

Role of Epidemic Tumor Vaccine in Cold Tumors to Hot Tumors

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Abstract. Small cell lung cancer (SCLC) is a type of highly aggressive, prone to early metastasis and recurrence, neuroendocrine malignant tumor. The treatment effect of second-line therapy for patients with recurrence/refractory cases is limited, and new therapeutic targets and strategies are urgently needed. Delta-like ligand 3 (DLL3) is abnormally highly expressed on the surface of most SCLC cells, while its expression is limited in normal tissues, making it a promising therapeutic target. The DLL3/CD3 bispecific antibody can redirect T cells to the vicinity of the tumor, inducing the formation of immune synapses, T cell activation, and tumor cell lysis. Tarlatamab, as the first bispecific antibody targeting DLL3/CD3 to enter clinical trials and obtain regulatory approval, has completed the transformation from preclinical validation to key clinical studies. Studies have shown that tarlatamab has durable anti-tumor activity and manageable safety in patients with recurrent/refractory SCLC. This article reviews the biological basis of DLL3, the mechanism of action of DLL3/CD3 bispecific antibodies, as well as the preclinical and clinical research progress of tarlatamab, and assesses its efficacy and safety in heavily treated SCLC. Overall, DLL3/CD3 bispecific antibodies provide a new immunotherapy pathway for recurrent/refractory SCLC, achieving significant clinical relief and survival improvement. Future research needs to further optimize toxicity management, analyze resistance mechanisms, and screen for advantageous populations to promote its precise and sustainable clinical application.

Keywords: Small cell lung cancer; DLL3; CD3; bispecific antibody; Tarlatamab.

1. Introduction

Small-cell lung cancer (SCLC) is a highly aggressive neuroendocrine malignant tumor, accounting for 10% to 15% of all lung cancers. It is characterized by rapid growth, early metastasis, high recurrence rate, and poor prognosis [1]. Although platinum-based chemotherapy combined with immunotherapy has become the first-line standard regimen for extensive-stage SCLC in recent years, most patients still experience rapid disease progression after initial treatment, and the options for second-line treatment of relapsed/refractory SCLC are very limited, resulting in unsatisfactory overall efficacy and survival benefits [1-3]. Delta-like ligand 3 (DLL3) is a Notch signaling pathway-related molecule that is abnormally expressed on the surface of most SCLC cells, while its expression is limited in normal tissues. Therefore, it is considered a promising therapeutic target [4-6]. Tarlatamab, as a DLL3/CD3 bispecific T-cell engager antibody, can simultaneously bind to DLL3 on the surface of tumor cells and CD3 on the surface of T cells, redirecting T cells to the vicinity of tumor cells, thereby inducing T cell activation and tumor cell lysis. Preclinical and clinical studies have shown that tarlatamab exhibits good anti-tumor activity and manageable safety in relapsed/refractory SCLC.

In recent years, various DLL3-targeted monoclonal antibodies and antibody-drug conjugates have been studied in SCLC. For example, rovalpituzumab tesirine showed partial tumor shrinkage in early clinical trials, but toxicity and tolerability issues limited further application [7,8]. In contrast, tarlatamab achieves sustained immune activation through the dual-specificity T-cell redirecting mechanism, demonstrating significant anti-tumor effects in vitro and in vivo models, and showing sustained remission in early clinical studies, proving that DLL3/CD3 bispecific antibodies have high therapeutic potential in relapsed/refractory SCLC [9,10].

This article reviews the biological basis of DLL3 as a therapeutic target, the mechanism of action of DLL3/CD3 bispecific antibodies, and the research progress of tarlatamab in preclinical and clinical studies. The aim is to systematically summarize the efficacy, safety, and clinical application

experience of these antibodies, providing a theoretical basis for the optimization of treatment for relapsed/refractory SCLC and offering references for subsequent exploration of precise immunotherapy strategies.

