

Application of CAR-T cell therapy based on nano-delivery system in ovarian cancer

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Abstract. Ovarian cancer is one of the common malignant tumours in women. Approximately 70% of patients will still relapse within 2-3 years after conventional treatment. While tumor-associated glycoprotein-72 (TAG-72) is more expressed in ovarian cancer compared with normal ovarian tissue and is associated with a worse prognosis, and there are ongoing clinical trials of TAG-72-directed CAR-T cells. Chimeric antigen receptor T cell (CAR-T cell) therapy is a new and promising immune approach in hematologic tumors while for solid tumors faces problems, such as difficulty in targeting infiltration, tumor antigen evasion, immunosuppressive environment, and potential toxic side effects. These problems will directly affect the efficacy of CAR-T cells, and the PD-1 pathway seems to be the 'master switch' in all immunosuppressive signaling pathways, so we develop a model in which we propose to use Crispr-Cas9 technology to excise the PD-1 expressed by CAR-T cells in TAG-72-directed CAR-T, and at the same time, it will be encapsulated by nanomaterial IR780, which can be precisely located to the cancer nest through PTT (photothermal therapy), effectively supporting immune escape. Thereby, the reprogrammed CAR-T cells may effectively avoid cytokine storms. By developing this model, we hope to provide a new protocol for CAR-T therapy for ovarian cancer, which is expected to prolong overall survival.

Keywords: Ovarian cancer, CAT-T, PD-1, Nanomaterial

1. Introduction

The mortality rate of ovarian cancer ranks fifth among all female cancers and ranks first among malignant tumors in the female reproductive system [1]. Patients are typically discovered at advanced stages and have a dismal prognosis [2]. Some studies have found that TAG-72 is highly expressed in ovarian cancer tissues, which can understand the development of the disease and provide a useful

indicator for evaluating the prognosis of lesions and tumor recurrence. TAG72-CAR-T cells are effective against advanced ovarian cancer in in vitro cell models and preclinical animal models [3]. Traditional therapeutic approaches for ovarian cancer encompass radical surgery, chemotherapy, and targeted therapy. The majority of patients attain remission subsequent to first-line treatment; however, approximately 70% of patients continue to experience relapse within 2 to 3 years [4]. CAR-T therapy is a new, promising immune method that may cure malignant tumors. However, CAR-T therapy in solid tumors faces problems such as difficulty in targeted infiltration, tumor antigen escape, immunosuppressive environment, and potential toxic side effects. These problems will directly affect the efficacy of CAR-T cells [5]. The PD-1 pathway appears to be a "master switch" in all immunosuppressive signaling pathways, because in some cases, a single dose of anti-PD-1 therapy can change the tumor microenvironment from an initially highly suppressive state to a highly active inflammatory site [6]. Many cancer types overexpress PD-L1, impairing the anti-tumor response of T cells through PD-1/PD-L1/2 interactions to evade immune system attack. However, the PD1/PDL1 pathway is also very important for maintaining normal peripheral immune tolerance. Direct targeting of PDL1 may cause very serious off-target effects [7]. Therefore, how to adjust the affinity of CAR for different targets through structural modification of CAR so that CAR T cells can avoid the immunosuppressive signal of PD1 without causing off-target is a very valuable research topic. PTT (photothermal therapy, PTT) combined with CAR-T tumors is a treatment method that has attracted much attention recently [8]. It injects materials with photothermal conversion properties into the human body, uses target recognition technology to gather into tumor tissues, and irradiates them with light sources outside the body. Under this condition, photothermal materials generate heat to "burn" tumor cells. It is worth noting that multiple studies have shown that PTT can not only kill tumor cells to release more antigenic substances and cytokines, but also destroy the extracellular matrix in tumor tissue and promote the infiltration of immune active cells into the tumor, thereby remodeling Tumor microenvironment and improve the effectiveness of immunotherapy [9]. We hypothesize that KO-PD1 CAR-T cells can target ovarian cancer while reducing immune escape in CAR-T, and that targeted delivery using nanomaterials can effectively reduce CAR-T cell exhaustion while precise delivery

In this study we aimed to develop the concept for a new treatment for ovarian cancer. Our combined new approach is inspired by the following three studies each of which discussed different orthologous approaches:

2. Article 1: The STING agonist IMSA101 enhances chimeric antigen receptor T cell function by inducing IL-18 secretion [10]

CART therapy has proven successful in treating hematological malignancies. However, the treatment of solid tumors remains challenging because the immunosuppressive tumor microenvironment (TME) impedes the function of CAR T cells. Here, the authors demonstrate that IMSA 101 as a novel STING agonist, improves the effectiveness of CAR T cells. Demonstrating as if IMSA 101 is administered intratumorally in combination with CART therapy, it shows that this treatment significantly enhances the overall survival in murine tumor models. This improvement is associated with increased T cell activation and elevated levels of pro-inflammatory cytokines, specifically, IL-18 in the tumor microenvironment. These data suggest that IMSA 101 promotes CAR T cell infiltration into the tumor microenvironment.

