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Clinical application of adoptive T cell therapy in solid tumors

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Study Design A
Data Collection B
Statistical Analysis C
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Literature Search F
Funds Collection G

AEF **Yi-Wen Zang***
AEFG **Xiao-Dong Gu***
EF **Jian-Bin Xiang**
EF **Zong-You Chen**

Department of General Surgery, Huashan Hospital, Fudan University, Shanghai, China

Corresponding Author:

Source of support:

* Xiao-Dong Gu and Yi-Wen Zang have equally contributed to this work. They are joint first authors

Zong-You Chen, e-mail: zongyouc@sohu.com

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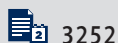
As an emerging therapeutic approach, adoptive T cell therapy shown promise in advanced solid malignancies. The results obtained in patients with metastatic melanoma and kidney cancer are encouraging because of the visible clinical benefits and limited adverse effects. Recently, the genetically-modified T cells expressing specific T cell receptors or chimeric antigen receptors are just now entering the clinical arena and show great potential for high avidity to tumor-associated antigens and long-lasting anti-tumor responses. However, continued investigations are necessary to improve the cell product quality so as to decrease adverse effects and clinical costs, and make adoptive T cell therapy a tool of choice for solid malignancies.

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Adoptive Transfer • Antigens, Neoplasm • Immunotherapy, Adoptive

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Background

Although progress has been made in improving the prognosis of advanced solid tumors, it still has relatively short-term benefits for most patients. Immunotherapy can result in long-term benefit, even after short-term treatment. Adoptive T cell therapy (ATCT) has emerged as a potent immunotherapeutic approach for the treatment of cancer in the past 2 decades. The whole process involves the identification and expansion *ex vivo* or *in vivo* of autologous T cells capable of tumor killing, which are then adoptively transferred back into the patients, often along with appropriate growth factors to stimulate their survival and expansion *in vivo*. This could be either non-specific such as lymphokine-activated killer cells and cytokine-induced killer cells, or specific recognition of tumor cells by cell culture or genetic modification techniques.

In this paper, we review the recent clinical research on treatment of advanced solid tumors with ATCT to provide a general update on this topic.

Non-Specific Immunotherapy

Lymphokine-activated killer cells

Lymphokine-activated killer (LAK) cells, so-called because the immune effector was induced by cytokines, were first described in the early 1980s. LAK cells are generated *in vitro* by the incubation of human peripheral blood leukocytes (PBLs) in interleukin-2 (IL-2), yielding populations with different sets of T cells and NK cells with cytolytic properties not specifically directed against tumor cells. The cytolytic activities of LAK cells are not restricted by the major histocompatibility complex (MHC) [1–3].

The first clinical trial of the systemic administration of autologous LAK cells was done by Rosenberg in 1985 [4]; 11 of the 25 patients with metastatic cancer had partial (regression of cancer with more than 50% of volume) or complete responses. Decades afterwards, it was demonstrated that LAK cells had efficacy against metastatic solid tumors such as melanoma, renal cell carcinoma, and other advanced solid tumors [5–10]. In 1993, a prospective randomized trial showed a trend toward improved survival in patients with melanoma receiving IL-2 plus LAK cells compared with IL-2 only, but no difference in survival was seen in patients with renal cell cancer (RCC) in the 2 treatment groups [11]. Subsequently, a phase-III randomized trial revealed that the dose and schedule of IL-2 resulted in a low level of antitumor activity against RCC, the addition of LAK did not improve the response rate, and more patients on the LAK arm experienced pulmonary toxicity, yet only 4 in 71 patients (6%) had major responses [9].

Postoperative adoptive immunotherapy of LAK cells could lower the frequency of recurrence of hepatocellular carcinoma, as demonstrated by a randomized trial [10]. The immunotherapy group had significantly longer recurrence-free survival (RFS) and disease-specific survival (DSS) than the control group. Adoptive immunotherapy reduced the risk of recurrence by 41%. Time to first recurrence in the immunotherapy group was significantly longer than in the control group (38% vs. 22% at 5 years). Intralesional injection of LAK cells and IL-2 also revealed a potential role in treating metastatic hepatocellular carcinoma and recurrent glioblastoma, with low incidence of severe adverse effects [8,12–14]. As summarized by Vauleon [15], 12 trials treating high-grade gliomas with LAK have been reported in the literature. There were 5 complete response (3 glioblastomas [GBM]), 13 partial responses (8 GBM), and 6 stable disease (6 GBM) in a total of 118 patients. Neurological toxicity such as brain edema and aseptic meningitis was observed in 6 of the 9 trials reporting this factor.