2. The Biological Basis of DLL3 as a Therapeutic Target for SCLC

DLL3 is one of the ligands related to the Notch signaling pathway and is closely associated with the neuroendocrine differentiation of SCLC [4-6]. Functionally, the abnormal expression of DLL3 is not merely a change in a surface marker; it can affect the neuroendocrine phenotype, cell proliferation, and invasion and metastasis ability of SCLC by regulating the activity of the Notch signaling. Studies have found that the change in DLL3 expression is closely related to the Notch1/HES1 signaling and Snail-related invasive phenotype, suggesting that it may be involved in the tumor progression process. On the other hand, the state of the Notch pathway is also related to the immunophenotype of SCLC. Notch activation can be accompanied by enhanced antigen presentation and tumor intrinsic immune response, while the neuroendocrine dominant state represented by high DLL3 expression may be related to insufficient immune infiltration and an immunosuppressive microenvironment. Current histological and translational studies have observed an association between the expression level of DLL3 and immune cell infiltration and the characteristics of immune treatment response; however, whether DLL3 directly drives the remodeling of the immune microenvironment still needs further mechanism research to confirm [4-6]. Abnormal expression of DLL3 is associated with the inhibition of the Notch signal and the maintenance of the neuroendocrine phenotype, promoting the proliferation, migration, and invasion of SCLC cells, and possibly participating in the remodeling of the tumor immune microenvironment by affecting antigen presentation, T cell infiltration, and immune suppression status. However, the rovalpituzumab tesirine antibody-drug conjugate (ADC) drug targeting DLL3 failed to translate into survival benefits in later clinical studies; its failure is more likely related to toxic adverse reactions of the cytotoxic payload, narrow treatment window, and insufficient efficacy persistence, rather than the lack of value of the DLL3 target. Subsequently, the clinical benefit obtained by tarlatamab further supported the rationality of the DLL3-targeted strategy. Future studies should combine the expression level of DLL3, immunological microenvironment characteristics, and drug resistance mechanisms to conduct stratified and combined treatment research to identify the advantageous population and optimize the treatment plan. However, the clinical development process has indirectly demonstrated that the DLL3 target itself has good tumor-enrichment characteristics, and the problem lies more in the drug platform rather than the failure of the target [7,8]. Therefore, developing DLL3-targeted therapy using an immune redirection strategy different from ADC has clear theoretical rationality [7-9].

3. The Structural Characteristics and Mechanism of Action of DLL3/CD3 Bispecific Antibodies

The core design concept of the DLL3/CD3 bispecific antibody is to bind to DLL3 on the surface of tumor cells at one end and to CD3 on the surface of T cells at the other end, thereby establishing a "molecular bridge" between the tumor cells and T cells [9]. This bridging process not only shortens the spatial distance between the two types of cells but also promotes the formation of immune synapses, triggering downstream signal transduction of TCR/CD3, and ultimately inducing T cell activation, proliferation, and the release of cytotoxic effector molecules [9]. Tarlatamab belongs to the half-life extended BiTE platform. It extends the in vivo half-life through the Fc structure, making intermittent administration possible and improving the limitations of traditional short half-life BiTE continuous infusion [9]. Preclinical studies have shown that AMG 757 can induce the upregulation of activation-related molecules such as CD69, CD71, PD-1, and PD-L1, and promote the release of granzyme B, perforin, and inflammatory cytokines, ultimately mediating the lysis of DLL3-positive tumor cells [9].

4. The Progress of the Preclinical Research of Tarlatamab

Previous clinical studies have shown that AMG 757 exhibits significant and specific cytotoxic activity against DLL3-positive SCLC cells [9]. In vitro experiments demonstrated that AMG 757 could effectively induce human T cells to kill various DLL3-positive SCLC cell lines, including NCI-H211 and SHP-77 cell lines with extremely low DLL3 expression ($<1,000$ molecules/cell), with the half maximal effective concentration (EC50) remaining within the range of 18–203 pmol/L, indicating its potent killing ability even under low target expression conditions [9]. In co-culture systems, AMG 757 could significantly activate T cells, induce the expression of activation markers such as CD69, CD71, and PD-1 on CD4⁺/CD8⁺ T cells, enhance the release of granzyme B and perforin, and promote the secretion of pro-inflammatory cytokines, suggesting that its anti-tumor effect is not only dependent on direct contact but also involves a sustained immune amplification effect [9].

In vivo experiments, AMG 757 demonstrated strong tumor suppression effects in patient-derived xenograft (PDX) models and orthotopic mouse models [9]. In the LXFS 538 PDX model, mice treated with AMG 757 showed 98% tumor regression after a three-week treatment course, with 8/10 mice achieving complete remission ($P < 0.0001$), significantly superior to the control group [9]. In the SHP-77 orthotopic lung tumor model and NCI-H82 liver metastasis model, AMG 757 also significantly inhibited tumor growth and promoted the infiltration of CD4⁺/CD8⁺ T cells into the tumor tissue, while enhancing their activation state ($P < 0.05$ – 0.0001), indicating its ability to effectively mobilize the immune response in different tissues and metastatic foci for tumor clearance [9]. These results suggest that AMG 757 not only can efficiently kill DLL3-positive SCLC cells in vitro but also can trigger a persistent and systemic anti-tumor effect through a T-cell-mediated mechanism, providing a solid scientific basis for its subsequent clinical development [9].

5. Clinical Research Progress of Tarlatamab in Relapsed/Refractory SCLC

5.1. Early Clinical Exploration

The DeLLphi-300 Phase 1 study conducted by Paz-Ares et al. was the first key clinical evidence of tarlatamab in relapsed/refractory SCLC [10]. This study included a total of 107 patients, and the results showed an objective response rate (ORR) of 23.4%, a median duration of response (mDOR) of 12.3 months, a median progression-free survival (mPFS) of 3.7 months, and a median overall survival (mOS) of 13.2 months [10]. This study suggested that tarlatamab has a good potential for durable remission in heavily treated SCLC patients, laying the foundation for subsequent dose optimization and expansion validation [10].

