

Current Status and Progress of CAR-T Cell Therapy in the Treatment of Small Cell Lung Cancer

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Abstract. Small cell lung cancer (SCLC), which takes possession of about 15% of lung cancers, is closely related to smoking and is more common in older men. It has a high degree of malignancy, rapid growth, early metastasis, low survival rate in five years, and unfavorable prognosis. Current treatments include targeted therapy, chemotherapy and immunotherapy, etc. Among them, immunotherapy has grown in popularity as a new technique these years for treating tumors in the overall setting of SCLC. The chimeric antigen receptor (CAR-T) cell therapy, which has already showed considerable success curing hematological malignancies, has attracted the attention of many scientists. According to the current experimental studies, CD56, DLL3, GD2, CD133/AC133, CDH17 and other CAR-T cell targets have been found. AMG 119, a DLL3-targeted CAR T-cell therapy, has successfully completed phase I clinical trial and demonstrated good efficacy. Because of the limiting factors of CAR-T such as antigen escape, cancer infiltration, CAR-T cell trafficking, immunosuppressive microenvironment and on-target, off-tumor effects, etc. Further research and exploration are still needed in the field of solid tumor application. In order to effectively treat SCLC, there was still a huge progress to be made for CAR-T cell therapy compared to conventional therapy approaches. Further basic research and clinical studies are required.

Keywords: Small lung cancer, CAR-T cell therapy, Target spot.

1. Introduction

Small cell lung cancer (SCLC) originated from neuroendocrine (NE) cells, with high malignancy, rapid growth, and early onset of lymphatic and hematologic metastasis. Although it is relatively sensitive to radiation and chemotherapy, it can be rapidly resistant to drugs and has a poor prognosis. Less than 5% of patients survive for five years, and those who do not undergo active treatment often only live for two to four months [1]. About 15% of all lung cancers are SCLC [1], which are more common in older males and are strongly correlated with smoking. Small cell lung cancer often spreads quickly to distant organs, with the exception of those with TNM stage I (T1-2N0M0) who can undergo surgery, other patients should be mainly combined with radiotherapy and chemotherapy [1]. Etoposide or irinotecan coupled with platinum is the first-line conventional chemotherapy; nevertheless, its effect is generally more successful in the first application, and the majority of patients will have recurrent disease or additional metastatic sites [1]. With advances in genetics and molecular medicine, targeted therapies have also become the first choice for SCLC. Many potential targets, such as enhancer of zeste homolog 2 (EZH2) and poly (ADP-ribose) polymerase (PARP), have emerged, but more comprehensive studies are still lacking [2]. The treatment of SCLC is extremely difficult to advance because of its complex pathological features and poor clinical prognosis when compared to non-small cell lung cancer (NSCLC) [2]. Therefore, more researchers are turning their attention to immunotherapy to find more effective treatment methods.

With a total recovery rate of over 80%, as newly developed immunotherapy, chimeric antigen receptor (CAR-T) cell treatment showed remarkable efficacy in curing hematological malignancies at this time [3]. According to some researchers, this therapy has demonstrated outcomes with a great process in clinical studies when used to treat lung cancer. This approach still leads the way in a new direction for the treatment of SCLC and other resistant lung cancers, despite the fact that there are still a lot of barriers to its implementation in the treatment of solid tumors [3].

In order to offer novel recommendations for the advancement and enhancement of immunotherapy in SCLC, this article will focus on the most recent advancements about CAR-T cell therapy research related to SCLC, as well as the present restrictions and difficulties.

2. Background of CAR-T Cell Therapy

Intracellular signal transduction domains, hinge and transmembrane domains, and extracellular antigen recognition domains make up chimeric antigen receptor (CAR) construct [3]. The single stranded variable fragment (scFv) of the extracellular antigen recognition domain can specifically recognize tumor surface antigens. For instance, CAR-T cells can be stimulated and able to send activation signals to the intracellular domain when scFv recognizes tumor-associated antigens (TAAs) [3,4]. The extracellular and intracellular domains are connected by hinges and transmembrane domains [3]. The stimulator CD3 ζ chain, which often binds to co-stimulatory molecules, such as CD27, CD28 to eventually stimulate T cells, makes up the intracellular signal transduction domain [3].