Cytokine-induced killer cells

To date, use of LAK cells as tumor immunotherapy is hampered by the limited expansion of LAK cells *in vitro*, low cytolytic activity *in vivo*, and relatively high toxicity, mainly due to the infusion of IL-2. Cytokine-induced killer (CIK) cells are an improvement of LAK cells by introduction of anti-CD3 antibody and IL-2 into the culture of PBLs. CIK cells possess non-MHC-restricted cytolytic activity which is increased by over 70-fold when compared with standard IL-2 stimulated LAK cell activity [16]. The lytic activity can be further increased by additionally adding other cytokines such as IL-1, IFN- γ , IL-7, IL-15, and CH-296 [16–20].

Several randomized studies have revealed that the combination of CIK cell therapy and standard therapy was superior to standard therapy alone in patients with solid tumors, including renal, hepatocellular and nasopharyngeal carcinoma, and the graft-versus-host effects were limited [20–23]. Sixty patients with metastatic nasopharyngeal carcinoma after radiotherapy were randomized and assigned to 2 groups to evaluate the efficacy of autologous CIK cells transfusion used in combination with gemcitabine and cisplatin (GC) chemotherapy [23]. The total effective rate of GC+CIK group was 70% (21/30), much higher than that of the GC group (46.7%, 14/30). Similarly, CIK cell therapy could remarkably decrease the recurrence rate of patients with hepatocellular carcinoma [21] and prolong the PFS [22]. Recently, 148 patients with metastatic clear cell renal cell carcinoma (RCC) were randomly divided into 2 groups: autologous CIK cell immunotherapy and IL-2 treatment combination with IFN- α -2a. The 3-year PFS and OS in the CIK group were 18% and 61%, significantly higher than in the IL-2/IFN- α -2a group (12% and 23%). Similar results could be seen in several retrospective studies investigating the therapeutic

effect of CIK cells in patients with non-small-cell lung cancer (NSCLC) [24] and advanced gastric cancer [25,26]. Furthermore, CIK cells can also enhance the killing activity of L-OHP on oxaliplatin-resistant gastric cancer cells *in vitro* and *in vivo* [27,28].

The international registry on CIK cells (IRCC) has been established to collect the clinical data and set standards on reports of clinical trials using CIK cells [29], which included 11 clinical trials. Of the 384 patients with reported clinical response, the total response rate (RR) was 91/384 reported patients, of which 24 patients showed a complete response, 27 patients showed a partial response, 40 patients showed a minor response. DFS was significantly higher in patients treated with CIK cells than in a control group without CIK treatment. CIK cells treatment had minor adverse effects; however, there is no reliable biomarker so far for estimating the clinical response of CIK therapy. It has been shown that CD4/CD8 ratio and percentage of NK cells are significantly increased in patients receiving CIK transfusion [21,25], but the exact relationship of these markers with clinical outcome is still unknown.

To date, the variations in methods and clinical evaluation among the studies hamper definite conclusions about the clinical efficacy of CIK cell therapy. More studies are needed to elucidate the best treatment schedule for CIK cell therapy.

Specific Immunotherapy

Various approaches have been used to obtain tumor-specific T cells to increase the efficacy of anti-cancer cell therapy protocols. One main approach is tumor-infiltrating lymphocytes (TILs) grown from metastatic tumor nodules, repeated in *in vitro* stimulation with tumor-associated antigens (TAAs). Another approach is genetic modification of T cells to express a T cell receptor (TCR) or a chimeric antigen receptor (CAR) to a known TAA.

Autologous expanded tumor-infiltrating lymphocytes

The adoptive transfusion of autologous TIL, first described in 1988 by Rosenberg [30], has been considered the best available treatment for patients with metastatic melanoma. However, the decisive improvement in efficacy came after 2000 with the introduction of an immuno-depleting preparative regimen given before the TIL infusion [31,32], resulting in clonal repopulation of circulating lymphocytes with anti-tumor activity.

