Engineered T Cell Therapy for Gynecologic Malignancies: Challenges and Opportunities

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Gynecologic malignancies, mainly including ovarian cancer, cervical cancer and endometrial cancer, are leading causes of death among women worldwide with high incidence and mortality rate. Recently, adoptive T cell therapy (ACT) using engineered T cells redirected by genes which encode for tumor-specific T cell receptors (TCRs) or chimeric antigen receptors (CARs) has demonstrated a delightful potency in B cell lymphoma treatment. Researches impelling ACT to be applied in treating solid tumors like gynecologic tumors are ongoing. This review summarizes the preclinical research and clinical application of engineered T cells therapy for gynecologic cancer in order to arouse new thoughts for remedies of this disease.

Keywords: gynecologic malignancies, engineered T cells, CAR-T, TCR-T, adoptive T cell therapy, immunotherapy

INTRODUCTION

Gynecologic malignancies are serious threats to women’s health worldwide. Although traditional procedures like surgery, radiotherapy and chemotherapy have effectively decreased mortality, researchers are seeking new ideas and strategies to reduce the recurrence and metastasis of tumors, alleviate adverse drug reactions, as well as further improve the life quality of patients.

Adoptive T cell therapy (ACT) is one of the most powerful weapons among a wide range of approaches focusing on our immune system. The basic principle of this treatment refers to reinflusing autologous lymphocytes which are expanded, screened and modified in vitro to patients for tumor regression mediated by T cells. Early preclinical research successfully proved that with a genetically transferred synthetic receptor targeting antigen CD19, which is a broad marker commonly expressed by B cell lymphoma cells, reinflused autologous T cells could eliminate...
established B cell tumors in mice (1). Based on multiple tried-and-true basic experiments, clinical trials later showed prominent advantages of this kind of engineered T cells named chimeric antigen receptor T cells (CAR-Ts) in patients with hematological malignancies (2−5). Promoted by these significant achievements, adoptive T cell therapy has proved to be the potential adjuvant therapy for tumor treatment.

The application of natural tumor-infiltrating lymphocytes (TILs) obtained from suspension or fragments of the resected tumor is the earliest achievement of ACT. In 24th May, 2019, a TIL product named LN-145 was granted as the breakthrough designation for cervical cancer (6), exhibiting remarkable objective response rate (ORR) and disease control rate (DCR) in treating cervical cancer (7). Although TILs have higher concentration of specific T cells comparing to peripheral T cells, the hostile tumor microenvironment attenuates the long-term survival of functional T cells, as TILs are sensitive to anergy, exhaustion and apoptosis. In addition, the gathering of TILs requires joint efforts of surgeons to obtain fresh tumor samples where effective lymphocytes could be extracted. Groundbreakingly, engineered T cells, including T cell receptor modified T cells (TCR-Ts) and CAR-Ts, currently have a promising advance in tumor immunotherapy since they could be genetically modified in structure to target specific tumor antigens or to express cytokines ameliorating immunosuppressive tumor microenvironment. Two CAR-T products have already been approved by the USA Food and Drug Administration (FDA) for refractory leukemia and lymphoma immunotherapy (8, 9).

In this review, we discuss the application of engineered T cells in gynecologic malignancies in preclinical and clinical trials, and explore further opportunities of implicating this therapy in clinical decision for gynecologic oncology. A brief timeline of milestones associated with this field is arranged (Figure 1). Pioneer clinical application of engineered T cells, critical clinical trials carried out for gynecologic cancers and commercial CAR-T agents and related synergist approved by the FDA are included (10–12).

**ENGINEERED T CELLS**

Based on the gene editing technology, engineered peripheral T cells with specific antigen binding receptors like TCRs or CARs could further facilitate ACT progress compared with TILs. These two therapies have different mechanisms and efficiency preference for treating distinct tumors. Currently, mainstream cell preparation methods include the following steps: (1) obtaining frozen apheresis white blood cell (WBC) product from patients; (2) the selection and enrichment of T cells by corresponding selection beads; (3) activation of T cells via addition of stimulating cytokines like interleukin (IL) 2 and...
T Cells (TCR-Ts) Therapy

However, the utility of TCR-Ts in treating potential of TCR-Ts dramatically outweighs CAR-Ts in treating to maintain their original regulatory mechanism, being more which can also be expressed by normal tissues to affect their surface antigens are often tumor associated antigens (TAAs) neoantigens was described (13), which exhibited advantages with lower price and risk of random insertional mutagenesis.