Since the absence of IL-18 receptor on CAR T cells diminishes but does not entirely eliminate the anti-tumor response, it indicates that IL-18 plays a critical role in the efficacy of this combination therapy.

3. Article 2: Antigen-dependent IL-12 signaling in CART cells promotes regional to systemic disease targeting [11]

This study focused on improving the efficacy of CAR T cell therapy for solid tumors, specifically targeting TAG72 in ovarian cancer. CAR-T cell therapy for solid tumors often faces challenges such as poor T cell migration and limited duration.

The authors tackled these challenges by engineering CAR T cells with a CD28 transmembrane domain and a 4-1BB co-stimulatory domain to enhance T cell functional IFN γ secretion, thereby augmenting the tumor cell killing effect. We incorporated membrane-bound IL-12 (mbIL12) into CAR-T cells to further boost their anti-tumor activity. Both in vitro and in vivo ovarian cancer models indicated that mbIL12 CAR T cells enhanced antigen-dependent proliferation and more effectively eliminated tumor cells. Local administration of these engineered CAR T cells showed a response to the region and systemic diseases in mouse models, demonstrating their safety and efficacy. In summary, this study suggests that CAR T cells engineered with IL-12 may improve treatment of solid tumors by promoting local and systemic disease targets.

4. Article 3: CAR affinity modulates the sensitivity of CAR-T cells to PD-1/PD-L1-mediated inhibition [12]

Despite the success of CAR T-cell therapy in haematological tumours, the treatment of solid tumours still faces significant challenges, including PD-1/PD-L1-mediated immune escape. In this paper, the authors hypothesised that CAR affinity could be a key factor in regulating T cell sensitivity to the PD-1/PD-L1 axis, but this factor has not been fully explored. Therefore, the authors established preclinical models of tumour cell lines expressing different PD-L1 densities in in vivo and in vitro experiments to detect different CAR structures. Also, to better control PD-L1 expression, the authors further established a synthetic model of glass-supported lipid bilayer (SLB). In the established model, the authors found that the homologous antigens targeted by CAR - T cells inhibiting PD-1/PD-L1 had a lower LA affinity or LA affinity (HA) for their cognate antigens and were also found to contain different co-stimulatory structural domains (CD28, ICOS and 4-1BB). The authors found that LA CAR - T cells were more susceptible to immunosuppression than HA CAR - T cells. It could be found that PD-1 destruction enhances LA CAR - T cell function, whereas among the three structural domains, the authors found that the CD28 and ICOS co-stimulatory structural domains of HA CAR - T cells could produce similar effects. Above all, the authors provide new options for CAR-T cell therapy in combination with immune checkpoint inhibitors.

5. Working model

Here, we developed a model in which we first collected blood sample from a patient and extracted T cells, then knocked out programmed death 1 (PD-1) of T cells with CRISPR case9 technology to have an effect similar to the immune checkpoint inhibitor, then reprogrammed the T cells, and loaded IR780 nanoparticles onto CAR-T cells, subcultured, and then transfused back into the body (Figure 1).