The adoptive therapy of TIL can mediate the dramatic regression of metastatic cancer in patients with melanoma, with over 50% clinical responses, many of which are lasting for years [33]. ATCT of TIL can mediate complete and durable regression of melanoma brain metastases [34], indicating that

TIL can cross the blood-brain barrier and might be a new approach for brain tumors.

The great progression of TIL therapy in metastatic melanoma suggests that this approach might be used for other malignancies. Increasing lymphocytic infiltration in the solid tumors always infers a good prognosis; however, the difficulty in identifying antigen-specific T cells in other cancers is a major barrier to widespread use of TIL therapy. Currently, not all patients with advanced melanoma can be candidates for surgical excision of a tumor metastasis necessary to generate TIL. Techniques have been developed to modify peripheral lymphocytes recognizing tumor antigens.

Genetically-modified autologous peripheral blood lymphocytes

T cell therapy with modified T cell receptor genes

T cell receptor α and β chain genes can be identified and isolated from the T cells of the rare patients who respond to tumors [35]. By means of viral or non-viral technologies, large numbers of antigen-specific T cells can be rapidly generated [36,37]. The modified T cells can proliferate and respond to tumor cells expressing the target TAA presented by MHC molecules.

The first clinical trial using TCR-T cells targeting MART-1 (melanoma antigen recognized by T cells, DMF4 clone) for the treatment of metastatic melanoma was performed by Rosenberg's group [38]. The TCR genes were isolated from a patient who had received TIL therapy with excellent clinical response. The infused MART-1 TCR T cells sustained for over 1 year, and 2 of 17 patients demonstrated a sustained objective regression of metastatic lesions. Later on, T cells with TCR recognizing MART-1 (DMF5 clone) or gp100 were generated and transfused into patients with advanced melanoma and showed a relatively enhanced avidity [39]. Six of 20 patients (30%) treated with DMF5 TCR and 3 of 16 (19%) treated with gp100 TCR experienced an objective antitumor response. Tumors regressed in multiple organs, including the brain, lung, liver, lymph nodes, and subcutaneous sites. However, 29 of 36 patients had a widespread erythematous skin rash, 15 patients developed anterior uveitis, and 15 patients developed hearing loss, caused by recognition of low-level expression of MART-1 and gp100 antigens on normal melanocytes, retina, and inner ear (also called the "on-target, off-organ" effects).

Carcinoembryonic antigen (CEA) is another promising TAA as a target of immunotherapies, which over-express in many epithelial cancers, most notably in colorectal adenocarcinoma. In a phase I trial produced by Parkhurst's group [40], 1 in 3 patients with metastatic colorectal cancer had an objective regression of cancer metastatic to the lung and liver after the

adoptive therapy using CEA TCR-T cells. However, all 3 patients suffered a severe transient inflammatory colitis as a result of the “on-target” effect, which emphasized the destructive power of small numbers of highly avid T cells and the limitations of using CEA as a target for cancer immunotherapy.

NY-ESO-1 is a member of the cancer-testis antigens family, expressed by a wide range of epithelial malignancies, but is restricted in its expression in normal adult tissues to cells in the testis that lack expression of MHC-I molecules. As a result, NY-ESO-1 is not susceptible to damage by T cells that recognize the gene products, with less possibility to induce “on-target” cytotoxicity. Transfer of NY-ESO-1 TCR engineered T cells mediated objective cancer regressions in 5 of 11 patients with melanoma and 4 of 6 patients with synovial cell sarcoma bearing tumors express NY-ESO-1 [41]. No “on-target” toxicities were seen in this trial. All patients experienced a transient neutropenia and thrombocytopenia induced by the preparative regimen and the transient toxicities associated with IL-2. Similar results were reported in another phase I trial [42], but 3 of all 9 patients suffered severe neurological toxicity. Other cancer-testis antigens such as LAGE-1, MAGE-A4, and SSX-2 have also been investigated for their potential role as target tumor antigens for T cells in active immunotherapies. Some anti-tumor activities towards several tumor cell lines and rodent tumor models have been reported [43–45]. Clinical trials in humans have not yet been published.