Compared with the antibody-binding-like principle of CAR-Ts, TCR-Ts can recognize target antigens more extensively since they not only identify cell membrane antigens but also intracellular tumor antigens presented by pMHC, inducing a more orderly and durable immunological synapse formation process. Particularly, the targeting of almost 90% solid tumors relies on tumor specific antigens (TSAs) inside tumor cells, while surface antigens are often tumor associated antigens (TAA) which can also be expressed by normal tissues to affect their function. Besides, TCR-Ts follow the natural signaling pathway to maintain their original regulatory mechanism, being more sensitive to low-copy antigens than CAR-Ts. Consequently, the potential of TCR-Ts dramatically outweighs CAR-Ts in treating solid tumors (14). However, the utility of TCR-Ts in treating solid tumors is progressing slowly. Currently, there is no market approval for any TCR-T products. Several clinical trials are still ongoing.

Chimeric Antigen Receptor T Cells (CAR-Ts) Therapy

The most obvious character of CAR-T cells in contrast to TCR-T cells is that CARs can directly bind antigens in an MHC-independent fashion, therefore they are potentially able to detect most of the surface-expressing targets in patients who have various HLA types. This is particularly important for immunotherapy because tumor cells losing MHC-associated antigens are probable to escape immune surveillance. A CAR is composed of an extracellular antigen-binding domain, most of which is an antibody-derived single-chain variable fragment (scFV), a transmembrane domain and an intracellular signaling domain of the TCR CD3ζ chain to activate T cells (15). The consisting improvements of CAR-T include the introduction of an additional co-stimulatory molecular CD28 or 4-1BB (CD137) intracellular domain (16), and inducers for transgenic cytokines like IL-12 and IL-15 (17) (Figure 2).

The landmark of CAR-T therapy is the commercial CD19 specific CAR-T approved by the FDA for relapsed or refractory acute lymphocytic leukemia (ALL). Two commercial agents, tisagenlecleucel (Kymriah, Novartis) (9) and axicabtagene ciloleucel (Yescarta, Kite Pharma) (8) were acknowledged in 2017. After this, brexucabtagene autoleucel ( Tecartus, Kite Pharma) (18), lisocabtagene maraleucel (Breyanzi, Bristol-Myers Squibb) (19) and idecabtagene vicleucel (Abecma, Bristol-Myers Squibb) (20) were approved successively by the FDA for marketing, further promoting the clinical implement of CAR-T therapy in hematological malignancies. Among these agents, only Abecma targets B cell maturation antigen (BCMA), others continue to focus on CD19.

STUDIES OF ENGINEERED T CELLS IN COMMON MALIGNANT GYNECOLOGIC TUMORS

Unlike the popularity of CAR-T therapy in hematological malignancies, studies for broader swaths in the field of gynecologic tumors are still in the bud. Antigen selection is crucial in deciding treatment programs which lead to TCR-T or CAR-T therapy and the treatment efficiency. Where the antigen is expressed at the cell and tissue level should be the first consideration by high-throughput, ultra-sensitive mass spectrometry and other means when ACT is carried out. Improvements could be reflected in the optimization of antigen selection for patients with different types of gynecological tumors in the future.

Ovarian Cancer

Ovarian cancer significantly jeopardizes the health of women with high lethality. With advanced surgical treatment and systematic care, the five-year relative survival rate of patients is slightly promoted, but still less than 50% (21). Armed with the knowledge that the melanoma-associated antigen 4 (MAGE-A4) and the New York esophageal squamous cell carcinoma 1 (NY-ESO-1) are commonly expressed by ovarian cancer cells (26.4% and 3.6% respectively) (22), CAR-T products targeting these two ideal antigens have been designed and applied in clinical research. MAGE-A4T cells are used in HLA-A*02:01 (A2+) patients with MAGE-A4 positive tumors including ovarian cancer in an ongoing phase I multi-tumor study (NCT03132922). In cohort 3/expansion (28 patients), 7 patients with synovial sarcoma had partial response (PR), 11 patients had stable disease (SD), 5 patients had progressive disease (PD) and the remaining 5 were non-evaluable. MAGE-A4 specific TCR-T exhibited therapeutic potential and manageable adverse effects at a dose range of (1.2~10) x10^6 (23). In further research, a CD8α co-receptor was introduced into CD4+ T cells alongside the engineered TCR (ADP-A2M4CD8). These modified CD4+ T cells could in turn
elevate the cytotoxicity and expansion of effector CD8+ T cells (24). NY-ESO-1 is the most broadly researched antigen with a panel of phase I/II clinical studies ongoing (NCT01567891, NCT03159585, NCT03691376, NCT03017131, NCT02869217). TBI-1301 is a cell product which is genetically modified to express NY-ESO-1 specific TCR. Butler et al. conducted a phase Ib clinical trial using TBI-1301 to treat HLA-A*02:01+ or A*02:06+ patients with NY-ESO-1+ solid cancers (NCT02869217). The ovarian patient had SD for 4.7 months and the standard dose infused was 5×10⁹ (25). Another study used affinity enhanced autologous NY-ESO-1+c259T cells for treating HLA-A*02:01, *02:05, or *02:06 positive recurrent ovarian cancer (NCT02869217). However, so far, no objective tumor response has been recorded for 6 patients who completed the research.

