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The Feasibility of Using Emerging Antibody-drug Conjugates for the Treatment of Small Cell Lung Cancer

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Abstract:

Antibody-drug conjugate (ADC) consists of three parts: antibodies, linkers, and cytotoxic payloads. With the deepening of research on ADC, the potential therapeutic effects of ADC on SCLC have also been emphasized. This study aims to evaluate and introduce five novel ADCs targeting small cell lung cancer (SCLC) and explore their anti-tumor activity, cytotoxicity, application prospects and so on. The first ADC drug is rovalpituzumab tesirine(Rova-T), currently, Rova-T has entered Phase III clinical trial. The second drug is ABBV-011, and a study found that a single dose of ABBV-011 can lead to significant dose-dependent tumor regression in LU64. The third drug is NN2101-DM1, which is found to exhibit anti-tumor activity in xenograft mouse models. The fourth drug is D3-GPC2-PBD ADC. A study treated mice carrying NCI-H526(H526) xenografts with D3-GPC2-PBD ADC and found that the drug can lead to robust and sustained tumor regression, significantly improving the survival rate of mice without any side effects. The fifth study was on ADC targeting junctional adhesion molecule 3 Gene (JAM3), which found that SCLC cell lines typically express high levels of JAM3. The study also found that knocking down JAM3 can inhibit the growth of SCLC cells. Although these five ADCs are in different clinical trial stages, they all have inhibitory effects on SCLC.

Keywords:. Antibody-drug conjugate (ADC); Small cell lung cancer (SCLC); DLL-3; SEZ6; K-cit.

1. Introduction

SCLC is an invasive, poorly differentiated neuroendocrine tumor that accounts for about 15-20% of lung cancer. SCLC has a high degree of malignancy and is the worst prognosis type among lung cancer. Typical respiratory symptoms of SCLC include cough, difficulty breathing, or hemoptysis. Imaging findings include a central lung mass, often accompanied by enlarged chest lymph nodes. Two-thirds of patients experience distant metastasis at the first diagnosis [1].Although SCLC is sensitive to chemotherapy and radiation therapy, patients with SCLC have a high recurrence rate within two years and are prone to developing resistance to treatment.

At present, the combination chemotherapy regimen of Atezolizumab and Durvalumab has been recommended by multiple authoritative guidelines as the first-line standard treatment for extensive SCLC [2]. Long-term clinical data indicates that immune checkpoint inhibitors and chemotherapy prolongation therapy can significantly improve the survival rate of SCLC patients, with a 3-year survival rate three times higher than that of chemotherapy alone [3]. Therefore, immunotherapy has shown promising results. ADC has made outstanding achievements in many anti-cancer fields, so many researchers are testing the feasibility of applying ADC to SCLC treatment. ADC is composed of antibodies, connectors, and payloads. Payloads are cell-killing drugs that can induce cell apoptosis through different mechanisms. Antibodies can specifically bind to highly expressed target antigens on the surface of tumor cells, allowing ADC to enter the interior of target cells through endocytosis. ADC forms early endosomes after entering target cells, which can release cytotoxic payloads by binding to lysosomes. Payloads inducing cell apoptosis [4]. ADC is an efficient therapy that combines chemotherapy and immunotherapy, which can achieve precise targeting and effectiveness at the same time. Compared with traditional chemotherapy, it has a larger therapeutic window and broader development prospects. This article aims to evaluate the research results of existing novel ADCs as SCLC therapeutic drugs and provide prospects for the future.

This article will provide a detailed summary and report on the ADC research directions proposed or popular in recent years for the treatment of SCLC, such as Rova-T, ABBV-011, NN2101-DM1, D3-GPC2-PBD ADC, etc. Comparing and discussing their composition, mechanism of action, research methods, and research results such as

the sensitivity, anti-tumor effect, and toxic side effects of specific ADC, discussing the current situation, and looking forward to the future.