Despite the promising therapeutic results of TCR-engineered T cells, the number of TAAs identified is relatively limited. During the past decade, a novel TAA named receptor-binding cancer antigen expressed on SiSo cells (RCAS1) has gained attention for its ability to induce cell-cycle arrest and/or apoptosis in RCAS1 receptor-bearing cancer cells [46–48]. Tissue RCAS1 expression was associated with important clinicopathological parameters for patient prognosis and tumor immune escape, which might become a potential target for immunotherapies. Another concern is that some TAAs are expressed on tumor cells and on activated T cells. Several studies demonstrated that survivin, an apoptosis inhibitor protein, belongs to this type of TAA. The expression of survivin and the subsequent presentation of the survivin TCR on the cell surface of activated T cells led to their recognition and fratricide killing by survivin-specific TCR-modified T cells in an MHC-restricted fashion [49], which indicates the limitation of survivin-specific TCR-T cells.

T cell therapy with modified CAR genes

One of the important mechanisms of immune evasion for malignant tumors is that tumor cells can frequently lose antigen expression through down-regulation of MHC expression and failure to process antigens to the cell surface. This process led to the restricted use of TCR-T cell therapies because T cells

specifically recognize a single TAA presented by MHC molecules only. In contrast, most CARs use an antibody-derived antigen-binding motif to recognize antigen or bind to the cognate ligand or receptor counterpart, and recognize native cell surface antigens in a MHC-independent manner [50]. Targeting of tumor antigens by CAR-modified T (CAR-T) cells is applicable to any cell surface antigen, including proteins, glycolipids, carbohydrates, or even intracellular antigen for which a monoclonal antibody (MoAb) can be generated. This allows CAR-T cells to affect a wider range of targets compared to TCR.

CARs provide both antigen-binding and T cell-activating functions. Based on the costimulatory activity, current CARs are classified into 3 generations. The **first-generation** CARs contain the single-chain variable fragments (scFv) fused to an intracellular signaling domain of CD3 ζ or Fc receptor γ chain without the intracellular signaling domains of costimulatory molecules.

Although the anti-tumor effect of CAR-T cells has been demonstrated in external and animal experiments, clinical trials have just started. Several centers have conducted phase I clinical trials testing the anti-tumor efficacy of first-generation CARs targeting folate receptor (FR) in ovarian cancer [51], carbonic anhydrase IX (CAIX) in renal cell carcinoma [52,53], L1-cell adhesion molecule (L1-CAM; CD171) [54], CD20 in indolent non-Hodgkin lymphoma [55], and diasialoganglioside GD2 [56,57] in neuroblastoma. The objective clinical responses in these phase I trials were weak since most patients did not receive a significant clinical benefit. Moreover, the transfused CAR-modified T cells showed limited peripheral persistence upon repeated antigen exposure.

One method to improve this weakness of first-generation CAR by providing costimulation to CAR-transduced T cells was tested by Brenner’s group [56]. They engineered Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes (CTLs) to express a chimeric antigen receptor directed to the diasialoganglioside GD2. Persistence of infused CAR-EBV-specific T cells was indeed improved compared to standard bulk-engineered T cells infused concurrently in all subjects. Four of the 8 patients with evaluable disease experienced tumor necrosis or regressions.

Receptors encompassing both CD3 ζ and 1 costimulatory signaling domain, mostly CD28, are the prototypes for **second-generation** CARs. Savoldo [58] demonstrated the superior persistence, expansion, and trafficking to a site of disease for CARs with dual signal domains to the first generation CARs through a side-by-side comparisons. More recently, triple-fusion receptors, so-called **third-generation** receptors, consisting of CD3 ζ , CD28, and CD134 (OX40) or CD137 (4-1BB) signaling region, have been reported. Triple fusion receptors are active *in vivo*, but comparisons to second-generation CARs have not yet been reported. Some researchers believed that the functional activity

Table 1. Characters of different ATCT in solid tumors.