Mesothelin (Msln) is another frontier antigen for ovarian cancer. Anderson et al. conducted a preclinical experiment with Msln specific TCR1045 T cells. These T cells exhibited tumor cytotoxicity both in ID8VEGF ovarian cancer cells and in murine model, but the function was on the wane within 21 days. To enhance the antitumor activity, engineered T cells were repeatedly infused to mice and a maintained effect was seen. The time to progression (TTP) for TCR1045 plus an irradiated peptide-pulsed splenocyte vaccine was longer than that of using T cells alone or no-treatment group (112 days, 91 days, 77 days) (26).

Findings for targeting mesothelin in CAR-T therapy are also of note. Haas et al. enrolled five patients with mesothelin expressing recurrent ovarian cancer in a phase I study (NCT02159716). The most significant result was seen in ovarian cancer among multiple mesothelin+ tumors involved. Patients received lentiviral transduced CART-meso cells with different doses: two were infused with (1~3)×10⁸/m² cells, and three were infused with (1~3)×10⁷/m² cells, both groups were evaluated as SD for 28 days. Although the function of tumor control was observed, these antitumor responses were transient and limited (27). A case of patient with refractory epithelial ovarian cancer after chemotherapy was reported recently. The patient received two infusions of CAR-Ts encoded by genes specific for mesothelin and the immune checkpoint inhibitors. An antiangiogenic drug inhibiting vascular endothelial growth factor receptor (VEGFR)-2 named apatinib was included in the treatment. The follow-up assessment showed partial response with attenuated diameter of liver metastatic nodules and a 17-month survival (NCT03615313). Only slight adverse reactions were observed (28). Zhao et al. revealed that humanized (hu)
CD19 specific CAR had 6-fold higher affinity compared with murine CAR (29). Murine CAR has different structure domains which tend to trigger adaptive immunity. Once immune recognition of murine scFv is established, the therapeutic effect would be considerably subdued. Improved strategy employing huCART-meso cells to treat cancers commonly express mesothelin is now recruiting candidates (NCT03054298). A research using the fourth generation CAR-Ts for refractory or relapsed ovarian cancer has just been initiated with outcomes remaining to be seen (NCT03814447).

Mucin 16 (MUC16) is a glycosylated mucin widely expressed in ovarian cancer, serving as a promising target for CAR-T therapy. A phase I clinical trial is ongoing with MUC-16ecto CAR-T cells to treat recurrent ovarian cancer (NCT02498912). 5 dose levels are planned for the assessment of the maximum tolerated dose ($3 \times 10^5, 1 \times 10^6, 3 \times 10^6, 1 \times 10^7, 3 \times 10^7$). Furthermore, these CAR-T cells are modified to secrete IL-12, which could improve T cell persistence and overcome various inhibitions from the tumor microenvironment (30).

Nectin is a cell adhesion molecule which belongs to the Ca$^{2+}$-independent immunoglobulin superfamily proteins. Nectin-4 is expressed in various organs during fetal development but barely expressed in adults other than placenta. In ovarian tumor tissues, nectin-4 is overexpressed and plays a key role in tumor cell adhesion, migration, aggregation and proliferation (31). Currently there is a phase I clinical trial using the CAR-T, which involves in various costimulatory domains and cytokines (IL-7 and CCL19, or IL-16, membrane bound IL-15 (mbIL-15) to promote persistence of T cells and the kill switch to ensure safety simultaneously. It was applied in a phase I clinical trial for patients with advanced and recurrent platinum-resistant ovarian cancer in 2019 (NCT03907527). This is a seminal gene and cellular therapy which owns a non-viral multigenetic transfer patent to produce UltraCAR-T cells without the need for in vitro proliferation, thus shortening the waiting period from several weeks to one day. This landmark study has the potential to allow the therapy accessible to common patients by reducing costs. It also holds promise for subverting the current pattern of CAR-T cell therapy by regulating the immune system and tumor targeting in a more precise fashion (33).