2. Background

2.1 The Mechanism of Action of ADC

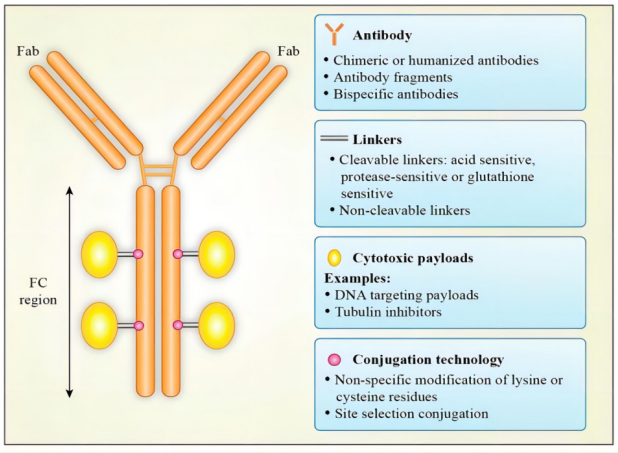

Fig. 1 ADC's Infrastructure [5].

ADC can utilize the specificity of antibodies towards targets to deliver effective cytotoxic drugs to cancer cells, consisting of three parts: antibodies or antibody fragments, cleavable or noncleavable connectors, and cytotoxic drugs, as shown in Fig.1. In practical applications, antibodies will bind to target cell antigens that are overexpressed on the surface of tumor cells. Once bound, the ADC antigen complex enters the cell through clathrin-mediated endocytosis and is encapsulated in the endosome. Endosome transport antibody-drug conjugates to lysosomes, and proton pumps on lysosomes provide an acidic environment to completely decompose ADCs, as shown in Fig.2. Through this process, the payload is released into the cytoplasm and exerts its cytotoxic effect by inserting DNA or inhibiting microtubule aggregation, thereby triggering cell apoptosis.

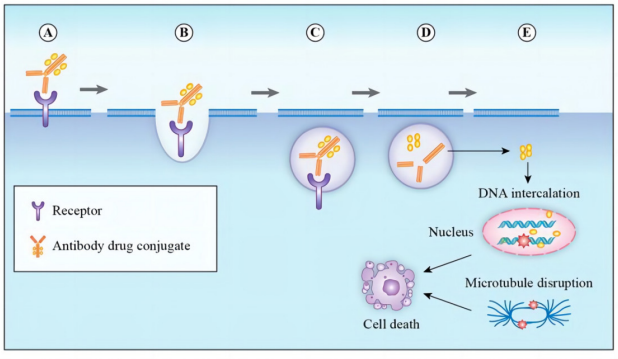

Fig.2 The mechanism of action of ADC [5].

ADC can also induce cell apoptosis in adjacent tumor cells through the bystander-killing effect.

The antibodies on ADC have low immunogenicity and high specificity. Low immunogenicity can evade the attack of the immune system, while high specificity can minimize the risk of off-target. There are mainly two types of cytotoxic drugs, namely microtubule inhibitors and DNA damaging agents. microtubule inhibitors can bind to microtubule proteins, interfere with the dynamic combination of microtubules, and cause cells to stagnate in the G2/M phase of the cell cycle, ultimately leading to apoptosis. Compared with microtubule inhibitors, DNA inhibitors can disrupt DNA through double-strand breaks, alkylation, chimerism, cross-linking, etc., acting on the entire cell cycle and producing cytotoxic effects.

2.2 Overview of SCLC, Existing Treatment Strategies and Limitations of SCLC

SCLC is a high-grade neuroendocrine carcinoma that mainly affects smokers and former smokers, with an abnormally poor prognosis. At the genetic level, Genome-Wide Association Studies (GWAS) have shown that the 15q25 locus is associated with smoking status and nicotine dependence, while smoking is significantly associated with the risk of SCLC [6]. Loss of dual alleles of two tumor suppressor genes Retinoblastoma1(RB1) and Cellular tumor antigen p53(Tp53) is a characteristic of SCLC. A study has shown that by inducing the inactivation of RB1 and Tp53 in the epithelial cells of experimental group mice, the mice can develop into small-cell lung cancer [6]. The biomarker Delta-like ligand 3(DLL-3) can be expressed on the surface of tumor cells, inhibiting the signal transduction of the Notch pathway [7]. In recent years, there have been ADCs targeting DLL-3 targets in the research stage, such as Rova-T, which will be described in detail in this article.