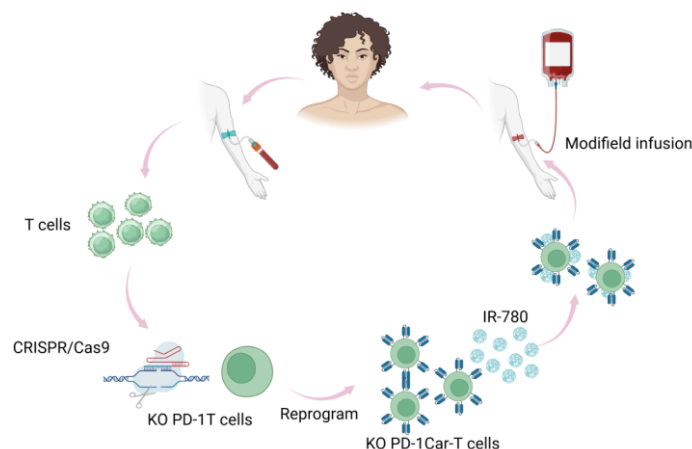


Figure 1. Establishment of the CAR-T model

In this model, we focus on expanding the use of TAG-72 as well as PD-1-guided CAR T cells on knockout T cells in ovarian cancer therapy. Since tumour cells do not recognise PD-1, immune escape is reduced and CAR-T cells can precisely kill tumour cells by NIR irradiation. At the same time, knockdown of PD-1 significantly inhibited the expression of vascular endothelial growth factor (VEGF) in tumour tissues, enhanced the infiltration of CD4⁺ T cells and CD8⁺ T cells, induced apoptosis of tumour cells, and inhibited tumour growth. In order to enhance the targeting ability of CAR T cells and modulate the immunosuppressive effect of the tumour microenvironment, we adopted an innovative nanotechnology strategy. This strategy enhances immunotherapy for solid tumours by combining CAR-T cells with ir-780. Through orthogonal coupling technology, ir-780 was combined with CAR-T cells to form CT-ir780. CT-ir780 has a mild photothermal intervention that disrupts the extracellular matrix, dilates blood vessels, relaxes dense tissues, and promotes remodelling of the tumour microenvironment. Therefore, on the one hand, the absence of PD-1 reduces immune escape, and on the other hand, CAR-T cells can accurately and effectively kill tumour cells by NIR irradiation (Figure 2).

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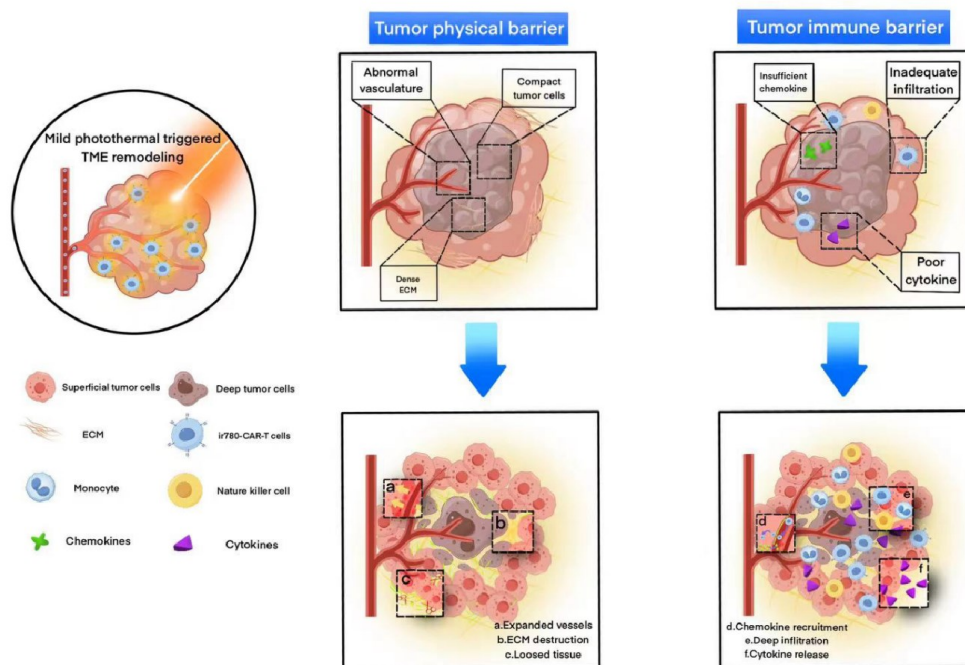


Figure 2. Therapeutic models in the immune microenvironment

6. Method and Result:

6.1. Establishment of a CAR-T Immunotherapy Tumor Mouse Model Using Human Ovarian Cancer Cells

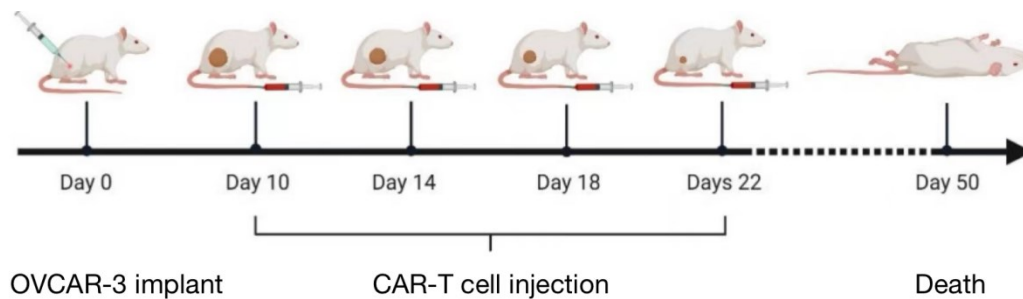
We propose to establish a murine aubcutaneous transplantation model with human ovarian cell OVCAR3 in NOD SCID IL2rgNULL (NSG) mouse and mice ovarian cell ID8 in C57BL/6 mouse for CAR-T immunotherapy^{7,8}. The NSG mouse model efficiently allows xenograft implantation, while the C57BL/6 mouse model better replicates the immune microenvironment [13]. The mice were randomly divided into six groups, with six mice in each group [11]. According to the different effector cells administered, the mice will be divided into the following groups:

- **Group A:** Control group
- **Group B:** Normal CAR-T cells

- **Group C:** PD-1/PD-L1 inhibitor group
- **Group D:** koPD1-CAR-T cells
- **Group E:** ir780-CAR-T cells
- **Group F:** koPD1-ir780-CAR-T cells

To better assess the performance in the immune microenvironment, the same grouping was also established in C57 mice.

The day of tumor implantation is defined as Day 0. On the 10th day, the average tumor volume is expected to reach approximately 50mm³. At this point, 200μL (1×10⁸/mL) of each CAR-T cell solution will be injected intravenously into the tail vein of the mice. Subsequent effector cell injections will be repeated every four days (e.g. Day 10, Day 14, Day 18, and Day 22) for a total of four injections. Additionally, PD-1/PD-L1 inhibitors will be intraperitoneally injected into Group C mice on the same days. After 26 days of treatment, Groups E and F will be subjected to daily laser irradiation of the tumors at a wavelength of 860nm for 15 minutes.



The condition of the mice, including tumor volume and body weight, will be monitored every day. When the maximum tumor diameter reaches 2cm or the body weight loss exceeds 20%, the mice will be sacrificed. Tumors will be completely excised, and tumor volume and mass will be recorded, along with the collection of blood samples. Tissue samples will be prepared for analysis using western blot and RT-qPCR to measure the expression levels of PD-1, PD-L1, and Vascular Endothelial Growth Factor (VEGF) mRNA and protein levels in tumor tissues. Immunofluorescence will be used to detect the expression of Ki67, PD-L1, and VEGF in tumor tissues to compare the tumor proliferation, immune evasion, and metastasis ability after treatment. ELISA will be used to detect the levels of TNF-α and IFN-γ to measure T cell activity, IL-12, and IL-18 cytokines in the blood to detect changes in the immune microenvironment. To assess the impact on the immune microenvironment, tumor samples were selected from the control group, koPD1-CAR-T group, and koPD1-ir780-CAR-T group in the C57 mouse model for single-cell sequencing and RNA-seq analysis to examine changes in the immune microenvironment and specific transcription factors in the tumor.

6.2. Experimental Results

Compared with other groups, the average tumor volume and mass in Group F are expected to be smaller, both in the NSG and C57 mouse models. The therapeutic effect of using koPD1-CAR-T cells alone is expected to be better than using PD-1/PD-L1 inhibitors alone. Furthermore, we anticipate that in Group F expression of VEGF, PD-1, and its primary ligand PD-L1 will be significantly reduced in tumor tissues and increased cell death and the expression of tumor-killing factors (TNF-α, IFN-γ) in tumor tissues. However, due to the modification of T cells, more cytokine production and release are likely to be observed. Therefore, we will also measure the expression levels of key cytokines such as IL-12 and IL-18 in mouse blood, especially comparing the differences in cytokines between the IR780 group and other groups to explore whether the addition of nanomaterials can reduce the cytokine storm.

7. Discussion

Ovarian cancer is characterized by a high incidence, high mortality rate, high recurrence rate, and poor prognosis [1]. Currently, the standardized treatment for ovarian cancer is optimal cytoreductive surgery

combined with platinum-based chemotherapy [14]. However, due to drug resistance and the intractability of the disease, long-term survival rates are reduced, and recurrence rates increase. Conventional treatment for ovarian cancer has reached a bottleneck. In efforts to develop more targeted therapies in recent years, several studies have found that TAG-72 is highly expressed in ovarian cancer tissues [15]. Therefore, detecting TAG-72 levels in early ovarian cancer tissues or serum can help understand disease progression and provide a useful indicator for evaluating prognosis and tumor recurrence. Additionally, research has shown that genetically modified CAR-T cells that specifically target TAG-72 (TAG72-CAR-T cells) are effective against advanced ovarian cancer in vitro and in preclinical animal models [16].