Studies have demonstrated that the combination of ACT and immune checkpoint inhibitor (Pembrolizumab and Nivolumab) can fight against T cell exhaustion induced by immune checkpoints and augment the antitumor activity in the treatment of advanced, recurrent or metastatic programmed cell death protein ligand 1 (PD-L1) expressing gynecologic malignancies (34). Accordingly, a programmed cell death protein 1 (PD-1) gene-knocked out transferred T cell product has been promoted recently via gene editing technology (CRISPR-Cas9, lentivirus technology, etc.). A phase I clinical study evaluating the safety and efficiency of PD-1 gene-knocked out CART-meso cells for treating mesothelin positive multiple solid tumors is currently ongoing (NCT03747965). A clinical trial of advanced refractory ovarian cancer using αPD-1 CART-meso cell therapy combined with apatinib was also observed with potential therapeutic effect, which is detailed mentioned above (NCT03615313).

### Cervical Cancer

Cervical cancer is one of the most common gynecologic malignancies bothering middle-aged women, especially in developing countries. Although the incidence and mortality of cervical cancer have declined in recent years, the morbidity crowd tends to be younger, which is still worthy of vigilance (35).

The infection with high-risk human papillomavirus (HR-HPV) is a noted driver for the development of nearly all cervical cancers. E6 and E7 oncoproteins are highly expressed by HPV+ cervical cancer cells, becoming attractive therapeutic targets for engineered T cells. Preclinical research revealed that HPV-16 E6 (36)/E7 (37) specific TCR-Ts could detect and kill HLA-A2+ HPV-16+ tumor cells in vitro without cross-reactivity against human self-peptides. The antitumor avidity of E7 TCR-Ts against cervical cancer was also verified in a murine model.

A phase I/II study of HLA-A2 restricted E6 TCR-Ts for HPV-associated cancers (NCT02280811) was reported by Doran et al. Other interventions include common conditioning regimen, and systemic aldesleukin. Among 6 cervical cancer patients, 2 of them displayed SD, one for 6 months, another for 4 months. The percentage of E6 T cells in infused cells (range from $(1-170) \times 10^9$) were 51% and 71% respectively. In the phase I portion, no severe adverse effects were observed (38). A first-in-human, phase I clinical trial of HLA-A2 restricted E7 TCR-Ts to treat patients with metastatic HPV-16+ cancers has just uploaded its report (NCT02858310). Two in five patients with cervical cancer displayed PR for 8 months and 3 months, with T cell portion in infused cells (range from $(1-107) \times 10^9$) being 97% and 96%, respectively. One patient had SD for 3 months, and no response was observed in the remaining two patients. Researchers also proposed that genetic defects in the key elements of the antigen presentation and interferon response were responsible for treatment resistance of ACT (39). Some patients combined the PD-1 blockade therapy to improve T cell infiltration. In trial NCT03578406, five patients were treated with E6 TCR-T monotherapy: two of them received $5 \times 10^6$/kg dose and three received $1 \times 10^7$/kg dose. 28 days later, three patients had SD, one patient had PD, one patient was loss to follow-up. In another arm, two patients were infused with $5 \times 10^6$/kg and $1 \times 10^7$/kg of anti-PD-1 TCR-Ts respectively. The patient with lower dose was assessed as SD at both day 28 and month 2 post-infusion, showing promising efficiency for combining engineered T cell therapy with immune checkpoint inhibitor for cervical cancer patients (40).
New therapeutic targets of CAR products have been widely expanded via several preclinical researches which have progressed to the stage of animal experiments. CD47 specific CAR-Ts were proved to effectively kill ovarian, pancreatic, and cervical cancer cell lines and retard pancreatic tumor growth in mice (41). Recently, the antitumor efficiency of CART-meso cells was illustrated in SiHa cells in vitro by elevated levels of IL-4, IL-2, IL-5, tumor necrosis factor (TNF) α and interferon (IFN) γ secretion. The capacity in tumor control sustained for about 1 week in vivo. Better results were obtained following the second injection of T cells (42). Positive responses were also observed in Hela, SiHa, ME-180 and C-33A cell lines and in murine models through natural killer group 2D (NKG2D)/NKG2D-ligand pathway (43).