Prior to genetically modifying T cells laboratory-based to express the CAR, this therapy first collects T cells through peripheral blood from patients or contributors [4]. Subsequently, a sizable number of genetically altered T cells were amplified, and the transformed CAR-T cells were then reinjected into the sick person, but before this, the patient needed to receive lympho-depleting chemotherapy to facilitate the body to accept the injection of CAR T cells [4]. Eventually, these cells will specifically recognize the target antigen in the patient and rapidly proliferate within a short time to play an anti-tumor role [4].

The internal domain structure of CAR-T cell has been artificially classified into four generations [5]. Tyrosine activation patterns on the CD3 ζ chain or Fc ϵ RI γ enable the first generation, the main transmitter of endogenous T cell receptor (TCR) signaling [5,6]. However, it is challenging to produce the anticipated anti-tumor effect due to the first generation cells' limited proliferation [5]. The intracellular signaling domain of co-stimulatory protein receptors was added in the second generation of CAR to provide additional signals for T cells. Common co-stimulatory molecules include CD28, CD16, and CD17, but they are gradually replaced by CD137(4-1BB and CD134(OX40)) [6]. When compared to the first generation, the second generation's ability to proliferate was improved, it secreted more cytokines, and it took longer for CAR-T cells to die [5]. The third generation can bind multiple signal domains and has stronger cytokine production and killing capabilities [5]. T cell redirected Universal cytokine mediated Killing (TRUCKs) is the name given to the fourth generation, which is created by adding IL-2 to the second generation design with [5]. Currently, numerous clinical trials are investigating the therapeutic usefulness of CAR constructions since IL-2, IL-15 and IL-17 are almost the most often combined cytokines for the development of this therapy [7]. The function and duration of T cells can be improved by these cytokines as well [7].

3. The Target Spot of CAR-T Cell Therapy in SCLC

Up to now, through a large amount of experimental studies, targets of CAR-T cells that have been observed to act on SCLC include CD56, DLL3, GD2, CD133/AC133, CDH17, etc [8-10]. This article will focus on the specific principles of these target spots.

3.1. CD56

Many experts are optimistic that CAR-T cell treatment's use in the medical disposition of solid tumors will result in a substantial advancement given the therapy's enormous success. Natural killer cells (NK cells), neurons and glial cells, dendritic cells and T cell subpopulations all express CD56 on their surfaces. For the time being, CAR-T cells targeted by CD56 have been involved in treating SCLC. Co-culture experiments in vitro by Crossland et al. showed that CD56-expressing neuroblastoma, glioma, and SCLC tumor tissue can all be killed by these cells [9]. With a specific

lysate rate of 64.9% at a 20:1 effect target ratio, experimental evidence demonstrates that CD56R-CAR T cells strongly dissolved all CD56-expressing targets [9]. In preclinical investigations, these cells may also prevent tumor proliferation in vivo since NSG mice with CD56+ H526 tumors started to exhibit a significant decrease in burden of cancer on day 20 following tumor cell insertion [9]. However, despite the high expression of CD56 in SCLC, because the expression of CD56 is not limited to tumors, the CD56-CAR T cell therapy may activate other lung tumor tissues at the same time, resulting in obvious side effects, so the immunotherapy targeting CD56 still needs to be further improved and explored.

3.2. DLL3

Delta-like ligand 3 (DLL3) is known to be a tumor-specific cellular surface marker of SCLC, which is abnormally exposed on the cell surface of neuroendocrine tumors while rarely expressed on the surface of normal tissues. Notch pathway is related to various body development, and DLL3 has an inhibit impact on this pathway. Notch signaling is down-regulated during the growth of neuroendocrine tumors, and the expression of DLL3 is inhibited [11]. Neuroendocrine transcription factor (ASCL1), which is also a cancer-causing factor in SCLC [11], controls the production of DLL3. With regard to the utilization of CAR-T cell treatment, in comparison to CD56, DLL3 has trails that make it more compatible with the fundamental requirements.