At present, the therapies for SCLC include chemotherapy, surgery, immunotherapy, etc. Platinum-based chemotherapy is the first-line standard treatment for SCLC, with platinum-based drugs combined with etoposide being the preferred option. Despite actively developing chemotherapy drugs, the prognosis of SCLC treated with chemotherapy drugs is still poor due to the lack of early detection techniques, limited treatment options and efficacy [8]. SCLC is initially particularly sensitive to concurrent chemoradiotherapy (CRT), with a response rate of over 60%, but this effect is generally short-lived. A study showed that the median survival of early patients was less than 2 years, and only about 1 year was observed in patients with metastasis [9]. In addition, immunotherapy has also shown promising prospects in the treatment of SCLC. However, in evaluating the efficacy, mechanism, kinetics, and toxicity of immunotherapy, there is a lack of the best and ap-

propriately standardized in vitro and in vivo experimental, preclinical, and clinical model systems, so it is still in the early stages of development [8].

3. Frontier Research on the Application of ADC in SCLC

3.1 The Therapeutic Potential of ADC in SCLC

Traditional chemotherapy has a narrow treatment window, and the minimum effective dose of the drug is relatively close to the maximum tolerable dose. If the dosage used is too large, it can easily lead to toxic reactions. ADC precisely transports drugs to the tumor site, reducing systemic toxicity by reducing the distribution of drugs in normal tissues, increasing to the maximum tolerated dose of drugs. At the same time, due to the specific binding between ADC and target antigens, the therapeutic effect of ADC is improved, and the minimum effective dose is reduced.

Compared with targeted drugs, the special structure of ADC gives it a certain degree of flexibility, and modifying its components can help reduce the resistance or self-resistance issues of targeted drugs.

In addition, the bystander killing effect can kill adjacent tumor cells, so ADC may greatly improve the quality of life of SCLC patients in the future.

3.2 Development of ADC for SCLC-specific Biomarkers

3.2.1 Rova-T

DLL-3 is regulated by achaete-scute family bHLH transcription factor 1 (ASCL1) and specifically expressed on the surface of SCLC tumor cells, inhibiting the Notch pathway. A study showed that over 80% of SCLC tumor cells express DLL3 mRNA and protein, while only a few normal cells express DLL3 in the cytoplasm.

Rova-T is composed of DLL3-specific human monoclonal antibody SC16, a cleavable dipeptide linker, and pyrrole Pyrrolobenzodiazepine (PBD) dimer toxin, which can be internalized into secondary endosomes by DLL3 expressing cells. In patient-derived xenograft (PDX) studies, Rova-T showed stronger and longer-lasting effects compared to mice treated with standard chemotherapy such as cisplatin and etoposide.

Based on the above research, a phase one clinical trial evaluated the safety, tolerability, and maximum tolerability of Rova-T in recurrent or progressive SCLC and Large cell neuroendocrine carcinoma of the lung (LCNEC). This study was conducted across ten cancer centers in the United States, screening patients aged 18 and above who had SCLC or LCNEC and had previously received one or two chemotherapy regimens. Patients were included in a dose escalation or dose expansion design, with medication ranging from 0.05mg/kg to 0.8mg/kg, administered intravenously every three or six weeks. Rova-T showed overall good tolerability among 74 patients, with 65 patients experiencing varying degrees of treatment-related adverse reactions, and over 28 patients experiencing grade 3 or higher treatment-related adverse reactions. The most common were thrombocytopenia, pleural effusion, and elevated lipase. The main reason for the toxicity characteristics of Rova-T is PBD. Among the 74 patients who received any dose of Rova-T, 65 patients were able to evaluate its activity, of which 7 patients achieved confirmed objective remission and 35 patients had stable conditions [10].

A second-phase clinical trial targeting second-line SCLC treatment included 339 patients receiving Rova-T, and the results showed toxicity characteristics consistent with the first-phase results. However, a phase III clinical trial targeting Rova-T as a second-line treatment for SCLC was halted a few years ago due to the shorter overall life (OS) of the Rova-T group compared to topotecan.

Although the Phase III experiment has been discontinued, DLL3 remains a high-value target for the treatment of SCLC.

3.2.2 ABBV-011

The main components of ABBV-011 are a monoclonal antibody SC17 against seizure-related homolog 6(SEZ6), an unclearable LD19.10 linker, and a calicamycin payload

A study presented in detail the development process of ABBV-011 and the therapeutic effects of the first phase of the study.