Currently, a phase I/II study of CART-meso cells in treating metastatic cancers including cervical cancer and ovarian cancer has been terminated with only one patient assessed as SD for > 3.5 months (NCT01583686). There is an ongoing phase I/II clinical trial using CARs targeting antigens such as GD2, prostate specific membrane antigen (PSMA), MUC-1, mesothelin or other markers positive to cervical cancer (NCT03356795). CD22 is often selected as the target for B cell malignancy. Recently, a phase I study employed CD22 specific CAR-Ts to treat solid tumors, including cervical cancer (NCT04556669). They also introduced the anti-PD-L1 monoclonal antibody to the CAR structure. More clinical evidence regarding the efficiency of CAR-T therapy for cervical cancer is required.

**Endometrial Cancer**

Endometrial cancer (EC) is the sixth most common cancer in women, and this ranking may rise especially in western countries (44). Although the 5-year survival rate of patients in the early stage is 95%, it would sharply decrease to 16% to patients with advanced or recurrent metastatic tumors (45).

There are not enough reports for the clinical assessment of ACT in EC until now. Only one patient treated with 5x10^7 TBI-1301 showed SD for 3.6 months without cytokine release syndrome (CRS) in a phase I/II clinical trial which has been mentioned above (NCT02869217). On 13 Nov 2020, a phase I/II clinical trial has just been initiated using CAR-Ts targeting alkaline phosphatase, placental (ALPP) for endometrial cancer and ovarian cancer (NCT04677740). The primary outcome measures related adverse events and the secondary outcome measures ORR, progression-free survival (PFS) and the number of transferred T cells.

**Vulvar Squamous Cell Carcinoma**

High-grade squamous intraepithelial lesion (HSIL) is a precancerous lesion of vulvar squamous cell carcinoma (VSCC) caused by HPV infection (46). The risk of cancer development can be reduced by treating HSIL. TCR-Ts targeting HPV-16 E6 protein thus provide a therapeutic window for HSIL to further prevent VSCC. A related phase I clinical trial was closed due to the lack of perceived clinical activity observed in the study (NCT03197025). A phase II study of HPV-16 E7 TCR-Ts for treating HSIL was also terminated without concrete results (NCT03937791). In a clinical study of E7 specific TCR-Ts mentioned above, vulvar diseases are included (NCT02858310).

**THE CHALLENGES WITH ENGINEERED T CELLS IN GYNECOLOGIC ONCOLOGY**

Several challenges become apparent when it comes to the promotion of engineered T cells. The major concern with this therapy is the severe adverse effect. TAAs can also be expressed by normal tissues, causing undesired on-target/off-tumor toxicity. CD19 CAR-Ts could induce the deficiency of normal CD19+B cells and cause weakened immunity. Besides, some TCRs or CARs are not specific to target antigen, but cross-react to other self-antigens. Taking MAGE-A3 specific TCR-Ts as an example, in previous studies, there were fatal events associated with injury in MAGE-A13 expressing tissues like the nervous system (47) and titin of cardiac cells (48, 49). MAGE-A13 was marginally expressed but unexpected and deadly destructive. Antigen selection is the first consideration in designing an ACT protocol. It is critical to choose ideal antigens that are tumor-specific, carcinogenic and immunogenic in order to strengthen the antitumor efficiency and reduce related toxicity simultaneously. In clinical trials using TCR-Ts to treat gynecologic malignancies, the target antigens involve: HPV16-E6/E7, NY-ESO-1, MAGE-A3, MAGE-A4, mesothelin. Antigens used as CAR-T therapeutic targets include: mesothelin, CD70, CD22, CD133, GD2, PSMA, MUC1, MUC16, human epidermal growth factor receptor 2 (HER-2), nectin-4, anti-alpha folate receptor (FR-α), ALPP, B7-H3, TnMUC1 (Table 1). In recent years, neoantigens have also emerged as a potential therapeutic option for gynecologic tumors since they are induced by somatic point mutations in tumor cells instead of co-expression with normal tissues. Matsuda et al. have successfully generated 3 neoantigen-specific TCRs through whole-exome sequencing (WES) of 7 ovarian tumors and the induction of peripheral blood mononuclear cells (PBMCs) isolated from healthy donors. These T cells could recognize their corresponding neoantigens although cross-reactivity to the wild-type peptide was observed in one of them (50). As an infant in the field of immunotherapy, it warrants further investigation whether these neoantigens will continue to be stably expressed by tumor cells.