AMG 119 is a CAR-T cell treatment related to relapsed/refractory (R/R) SCLC that specifically targets DLL3. At present, AMG 119 has entered the phase I clinical trial and shown good therapeutic effect. Some experiments have shown that AMG 119 has a strong eradicating effect on DLL3 positive cells in vitro and can significantly promote the stimulation and expansion of T cells [11]. Five individuals with R/R SCLC participated in the AMG 119 Phase I clinical trial, one patient had a 43% reduction in total target lesion diameter from baseline, while the other had a 16% reduction in total target lesion diameter and multiple liver metastases disappeared [12]. These preclinical findings imply that AMG 199 may be extremely effective and selective for SCLC tumor cells that are DLL3-positive. At the same time, the CAR-T cell clinical trial for DLL2 (NCT03392064) has also entered phase I, which offers a strong clinical foundation for the further improvement of CAR-T cell therapy.

3.3. GD2

Human cancer cells of different neuroectodermal ancestries can express the non-protein target disialoganglioside GD2. GD2 expression in SCLC has been verified by numerous investigations, and it is associated to the aggressiveness and growth of cancer cells [13]. It is also appropriate for developing CAR-T cell treatment for SCLC due to the restricted expositions of GD2 in healthy cells [13]. Reppel and colleagues designed GD2-CAR co-expression of IL-15 to encourage T cell growth and survival [13]. The practical potential of GD2 targets in CAR-T cell treatment was confirmed by experimental data showing that 26.7% of SCLC and NSCLC cell types expressed GD2 on their surfaces and that CAR-T cells demonstrated antigen-dependent cytotoxicity through both in vivo and in vitro lung tumor xenotransplantation mice [13]. Anti-GD2 CAR-T cells have also applied to the treatment of many illnesses, including glioblastoma, Ewing's sarcoma, and melanoma.

3.4. CD133/AC133

CD133 is reported to be a marker of tumor stem cells (CSCs) in certain cancers. CSCs are a group of undifferentiated cells that are responsible for the formation, maintenance and spread of tumors and have been considered to be one of the causes of tumor treatment resistance [14]. Moreover, human SCLC and NSCLC were found to contain CD133-positive cells [14]. Taromi and colleagues used orthotopic xenotransplantation mice models and human primary SCLC to examine the therapeutic effects of AC133-specific CAR-T cell treatment following chemotherapy. The experimental results showed that in the humanized in situ SCLC model, AC133-tageted CAR-T cells could reach the cancerous location, decrease tumor burden and prolong overall survival, but could not completely eliminate the cancer [8]. In the experiment of triple immunotherapy, namely PD-1 inhibition, CD73

suppression and CAR-T cell combination therapy, 25% of mice were cured, and no other serious side effects occurred after cure [8]. Meanwhile, AC133+ CSCs and PD-L1+CD73+ bone marrow cells were also detected in original human tumor tissues [8]. Although clinical trials have not yet been conducted, these final data illustrate that triple immunotherapy has the capacity to be effective in patients with SCLC.

3.5. CDH17

Cadherin 17 (CDH17) is one of the 7D-cadherin superfamily members and has been identified in colorectal, gastric, and human hepatocellular carcinomas. Moreover, metastasis, cell proliferation, and poor prognosis are linked to it. Tian W et al. assessed the living samples performance of CDH17+CAR-T cells using a pattern of SCLC xenotransplantation [10]. Research has revealed that tissues and cell lines have considerably higher levels of CDH17, and that CDH17-positive CAR-T cells have potent cytotoxicity action on SCLC cells in vitro [10]. Additionally, xenograft tumors of SCLC origin can have their development rate greatly slowed down in vivo by receiving therapy with CDH17+CAR-T cells [10]. Experimental data suggest that CDH17 has potential as an indication for CAR-T cell therapy, but more experimental evaluation is needed.

4. Limitations of CAR-T Cell Therapy

4.1. Immunosuppressive Microenvironment

The immunosuppressive microenvironment in tumors has particular histopathological characteristics, including high vascular density, extensive vascular leakage, and poor tissue structural integrity, etc. These changes lead to hypoxia, low pH, increased immunosuppressive cells, inhibitory checkpoints, and more tumor-derived cytokines [6]. Tumor-associated macrophages (TAM), regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs) are only a few of the cell types that can invade lung tumors in the microenvironment and induce immunosuppression [15]. The tumor infiltrates and cancer cells stimulate the creation of growth factors, tumor-promoting substances, and chemokines, which shield lung tumor cells from the host immune system's attack [15]. In addition, multiple investigations have demonstrated that insufficient T cell proliferation and transient T cell survival are two important contributors in the non-response or limited response of CAR-T cells [15].

