanoma (146,147), renal cell carcinoma (148,149), glioma (150), hepatocellular carcinoma (151-153),

Table 2 Published clinical studies of CAR-T cell immunotherapy

Year	CARs	Cancer type	# of patients	Therapy	Clinical response
2010 (118)	CD19	FL	2	FLU (post T-cell infusion) and IL-2	2 NR
2010 (119)	CD19	FL	1	Lymphodepletion (CTX/FLU) and IL-2	1 PR
2011 (120)	CD19	DLBCL, transformed FL	6	None	2 SD, 4 NR
2011 (120)	CD19	CLL	3	Lymphodepletion (BEN or CTX/PTS)	2 CR, 1PR
2011 (121)	CD19	CLL, ALL	9	None or lymphodepletion (CTX)	1 PR, 2 SD, 1 cCR, 4 NR, 1 death
2012 (122)	CD19	FL, CLL, SMZL	8	Lymphodepletion (CTX/FLU) and IL-2	1 CR, 5 PR, 1 SD, 1NE
2013 (123)	CD19	ALL	5	Lymphodepletion (CTX)	4 CR, 1cCR
2013 (124)	CD19	ALL	2	None or etoposide/CTX	2 CR
2013 (125)	CD19	ALL, CLL, transformed CLL	8	Allo-HSCT preparative regimen; none immediately before T cell infusion	1 CR, 1 PR, 1 SD, 2 Ccr, 3NR
2013 (126)	CD19	CLL, DLBCL, MCL	10	Allo-HSCT preparative regimen, DLI; none	1 CR, 1 PR, 6 SD, 2 NR
2014 (127)	CD19	ALL	27	None or FLU/CTX/Etoposide	27 CR
2014 (128)	CD19	ALL	16	Leukapheresis	9 CR
2015 (13)	CD19	ALL	20	FLU/CTX (post T-cell infusion)	14 CR, 3 SD, 4PD
2008 (117)	CD20	Relapsed indolent NHL and MCL	7	Autologous CD20-specific T cells and IL-2	2 CR , 1 PR, 4 SD
2010 (117)	CD20	DLCL	2	CD8+ CTL expressing a CD20-specific CAR following autologous HSCT	–
2012 (129)	CD20	Relapsed indolent NHL and MCL	3	Lymphodepletion (CTX/FLU) and IL-2	2 CR, 1PR,
2014 (130)	CD20	DLBCL	7	–	1 CR

CAR, chimeric antigen receptors.

and pediatric solid tumor (154,155) have been reported. Although some of these trials did not reach the end point of primary study, others have reported positive results. In one notable trial, Provenge, monocyte-derived dendritic cells (moDCs) pulsed with fusion antigen protein consisting of prostatic acid phosphatase (PAP) and GM-CSF, the first therapeutic cancer vaccine to be approved by the U.S. Food and Drug Administration in 2010, showed to prolong median OS by 4.1 months for metastatic castration resistant prostate cancer (4). Tecemotide vaccine, DCs pulsed with MUC1 for inoperable stage III NSCLC as a maintenance therapy following either concurrent or sequential chemoradiotherapy (156) showed 25.6 months of median OS in tecemotide versus 22.3 months in placebo, but the OS had no significant difference in administration of tecemotide after chemoradiotherapy compared to placebo.

Tecemotide might have a role in patients who initially receive concurrent chemoradiotherapy. NY-ESO-1 protein to target DCs vaccine was assessed in 45 patients with advanced malignancies and 13 patients experienced stabilization of disease, with a median duration of 6.7 months and 2 patients had tumor regression. There was no dose-limiting or grade 3 toxicity observed (157).

Since 2001, numerous DC vaccine clinical trials have been reported. Schadendorf *et al.* (158) had demonstrated that DC vaccine could not be more effective than DTIC chemotherapy in stage IV melanoma patients, but the follow-up data confirmed that melanoma patients did get clinical benefits after DC vaccines. Other two phase II clinical studies showed a clinical benefit (PR + SD) in 55.5% of evaluable cases to date (159,160). Furthermore, beyond large sample size clinical trials with autogeneic DC vaccines,

numerous early phase clinical studies using autogeneic/allogeneic DCs with allogeneic tumor cells continue to be progress (161-164). It has been suggested that allogeneic DCs are more effective in both *in vitro* and *in vivo*. For example, in a phase I/II trial of metastatic melanoma patients undergoing DCs loaded with an allogeneic tumor cell lysate (165), 4 out of 9 patients survived for more than 20 months, 2 patients showed signs of clinical response, all of whom didn't show any grade 3 or 4 adverse events related to the vaccines. In a sequential clinical trial reported allogeneic tumor cell vaccine with TGF- β in IV NSCLC patients, OS was 562 days and median survival was 660 days. Patients didn't have significant toxic effect (166,167). However, the majority of such studies still remain in the pre-clinical models or Phase I/II clinical trials.

Genetic modification is another way to improve the effective of DC vaccines, which includes overexpression of positive regulators (e.g., cytokine, chemokine and co-stimulatory molecules) and inhibition of negative regulators [e.g., suppressor of cytokine signaling-1 (SOCS1), programmed death ligand 1 (PD-L1), or A20]. For example, GVAX, a GM-CSF gene-transfected tumor cell vaccine (168) can extant the time to progression and the median OS and improve quality of life of patients with metastatic melanoma, pancreatic cancer, prostate cancer, or other tumors (169-172). DC vaccine would clearly enhance host antitumor immune responses. Nevertheless, numerous phase I/II studies had an overall limited clinical benefit. Therefore, further improvement is required, which may be achieved by understanding of DC biology and combination of DC-based vaccination with traditional therapy.

Summary and future directions

In this review, we summarized up to date advancement in ACT, which indeed improves PFS, OS, DFS, and/or quality of life of cancer patients, especially melanoma patients. Compared to other standard therapies, ACT can trigger immune response within cancer lesion to elicit persistent antitumor immune response for eliminating tumor cells and such treatment has nearly no serious side effect, although improvement of the clinical efficacy is warranted in future studies. Most recently, antitumor immunotherapy is very hot and different investigators have evaluated artificial antigen presenting cells, enhanced immunogenicity or generate genetically engineered T cells in preclinical models and in clinical trials. Moreover, combination of immune therapy together with surgery,

chemotherapy and radiotherapy could be an effective way in control cancer progression.

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Footnote

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