CRS is another common threat particularly for CAR-T treatment. The excessive stress reaction of immune system would release superabundant cytokines such as TNF-α, IL-1, IL-6, IL-12, IFN-α, IFN-γ, leading to systemic inflammatory response syndrome (SIRS) and multiple organ failure. Grade 3 and 4 CRS can be life-threatening. In a multicenter clinical trial using CD19 CAR-Ts to treat refractory diffuse large B-cell lymphoma, 20% patients had grade ≥3 CRS events. More seriously, a rare case of fulminant haemophagocytic lymphohistiocytosis was reported (51). In another trial of CD19 CAR-Ts treating refractory ALL, 3 cases...
### TABLE 1 | Clinical trials of engineered T cells in gynecologic cancer immunotherapy (www.clinicaltrials.com).

| Cancer Type | Antigen | Stage and Result | Host | NCT        |
|-------------|---------|------------------|------|-----------|
| Ovarian cancer | TCR-T | MAGE-A4 | Phase I (recruiting) | University of Miami, USA | NCT03132922 |
|             |        |                  | 7 pts had PR, 11 had SD, 5 had PD |               |           |
|             | TCR-T | NY-ESO-1 | Phase Ia (completed with results) | City of Hope National Medical Center, USA | NCT01567891 |
|             |        |                  | No objective effects have been reported |               |           |
|             | TCR-T | NY-ESO-1 | Phase I (completed without results) | Zhuijiang Hospital of Southern Medical University, China | NCT03159585 |
|             | TCR-T | NY-ESO-1 | Phase I (recruiting) | Roswell Park Cancer Institute, USA | NCT03691376 |
|             | TCR-T | NY-ESO-1 | Phase I (active, not recruiting) | Roswell Park Cancer Institute, USA | NCT03017131 |
|             | TCR-T | NY-ESO-1 | Phase Ib (recruiting) | Princess Margaret Cancer Centre, Canada | NCT02869217 |
|             |        |                  | One patient had SD for 4.7m with grade 2 CRS |               |           |
|             | TCR-T | NY-ESO-1 | Phase I (unknown) | Shenzhen Second People’s Hospital, China | NCT02457650 |
|             | TCR-T | Neoantigen | Phase II (suspended) | National Institutes of Health Clinical Center, USA | NCT04102436 |
|             | CAR-T | Mesothelin | Phase II (suspended) | National Institutes of Health Clinical Center, USA | NCT03412877 |
|             | CAR-T | Mesothelin | Phase I (completed with results) | Abramson Cancer Center of the University of Pennsylvania, USA | NCT02159716 |
|             | CAR-T | Mesothelin | Phase I (recruiting) | Shanghai 6th People’s Hospital, China | NCT03054298 |
|             | CAR-T | Mesothelin | Phase I (terminated) | National Institutes of Health Clinical Center, USA | NCT01583686 |
|             |        |                  | Only one patient had SD for > 3.5m |               |           |
|             | CAR-T | Mesothelin | Phase I/Ii (recruiting) | The Second Affiliated hospital of Zhejiang University School of Medicine, China | NCT03916679 |
|             | CAR-T | Mesothelin | Early Phase I (recruiting) | The Second Affiliated hospital of Zhejiang University School of Medicine, China | NCT03799913 |
|             | CAR-T | Mesothelin | Phase I (recruiting) | Shanghai East Hospital, China | NCT04562298 |
|             | CAR-T | Mesothelin | Phase I (recruiting) | National Cancer Institute, USA | NCT03608618 |
|             | CAR-T | Mesothelin | Phase I (unknown) | Biotherapeutic Department and Pediatrics | NCT02580747 |
|             |        |                  | Department of Chinese PLA General Hospital |               |           |
|             | CAR-T | Mesothelin | Early Phase I (recruiting) | Shanghai 10th people’s Hospital, China | NCT04503980 |
|             | CAR-T | MUC16 | Phase I (active, not recruiting) | Memorial Sloan Kettering Cancer Center, USA | NCT02498912 |
|             | CAR-T | Necitum/FAP | Phase I (recruiting) | The Sixth Affiliated Hospital of Wenzhou Medical University, China | NCT03932565 |
|             | UltraCAR-T | MUC16 | Phase I (recruiting) | Fred Hutch/University of Washington Cancer Consortium, USA | NCT03907527 |
|             | CAR-T | HER-2 | Phase I (not yet recruiting) | Lineberger Comprehensive Cancer Center, USA | NCT04670068 |
|             | CAR-T | ALPP | Phase I (not yet recruiting) | Xinqiao Hospital of Chongqing, China | NCT04627740 |
|             | CAR-T | FRα | Phase I (recruiting) | University of Pennsylvania Health System, USA | NCT03585764 |
|             | CAR-T | CD133 | Phase I (completed without results) | Biotherapeutic Department and Pediatrics | NCT02541370 |
|             |        |                  | Department of Chinese PLA General Hospital |               |           |
|             | CAR-T | HER-2 | Phase I (recruiting) | Zhongshan Hospital Affiliated to Fudan University, China | NCT04511871 |
|             | CAR-T | HER-2 | Phase II (withdrawn) | Southwest Hospital of Third Military Medical University, China | NCT02713984 |
|             | CAR-T | CD70 | Phase I/II (completed with results) | National Institutes of Health Clinical Center, USA | NCT02830724 |
|             | CAR-T | TrnMUC1 | Phase I (recruiting) | The Angeles Clinic and Research Institute, USA | NCT04025216 |
|             | CAR-T | HPV-E6 | Phase I (recruiting) | National Institutes of Health Clinical Center, USA | NCT02280611 |
|             |        |                  | One patient had SD for 6m, one had SD for 4m |               |           |
|             | CAR-T | HPV-E6 | Enhanced SD in combination with anti-PD-1 therapy | Qingzhu Jia, Chongqing, China | NCT03578406 |
|             | TCR-T | HPV-E7 | Phase I (recruiting) | National Institutes of Health Clinical Center, USA | NCT02868310 |
|             | TCR-T | HPV-E7 | Early Phase I (suspended) | National Institutes of Health Clinical Center, USA | NCT04476251 |
|             | TCR-T | HPV-E7 | Phase I (suspended) | National Institutes of Health Clinical Center, USA | NCT04411334 |
|             | TCR-CD4+ T | MAGE-A3 | Phase I (withdrawn) | National Institutes of Health Clinical Center, USA | NCT02111850 |
|             | TCR-T | MAGE-A3 | Phase I (terminated) | National Institutes of Health Clinical Center, USA | NCT02153905 |
|             |        |                  | One patient had PR after 6w and 12w |               |           |
|             | CAR-T | Mesothelin | Phase I (terminated) | National Institutes of Health Clinical Center, USA | NCT01583686 |