4.2. Tumor Infiltration and CAR-T Cell Trafficking

For immunotherapy to work as intended, CAR-T cells need to be drawn into the cancer locations so as to attach to target proteins on the tumor surface [6]. In contrast to hematological malignancies, the immunosuppressive microenvironment significantly restricts the transport and penetration of T lymphocytes to the tumor lesions [15]. Similar to this, unlike hematological malignancies that are readily identifiable and addressed by CAR-T cells, a lot of chemokines generated by tumors can stop T cells from metastasizing and penetrating into cancerous sites [6]. Because of the absence of matching chemokine receptor exposition on T cells, CAR-T cells' capacity to destroy cancer tissues with their intended immunocytotoxicity is substantially hindered since they are difficult to transport and infiltrate into the tumor location [6]. Once CAR-T cells have easily reached the cancerous site, entry into the immunosuppressive microenvironment is a crucial process needed to exhibit antitumor effects [6]. The unique histopathological characteristics of solid tumors are conducive to their growth, but they also make it hard for T cells to get into the area where the tumor is located, thus preventing T cells from continuing to interact with cancer cells, which is required for T lymphocytes to have capable of cytotoxic anti-tumor impacts [15]. T cell invasion of tumors associated with SCLC still needs to be further explored.

4.3. OTOT Toxicity

The binding of CAR-T cells to target antigens usually express themselves at various levels in normal tissue, is a factor that cannot be ignored to the progress of CAR-T cell treatment for SCLC. This is because of the on-target, off-tumor (OTOT) toxicity that brings about the breakdown of ordinarily healthy cells and organs, posing a high risk of morbidity [15]. Take CD56 as an example, it may not be a good target antigen as evidence by the possibility of OTOT toxicity in studies targeting CD56 for SCLC when CD56-CAR T cells be exposed to some CD56-positive immune cells, for example NK cells, neurons and CAR-T cells themselves [9]. Most of CAR-T cell targeted antigens are not tumor specificity since they are present in both healthy and cancerous cells [6]. As a result, antigen specificity plays a vital part in this therapy, and antigen selection is essential to make sure of therapeutic efficacy as well as to avoid OTOT damage [6]. This OTOT effect can be reduced in the way of limiting CAR-T cell activity, utilizing double CAR targeting or varying the responsiveness of scFv [6].

4.4. Antigen Escape

Tumor resistance to single-antigen-targeted CAR constructions is also one of the arduous restrictions of this treatment. Even though this kind of CAR-T cells initially provide a high rate of response, antigen escape occurs when a considerable portion of the patient's malignant cells exhibit either a complete or partial absence of target antigen expression [15]. Tumor antigen heterogeneity is an achievable source of treatment resistance when targeting a single antigen [13]. Taking GD2 as an example, the evaluation of GD2 expression in SCLC patient samples biopsies found a certain degree of heterogeneity, indicating the possibility of immune escape[13]. Antigen loss following this therapy is one of the primary causes of relapse, and the most frequent way is antigen mutation under the stress of this therapy, which includes splicing variation, lineage shift, and biallelic mutation [4]. Tumor immune evasion can potentially be aided by antigen mutation and the decrease in surface antigen density brought about by CAR-T cell endocytosis [4].

Owing to the numerous drawbacks previously highlighted, further clinical evidence is still required to justify the broad promotion of this new therapy in the treatment of SCLC.

5. Conclusion

Nowadays, immunotherapy has became one of the viable treatment options for SCLC. As a novel approach to treating SCLC, CAR-T cell treatment has advanced significantly and got into a phase of considerable progression. Despite there are still many limiting factors in clinical application, compared with traditional chemotherapy methods, CAR-T cell therapy still has many advantages. Because SCLC is difficult to cure, treatment options at different stages of disease progression also deserve more attention. In summary, based on current experimental studies, the application of this immune therapy in SCLC can be further improved. In future experiments to overcome SCLC, the sample size need be expanded to improve the evidence-based quality, and more fundamental studies and clinical evaluations should be invested.

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