Firstly, the study found that LD19.10 does not rely on acid decomposition, LD19.10 dissociates only upon reduction of the hindered disulfide. Therefore, using LD19.10 as a linker can eliminate the toxic dimethylhydrazine (DMH) decomposition products in acid-unstable calicheamicin. To verify the sensitivity of SCLC to LD19.10 conjugated drugs, researchers coupled LD19.10 to an antibody that is specific to CD46 (a widely expressed tumor antigen). They found that although CD46 is expressed lower in SCLC, anti-CD46-LD19.10 ADC still has strong anti-tumor effects, indicating that SCLC is a solid tumor sensitive to CD46-LD19.10 ADCs. In the process of searching for antigens, it was found through DNA microarrays that SEZ6 is highly and uniquely expressed in neuroendocrine carcinoma in a set of PDX samples covering multiple indications for solid tumors. In the RNA-seq data of primary SCLC tumors, low expression of SEZ6 was found in normal tissues. In the study on whether SEZ6 affects the prognosis of SCLC, it was found that patients with high expression of SEZ6 have poorer survival outcomes. The

above research fully confirms the therapeutic potential of this type of ADC in SCLC.

The study aims to couple LD19.10 drug with humanized monoclonal antibody SC17 through site-specific coupling, while also coupling with non-targeted Immunoglobulin G (IgG) antibody to generate ABBV-011 with non-specific ADC activity.

To determine the anti-tumor activity of ABBV-011, researchers used the PDX model of SCLC for testing. Research has found that a single dose of ABBV-011 can lead to significant dose-dependent tumor regression in LU64 (a SEZ6-positive PDX cell line). Compared with ABBV-011 at a dose of 0.5mg/kg, single-cycle cisplatin and etoposide showed less sustained tumor dissipation.

As shown above, ABBV-011 is an ADC targeting SCLC and is currently being tested in Phase I clinical trials[11].

3.2.3 NN2101-DM1

NN2101 is an ADC composed of an fully humanized antibody IgG1 coupled with microtubule inhibitor Mertansine (DM1) via N-succinimidyl-4 (N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC).

A study used humanized anti-K-cit antibody LMJ729 to develop an ADC (LOP628), which found that LMJ729 can bind to K-cit from humans and monkeys, but does not cross-react with K-cit from mice. The safety of LMJ729 has been confirmed in the subsequent Good Laboratory Practice of Drug (GLP) monkey study. A report shows that 70% of SCLC patients have an increase in K-cit expression.

Based on the above results, a study has developed a fully humanized antibody NN2101 targeting K-cit, which can inhibit K-cit activation mediated by SCF through competitive binding with SCF.

Based on the above results, a study has developed a fully humanized antibody NN2101 targeting K-cit, which can inhibit K-cit activation mediated by SCF through competitive binding with SCF. After knocking down K-cit using siRNA, the binding rate of NN2101 to tumor cells also decreased. Through confocal microscopy analysis using lysosomal-associated membrane protein 1 (LAMP-1), it was confirmed that the NN2101/K-cit complex can be effectively endocytosed.

Next, the researchers connected DM1 to NN2101 through SMCC to generate NN2101-DM1 and found that NN2101-DM1 has a similar affinity for K-cit as NN2101. To verify the cytotoxicity of the ADC, NN2101-DM1 was applied to imatinib-sensitive and imatinib-resistant cell lines, respectively. The results showed that the proportion of S phase and G2/M phase cells in the cell lines treated with NN2101-DM1 was significantly higher than that in the control group, NN2101 treated, and IgG-DM1 treated imatinib sensitive and imatinib-resistant cell lines.

The study also found that NN2101-DM1 exhibited anti-tumor activity in mouse xenograft models [12].

This study suggests that NN2101-DM1 is a potential treatment for c-Kit-positive cancer, and further clinical trials can be conducted for SCLC in the future.

3.2.4 D3-GPC2-PBD ADC

A report suggests that GPC2 (glycan-2) is a cell surface oncoprotein transcriptionally regulated by V-Myc Avian Myelocytomatosis Viral Oncogene Neuroblastoma-Derived Homolog (MYCN), and is highly expressed on the surface of SCLC tumor cells.