(Continued)
of death induced by refractory CRS were reported (52). Management methods of CRS include: monoclonal antibodies against IL-6 (siltuximab, clazakizumab) and its receptor (tocilizumab), IL-1 receptor (anakinra), glucocorticoids, against IL-6 (siltuximab, clazakizumab) and its receptor (tocilizumab). In trial NCT02869217, the patient expressing IL-7 and CCL19 in CAR-Ts in mice, the immune system network effect on CD123, a stem cell marker expressed in CD19-negative relapses, to prevent possible antigen loss (54).

The immunosuppressive microenvironment is a contributing factor to the proliferation, metastasis and drug resistance of gynecologic tumor cells. Particularly, abdominal cavity metastasis is a common pathological feature of ovarian cancer, and the formation of ascitic fluid provides a favorable microenvironment for affecting tumor growth and invasiveness. It promotes vascular and lymphangiogenesis in tumor tissues and enables tumor cells to evade immune surveillance via several pathways: 1) offering ligands for immune checkpoint proteins, such as PD-1 and cytotoxic T lymphocyte associate protein-4 (CTLA-4); 2) providing an immune suppressive setting through cytokines such as IL-10, IL-6, TGF-β vascular endothelial growth factor (VEGF) and so on, extracellular matrix components like matrix metalloproteinases (MMPs) or suppressive cells such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs); 3) interaction with multiple active substances in stromal cells, such as tumor-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), and endothelial cells; 4) creating a physically and chemically hostile metabolic environment that is hypoxia, glucose-deficient, acidic, full of indolamine-1-oxidase and arginase (55).