Type of ATCT	Characters	Advantages	Disadvantages	Comments
LAK cells	Non-specific. Non-MHC-restricted	Modest efficacy in melanoma, RCC, and glioma through systematic administration or intralesional injection	High toxicity. Limited expansion <i>in vitro</i> Low cytotoxicity	Almost abandoned
CIK cells	Non-specific. Non-MHC-restricted	Improved cytotoxicity VS LAKs. Low toxicity	The definite conclusion about the clinical efficacy of CIKs is unclear	Potential applicability in various solid tumors
TILs	Specific	Most effective treatment for metastatic melanoma	Candidature only accepted when the tumor is resectable Requirement of immunodepleting preparation	Limited application in other solid tumors
TCR-T cells	Specific MHC-restricted	High avidity to tumor cells Long-lasting responses	Limited identified TAAs “On-target, off-organ” effects	Potential applicability in various solid tumors
CAR-T cells	Specific Non-MHC-restricted	Wide range of potential target TAAs High avidity to tumor cells Long-lasting responses	“On-target, off-organ” effects	Potential applicability in various solid tumors

induced by CAR-T cell is dependent upon endogenous “natural” receptor interactions [59].

Current clinical trials using T cells modified with second generation CARs are mainly focused on CD19 antigen in hematogenous malignancies such as CD28- or CD137-containing CARs in non-Hodgkin lymphoma and lymphocytic leukemia [58,60–63]. Although all the studies were phase I trials, in which safety and feasibility were the biggest concerns, some candidates did show evidence of tumor regression to an extent. The therapeutic effect of the second-generation CARs in treating other solid tumors is still under investigation through *in vitro* studies and animal experiments [64].

Tc1 and Th1 cells with chimeric receptor fused to CD28 and CD3ζ specifically recognizing CEA showed strong antitumor activity and produced IFN-γ in response to CEA-expressing human lung cancer and colon cancer cells, and inhibited tumor growth RAG2-/- in mice [65,66]. Similarly, treatment of breast cancer-bearing mice with scFv-erbB2-CD28-CD3ζ-modified T cells resulted in significant inhibition of tumor growth and long-term, tumor-free survival in the hosts. More importantly, the surviving mice developed a host memory response to tumor cells, and this memory response could protect from rechallenge with parental breast cancer cells [67,68], indicating that the CAR-T cells transfusion will become a promising strategy for adoptive immunotherapy of human cancer.

Two reports of death following administration of CAR-T cells emphasize the dangers of this approach. The first report described a patient with widely metastatic colon cancer treated with a third-generation CAR (CD28/4-1BB/CD3ζ) – engineered T

cell therapy targeting ERBB2 [69], and the other trial described a patient with bulky chronic lymphocytic leukemia treated with a second-generation CAR (CD28/CD3ζ) that recognized CD19 [70]. The deadly toxicity may be due to the “on-target” effect – the low levels of ERBB2 on lung epithelia were recognized by transgenic T cells, and severe underlying infection followed by a cyclophosphamide-induced “cytokine storm” resulting in multiple organ failure.

In other trials, grade 2–4 liver toxicity was observed in CAIX-CAR-T cell therapies because of the specific attack of the modified T cells against the CAIX+ bile duct epithelial cells [52,53]. This toxicity can be prevented by blocking antigenic sites in off-tumor organs and allowing higher T cell doses [71]. FR, on the other hand, seems to be a promising candidate for the bio-target of CAR-T cells therapies in solid tumors. It is overexpressed in ovarian, lung, renal, and breast cancers, but restricted in normal conditions to the apical surfaces of polarized epithelia, where it may be inaccessible to circulation-redirectioned T cells [72].

Most scFvs are derived from mouse monoclonal antibodies, which may trigger a host immune response [51,53]. The immunogenicity of conventional CARs may lead to its restricted clinical use. CARs encompassing humanized scFvs or scFvs derived from human monoclonal antibodies will probably reduce this concern.

Summary and Future Direction

With similar active mechanism, LAK cells are nearly abandoned now because of high toxicity and low cytolytic effect, whereas

CIK cells seem have the therapeutic potential for solid tumors with good preclinical results. Specific immunotherapies, on the other hand, have a higher avidity and efficacy. To date, adoptive transfer of TIL is the most effective treatment for patients with metastatic melanoma. At the Surgery Branch, NCI, the efficacy of TIL therapy is currently being tested for patients with metastatic digestive tract adenocarcinomas. Genetically engineered T cells have a long-lasting effect towards a wide range of solid cancers. In mutant experiments, the host might develop a memory response to tumor and revive from the reexposure of the tumor cells. It is crucial to choose the target antigen. High-affinity TCRs or CARs have the potential to cross-recognize normal cells as well as tumor cells. NY-ESO-1 and folate receptor appear promising because low incidence of “on-target” adverse effects was been observed.

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