To verify the expression of GPC2 on the surface of SCLC cells, a study compared the expression of GPC1-GPC6 in SCLC with normal lung tissue and found that the tumor-normal differential expression of GPC2 was most significant in the GPC family, and the expression level of GPC2 was related to the degree of tumor malignancy. The higher the expression of GPC2, the higher the degree of malignancy of the tumor. The researchers also investigated the impact of the phenotypic consequences of depleting GPC2 on SCLC cell lines and found that GPC2-depleted cells exhibited strong apoptosis induction and growth inhibition effects. It was found that GPC2 is a crucial regulatory protein for the growth of SCLC.

Due to the effective endocytosis of antibodies that bind to GPC2, researchers combined the whole humanized antibody D3 with the DNA-damaging agent PBD (pyrrolobenzodiazepine) to form a novel ADC.

The sequence similarity between the GPC2 homologs in humans and mice is 83%, and all D3-GPC2 antibody epitope residues are conserved. To verify the rationality of the mouse model as a safety analysis species for D3-GPC2-PBD ADC, the study aims to compare the binding ability of D3-GPC2-IgG1 with GPC2 on the surface of mouse and human cells. Researchers transfected human and mouse GPC2 cDNA into Human Embryonic Kidney 293T (HEK293T) cells and found that the binding of GPC2 to IgG1 was very similar and the sensitivity was almost the same in both mice and humans. This indicates that D3-GPC2-IgG1 has high specificity for both humans and mice, making mice a suitable initial species for verifying the safety of D3-GPC2-PBD ADC.

In Western blot tests, D3-GPC2-PBD ADC can enhance DNA damage and cell death in H526 and H1930 SCLC cells. Subsequently, researchers conducted D3-GPC2-PBD ADC treatment on mice carrying H526 xenografts, which resulted in robust and sustained tumor regression, significantly improving survival rate with no side effects [13].

3.2.5 JAM3 can serve as a potential target for ADC re-

search in the treatment of SCLC

A study focused on the feasibility of ADC targeting JAM3 found that SCLC cell lines typically express high levels of JAM3 and Synaptophysin (SYP) mRNA, and the expression of JAM3 is positively correlated with SYP. At the molecular subtype level, JAM3 can be expressed at the mRNA and protein levels of two SCLC subtypes (SCLC-N, SCLC-Y), both of which are inhibited by siRNA.

To verify the response of ADC to JAM3-positive SCLC cells, researchers conducted experiments using HSL156-DT3C. HSL156-DT3C conjugate is an ADC analogue that sustainably induces apoptosis in NC SiRNA-transfected Lu-135, SBC-5, and Lu-134A cells. In contrast, the ADC analogue has a poorer effect on JAM3 silenced SCLC cells.

Meanwhile, the study also found that knocking down JAM3 can inhibit the growth of SCLC cells. This study suggests that ADC targeting JAM3 may be a novel and effective therapeutic option for SCLC. The study has not yet proposed a clinically applicable ADC. In future research, efforts can be made to develop a targeted JAM3 for the treatment of SCLC ADCs [14].

4. Conclusion

In the current medical environment where standard treatment is not ideal, the emergence of ADC undoubtedly provides a lot of exploration space for the treatment of SCLC. There is also an endless stream of research in this area, and many studies have proposed ADC with hope for clinical application in the treatment of SCLC. However, most of the current research is still at the mechanism level, such as the study of the high-expression protein molecule JAM3 target on the surface of SCLC cells. This study suggests that JAM3 may be a potential target for the treatment of SCLC, but has not yet developed a drug that is truly suitable for clinical trials. A few studies that have proposed more mature ADC drugs have mostly focused on mouse model experiments and phase I clinical trials. The NN2101-DM1 and D3-GPC2-PBD ADC mentioned in this study have only been studied in mouse models. ABBV-011 has undergone phase one clinical trials but has not undergone further clinical exploration. Currently, only Rova-T targeting DLL3 has undergone phase three clinical trials but was ultimately discontinued.

In future research, further exploration can be conducted on targets overexpressed on the surface of SCLC tumor cells, or more comprehensive clinical trials can be conducted on the proposed ADC. Explore whether different ADCs have the same effect on different subtypes of SCLC, the reasons for their different effects, the cytotoxicity, safety, and human tolerance of different ADCs, and explore the most effective administration methods and nursing principles.

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