The application of CAR-T therapy has long been constrained with unsatisfactory results in solid tumors including gynecologic tumors. A major hindrance for the broader use of CAR-Ts is attributed to the resistance of tumor microenvironment. Researchers found that by expressing IL-7 and CCL19 in CAR-Ts in mice, the immune cell infiltration in tumor tissues increased, thus reinforcing antitumor effects (56). In addition, chemokines e.g. CCR2b (57) and CCR4 (58) are factors affecting the progression and metastasis of tumor. Conversely, they can also facilitate the tumor infiltration of CAR-Ts when co-expressed with T lymphocytes. Although attempts in the combination of immune checkpoint blockades and ACT seem to make reversing the inhibitory microenvironment a reality, this strategy is still flawed due to neglect of the systemic network comprised of multiple immune suppressive mechanisms. A more concentrated attack on solid tumors is to use lipid nanoparticles to ferry immune-modulatory agents that are pertinently combined into components of tumor microenvironment. Compared with monotherapy, the level of TAMs, MDSCs and Tregs all reduced (9.4-fold, 4.6-fold, 4.8-fold), and the concentration of antitumor cells like CD8+ T cells and invariant natural killer T cells (iNKTs) increased (6.2-fold, 29.8-fold) (59). It seems to be a promising method with less cost, labor and fewer adverse effects.

The transient persistence of transferred T cells also makes it challenging to achieve optimal clinical results. Increasing the number of long-term memory T cells is a feasible way in obtaining sustained immunity. Stem memory T cells (Tscm) are superiorly potential in self-renewal, proliferation and long-last existence compared with T cells in other stages (60).

Replacing approaches to induce Tscm-like T cells has been a hot spot of tumor immunology in recent years. Productive methods include cancer vaccines with regulated TCR signaling (61), co-culture with cytokines like IL-7, IL-15, IL-21 (62), and the addition of co-stimulation domains (63).
THE FUTURE OF ENGINEERED T CELLS IN THE FIELD OF GYNECOLOGIC TUMORS

An essential contributing factor for the broader application of engineered ACT technology is a systematically manufactured process. The whole process should be strictly controlled with quality testing to obviate contamination and satisfy clinical demand. Although multiple CAR-T agents have been permitted into the market, the preparation of T cells before treatment is still performed in a personalized pattern, which is time-consuming for 12 days on average with small scale (64). The protocol is now embracing a more automatic and universal fashion called ‘off-the-shelf’ ACT manufacture using allogenic T cells that are modified to be mildly immunoreactive to the host (65). Importantly, the depletion of allogenic TCR, class I HLA molecule of donor T cells with CRISPR-Cas9 system would make ‘off-the-shelf’ CAR-Ts come true by reducing the risk of graft-versus-host disease (GVHD) (66).

The efficiency of engineered T cells in treating gynecologic tumors is currently not fully supported by sufficient clinical data and warrants further attempts in the clinical setting. Efforts to break barriers discussed above such as antigen selection, toxicities, the immune-unfavorable microenvironment in gynecologic tumors, the persistence of infused cells are making headway. Future investigation should provide update on these topics: (1) carrying forward clinical and preclinical trials; (2) more appropriate antigen binding sites; (3) how to break barriers to produce engineered T cell in a larger scale without toxicity; (4) how to maintain the cytotoxicity of engineered T cells in the tumor microenvironment; (5) synergistic treatment with immune checkpoint inhibitors or other substances. With further work to be done and deeper understanding of ACT, it would present a potential treatment for gynecologic oncology.

Another direction in engineered ACT technology is using natural killer (NK) cells as an alternative to T cells. NK cells have been proved to be safer in terms of CRS and GVHD risks than modified T cells with insensitivity to MHC and the presence of inhibitory receptor as a safety switch (67). A phase I study using mesothelin specific CAR-NK cells to treat epithelial ovarian cancer is ongoing (NCT03692637).

SUMMARY

Engineered T cells therapy for gynecologic cancer would inevitably face the existence of practical challenges such as safety concerns, difficult choices of appropriate antigen, the immunosuppressive tumor microenvironment, the short pharmacological duration and high finical cost. Based on a substantial number of preclinical researches with various models, series of phase I/II clinical trials are exploring the optimal route and dosage of ACT products, or whether a combination with surgery, radiotherapy, chemotherapy, or other immunotherapies would facilitate the treatment of malignant gynecologic tumors with decreased recurrence and metastasis rate, reduced adverse drug reactions, and improved life quality of patients.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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