This study adopted an open-label, dose escalation and dose expansion combined design, with the main purpose of evaluating the safety, pharmacokinetic characteristics, and preliminary anti-tumor activity at different dose levels. The patients included had received multiple lines of systemic treatment, and some patients had also received immune checkpoint inhibitors, thus being able to better reflect the clinical treatment challenges of patients with relapsed/refractory SCLC. The observed duration of response was long, indicating that the T-cell redirected killing induced by tarlatamab may not only cause early tumor shrinkage but also maintain relatively stable disease control in some patients. However, this study was a single-arm early trial, lacking direct control, and the number of patients at different doses was limited, so its efficacy signal still needs to be further verified in larger samples and randomized controlled studies. Future studies should focus on biomarkers for predicting benefits, treatment responses in patients with brain metastases, and early identification and standardized management of immune-related adverse reactions.

5.2. Dose Optimization and Key Phase II Study

The subsequent DeLLphi-301 study further compared the efficacy and safety of the 10 mg and 100 mg regimens [11]. The results showed that the ORR of the 10 mg group was 40%, while that of

the 100 mg group was 32%. Moreover, the overall benefit-risk ratio of the 10 mg regimen was better, so it gradually became the main recommended dose for subsequent development [11]. This result indicates that for DLL3/CD3 bispecific antibodies, a higher dose does not necessarily lead to better efficacy; instead, it may increase the burden of toxicity. Therefore, dose optimization is of particular importance in the development of such drugs [11].

DeLLphi-301, as a key phase II study, evaluated the efficacy and tolerability of different target doses in previously treated SCLC patients using a fixed dosing regimen, and comprehensively determined the appropriate dose through imaging efficacy assessment, disease remission duration, and adverse event monitoring. Unlike simply pursuing higher drug exposure, the results of this study suggest that the clinical efficacy of bispecific antibodies may depend on the balance between effective T-cell recruitment, sustained immune activation, and toxicity control. The 10 mg regimen can achieve a higher remission rate and demonstrate advantages in safety and treatment feasibility, making it more suitable as the recommended dosing regimen for subsequent research and clinical application. This result also suggests that future dose optimization should not only aim for the maximum tolerated dose but should also be evaluated comprehensively by considering pharmacodynamic changes, CRS occurrence characteristics, patient's underlying immune status, and tumor burden, thereby establishing a more individualized treatment strategy.

5.3. Continuous Benefits, Intracranial Activity, Phase III Validation and Regulatory Progress

The updated results of DeLLphi-300 published in 2024 further demonstrated that tarlatamab could maintain continuous clinical benefits in SCLC patients who had failed previous treatments and showed certain intracranial activity [12]. Considering the high incidence of brain metastasis in SCLC, this finding has strong clinical significance and suggests that tarlatamab may not only improve the control of systemic lesions but also bring additional value to central nervous system lesions [12].

This updated study supplemented the verification of the persistence of efficacy observed in the early trial by extending the follow-up period and further analyzing the disease control of patients with previous brain metastases. The emergence of the intracranial activity signal is of great value because brain metastasis is a common event in the course of SCLC and an important factor affecting quality of life and prognosis in subsequent treatment. Although the updated results suggest that tarlatamab may have potential advantages in controlling central nervous system lesions, the current relevant analysis is still limited by factors such as sample size, patient selection criteria, and imaging evaluation methods. In the future, further studies should be conducted in specialized research involving patients with active or stable brain metastases to clarify the intracranial remission rate, the duration of intracranial remission, and the optimal timing for combined application with local treatments such as radiotherapy.

The results of the DeLLphi-304 phase III clinical study in 2025 showed that for adult patients with extensive-stage SCLC who had received platinum-based chemotherapy and disease progression, the median overall survival in the tarlatamab treatment group was 13.6 months, while that in the chemotherapy control group was 8.3 months, with a death risk ratio of 0.60; in addition, the proportion of grade 3 or higher treatment-related adverse events in the tarlatamab group was significantly lower than that in the chemotherapy group (54% vs. 80%) [13]. Based on sufficient clinical efficacy and safety evidence, the US FDA granted accelerated approval for it in 2024 and converted it to traditional approval for patients with this indication in 2025 [14, 15]. This approval marks that tarlatamab has evolved from a potential candidate drug to a standard clinical treatment option with clear benefits for patients with extensive-stage SCLC and provides an important demonstration for the clinical application of DLL3-targeted immunotherapy strategies, verifying its effectiveness and controllable safety in real clinical settings [13-15].