CAR-T cell therapy has garnered significant attention as an important tool in cancer treatment, especially in hematologic malignancies, where it has shown promising therapeutic outcomes in recent years [17]. However, CAR-T cell therapy for solid tumors faces challenges, including difficulties in targeted infiltration, an immunosuppressive environment, and potential toxic side effects, making its efficacy suboptimal [18]. In solid tumors, CAR-T therapy faces an unfavorable immunosuppressive microenvironment, where the PD-1 and PD-L1 signaling axis plays a strong inhibitory role, severely affecting CAR-T therapy's effectiveness [19]. To reduce tumor cell immune evasion, we propose to use CRISPR/Cas9 to knock out the PD-1 gene in TAG72-CAR-T cells to mitigate immune escape by tumor cells [20]. Additionally, to improve targeting accuracy and achieve precise targeting of ovarian cancer tumors, we plan to employ a nano-delivery system to facilitate this possibility. By combining CAR-T cells with IR-780 and using PTT (photothermal therapy) as supportive treatment, it is hoped that precise induction of CAR-T cells targeting ovarian cancer will improve the tumor microenvironment by breaking down the extracellular matrix, expanding tumor blood vessels, and promoting immune cell infiltration, thereby enhancing CAR-T cell infiltration into tumors and minimizing off-target effects.

Based on these ideas, we established our IR780-KO-PD1-CAR-T cell model. On one hand, the deletion of PD-1 reduces immune escape; on the other hand, CAR-T cells can precisely and effectively kill tumor cells under near-infrared irradiation. We anticipate that our IR780-KO-PD1-CAR-T cells can overcome challenges such as tumor antigen escape, difficulties in cell homing, and colonization in ovarian cancer treatment while significantly inhibiting tumor growth and improving treatment efficacy.

To test our model, we propose to conduct experiments in NSG and C57 mice. By implanting ovarian cancer tumors in mice and injecting CAR-T cells, followed by measurements of indicators such as body weight, tumor volume, cytokines, and vascular endothelial growth factor, we hope to observe significant inhibition of tumor growth by IR780-KO-PD1-CAR-T cells, reduction of PD1/PDL-1 expression in tumor tissues, and increased tumor cell death, as well as the expression of tumor-killing factors such as TNF- α and IFN- γ .

While this multi-therapy approach shows promise, our model also has some shortcomings. First, since the CAR-T cells are heavily modified, the model may trigger a stronger immune response. Although we hope to reduce cytokine storms by encapsulating reprogrammed CAR-T cells in nanomaterials, the actual effects remain to be evaluated. Additionally, the metabolic toxicity of the materials in the body needs further assessment. Cytokine storms are a serious immune response that can cause fatal side effects in patients, if too many cytokines are released, it may lead to an uncontrolled immune response that attacks human organs. This phenomenon can trigger severe systemic inflammatory reactions, such as coughing, shortness of breath, renal failure, liver failure, neurological disorders, and even life-threatening conditions. So controlling this reaction is a topic that requires in-depth study [21]. This model involves the combined use of multiple therapeutic components, including nanomaterials, photothermal therapy, and immune checkpoint inhibitors, which complicates the treatment process. In clinical applications, this complexity may increase operational difficulties and costs, requiring careful dose management and treatment planning.

In conclusion, the combination of CAR-T, PTT, and PD-1/PD-L1 inhibition may offer a new, promising approach to ovarian cancer treatment. The innovative use of a nano-delivery system enhances the precision and efficacy of this multi-faceted therapy, potentially overcoming many challenges in treating solid tumors. However, to fully realize the potential of this therapy, it is necessary to carefully

consider risks, particularly regarding cytokine storms, and further research into clinical applications is needed.

8. Conclusion

This study discussed a novel approach to treating ovarian cancer using a combination of CAR-T cell therapy, CRISPR-Cas9 technology, and nanotechnology. It may address the limitations of conventional treatments, which could lead to recurrence due to drug resistance and immune evasion mechanisms within the tumor microenvironment. We proposed using TAG-72-directed CAR-T cells, modified by CRISPR-Cas9 to knock out the PD-1 gene, thereby reducing immune escape and enhancing therapeutic efficacy.

Additionally, the study incorporated IR780 nanomaterials to improve targeting and precision through PTT. The proposed model is expected to reduce tumor growth significantly and enhance CAR-T cell infiltration into tumors while minimizing off-target effects. We acknowledged potential risks at the same time, such as cytokine storms and the complexity of combining multiple therapies, and suggested further research to address these challenges. This approach aims to create a more effective and targeted treatment for ovarian cancer, improving patient outcomes and reducing recurrence rates.

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Hanyu Wang and Lang Ye contributed equally to this work and should be considered co-first authors.

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