The significance of the DeLLphi-304 study lies in its use of a randomized controlled design to directly compare tarlatamab with existing post-treatment regimens, thereby further converting the observed response signals in the earlier single-arm studies into clear evidence of survival benefits. The improvement in overall survival and the decrease in the proportion of high-grade adverse events

indicate that the drug not only has anti-tumor activity but also may improve the overall benefit-risk balance of post-relapse treatment for patients. At the same time, regulatory approval does not mean that related clinical issues have been completely resolved. In the future, further evaluation of efficacy and safety in different populations, especially for patients with brain metastases, elderly patients, and those with underlying diseases, should be conducted; the feasibility of its combination with immune checkpoint inhibitors, chemotherapy, or radiotherapy should also be explored, and the biological basis for acquired resistance should be clarified to promote continuous optimization of treatment strategies.

6. Safety Features and Management Points

The toxicity profile of Tarlatamab is highly correlated with its mechanism of action, with the most typical adverse reaction being cytokine release syndrome (CRS) [10,11]. The occurrence of CRS mainly results from Tarlatamab's dual specificity binding to DLL3 and T cell CD3, redirecting T cells in the body to tumor cells and triggering the release of a large number of cytokines, including IL-6, IFN- γ , and TNF- α , thereby causing a systemic inflammatory response [9,10]. Clinical studies have shown that CRS is most common in the first administration cycle, with an overall incidence of 50% to 56%, mostly mild to moderate (grades 1 to 2), usually appearing within 1 to 3 days, and reversible upon discontinuation or intervention [10,11]. To reduce the risk of CRS, a step-up dosing strategy is adopted in clinical practice, starting with a lower dose and gradually increasing to the target dose, while adjunctive prophylactic glucocorticoids, intravenous fluid replacement, and symptomatic treatment are provided [10,11]. When CRS reaches grade 2 or above, or when there is persistent high fever, hemodynamic instability, tocilizumab (a IL-6 receptor antagonist) can be used to quickly control the symptoms [10,11]. The timely identification and standardized intervention of CRS help to reduce the risk of serious adverse events and provide conditions for patients to continue treatment, allowing them to maintain significant tumor regression and T cell activation effects [10,11]. Overall, although CRS is the most common manifestation of immune-related toxicity of Tarlatamab, through scientific dose adjustment and intervention strategies, its risk is controllable, providing a reliable guarantee for the safe promotion of its clinical application [10,11].

Although Tarlatamab has achieved significant progress, the application of DLL3/CD3 bispecific antibodies in relapsed/refractory SCLC still has several issues. Firstly, although there is a certain correlation between DLL3 expression level and efficacy, the optimal threshold and standardized detection system have not yet been fully established [10-12]. Secondly, the molecular basis of resistance after initial sensitivity remains unclear, including antigen heterogeneity, T cell exhaustion, and immune suppression in the tumor microenvironment, which may all be involved. Thirdly, the optimal positioning of this type of drug in brain metastasis, frontline treatment, combination with PD-L1 inhibitors or chemotherapy still requires further research [12,13]. Future research should place greater emphasis on biomarker stratification, combined treatment strategies, real-world safety monitoring, and analysis of resistance mechanisms to promote DLL3/CD3 bispecific antibodies from "effective" to "precise and sustainable benefit".

7. Conclusion

Overall, this article systematically reviews the biological basis of DLL3 as a therapeutic target for SCLC, the mechanism of action of DLL3/CD3 bispecific antibodies, and the development process of tarlatamab from preclinical studies to clinical validation. The DLL3/CD3 bispecific antibodies represented by tarlatamab provide a new path for relapsed/refractory SCLC that is distinct from traditional chemotherapy, ADCs, and simple immune checkpoint inhibitors. Their core value lies in combining the tumor selectivity of DLL3 with the T-cell redirected killing mechanism, thereby achieving clinically significant durable remission and survival improvement in heavily treated patients. Currently, tarlatamab has completed key milestones from preclinical validation, early clinical exploration, to phase III confirmation and regulatory approval, demonstrating good

translational potential. This progress not only provides new treatment options for patients with limited treatment options in relapsed/refractory SCLC, but also further indicates that DLL3 does not lose its therapeutic value due to the setbacks in previous ADC research. The drug platform and immune effect mechanism may be important factors determining clinical benefits. However, current understanding of the relationship between DLL3 expression levels and efficacy, the impact of the immune microenvironment, and the mechanism of acquired resistance is still insufficient, and application evidence for some special patient groups also needs to be accumulated. In the future, if issues such as toxicity management, resistance mechanisms, and selection of advantageous populations can be further addressed, and combined treatment and real-world research are strengthened, DLL3/CD3 bispecific antibodies are expected to become an important component of precise immunotherapy for SCLC.

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