Editorial Commentary

DLL3-targeted CAR T-cell therapy in pre-clinical models for small cell lung cancer: safety, efficacy, and challenges

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Chimeric antigen receptor (CAR) T-cell therapy has achieved complete responses in patients with hematological malignancies (1). However, it has shown only limited success in patients with solid tumors, owing to the lack of an ideal antigen to target with no on-target, off-tumor toxicity and to poor infiltration, among other hurdles (2). In solid tumors, such as small cell lung cancer (SCLC), with metastases in multiple organs at the time of presentation, it has been difficult to achieve efficacy without generating on-target, off-tumor toxicity in the normal tissue of the organs that harbor the metastases (3).

Immunotherapies have been investigated to treat patients with metastatic SCLC. Many of these immunotherapies target delta-like ligand 3 (DLL3), which is a notch ligand that is overexpressed on SCLC cells but is expressed in limited amounts on normal cells (4,5). Some of these immunotherapies use bispecific T-cell engagers (BiTEs)—antibodies that can simultaneously bind to an antigen on tumor cells and a surface molecule on T cells to induce tumor lysis (6), thus harnessing the host's T-cell immune response to enhance antitumor activity. One such BiTE that targets DLL3 is tarlatamab (previously known as AMG 757; *Table 1*) (7). In preclinical models, tarlatamab showed significant antitumor efficacy against several SCLC cell lines and in patient-derived xenograft models and had manageable adverse events (8). To potentiate the clinical benefit of this agent, combination therapy that includes tarlatamab plus anti-programmed cell death protein 1 (PD1) or anti-programmed death-ligand 1 (PD-L1) checkpoint inhibitors, or both, is being investigated (NCT03319940, NCT04885998, NCT05740566, and NCT05361395). Another T-cell engager that is being investigated in SCLC is HPN328, which is a trispecific T-cell engager designed to engage albumin to increase the half-life of the molecule DLL3 on tumor cells and CD3 on T cells, with the goal of promoting activation and proliferation of T cells (NCT04471727). In an ongoing clinical trial, no grade ≥3 adverse events were observed in patients with SCLC who were treated with HPN328 who were experiencing a >30% decrease in tumor diameter, or in two patients with had stable disease (NCT04471727). Some of the disadvantages of BiTE therapies are a short half-life, the potential to induce cytokine release syndrome, and the inability to promote functional persistence or prevent or rescue T cells from exhaustion (9).

Alternatively, immune-effector cell therapies that target

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Table 1 Preclinical and clinical studies for T-cell engagers targeting DLL3 in small cell lung cancer

Status	Cell-based immunotherapy	Model	Reference/NCT#
Preclinical	Tarlatamab (AMG 757)	NSG mice administered with patient-derived SCLC cells were treated with AMG 757	Giffin MJ *et al*., *Clinical Cancer Research*, 2021 (7)
Clinical	Tarlatamab (AMG 757)	Phase I study of AMG 757 in combination with anti-PD1 therapy	NCT03319940
		Phase I study of AMG 757 in combination with anti-PD1 therapy	NCT04885998
		Phase III study of patients with SCLC given AMG 757 or chemotherapy	NCT05740566
		Phase Ib study of tarlatamab in combination with chemotherapy and anti-PD-L1	NCT05361395
		Phase II study of AMG 757	NCT05060016
	HPN328	Phase I/II study of HPN328 in combination with atezolizumab	NCT04471727

DLL3, delta-like ligand 3; NCT, National Clinical Trial; NSG, non-obese diabetic scid gamma; PD1, programmed cell death protein 1; SCLC, small cell lung cancer; PD-L1, programmed death-ligand 1.

DLL3 are also being investigated. Among the advantages of CAR T-cell therapies are that they can expand *in vivo*, can functionally persist, and can be engineered to resist exhaustion, in contrast to BiTE therapies (2).

In preclinical studies, DLL3-CAR-natural killer (NK) cells showed significant antitumor efficacy and good tumor infiltration in SCLC subcutaneous xenograft models (10). Nevertheless, flank tumors do not capture the complexity of the tumor microenvironment in the lung; therefore, orthotopic models would be more suitable for these studies. CAR-NK cells that target DLL3 were recently translated into a phase I clinical trial for patients with relapsed or refractory extensive-stage SCLC (NCT05507593). Another DLL3-targeted CAR T-cell therapy of note is AMG 119, which is being investigated in combination with tarlatamab in a phase I clinical trial (NCT03392064) (11). The group, Jaspers *et al.* (12), investigated DLL3-targeted CAR T cells and found that CAR T-cell efficacy was enhanced with the secretion of interleukin (IL)-18 in both orthotopic and metastatic models of SCLC. This study showed that IL-18-mediated pro-inflammatory response improves the median survival of tumor-bearing mice, compared with treatment with DLL3-targeted CAR T cells alone. Additionally, Chen *et al.* described *in vivo* studies evaluating the efficacy of DLL3 targeting CAR T cells and a DLL3 bispecific antibody in xenograft models (13). Interestingly, the group showed that in combination with anti-PD1 checkpoint blockade, their DLL3 bispecific antibody showed increased tumor suppression activity and prolonged survival while DLL3 targeting CAR T cells did not show a significant benefit from the addition of anti-PD1 checkpoint blockade (13) (*Table 2*).

A recently published study by Zhang *et al.* (14) demonstrated the safety and efficacy of DLL3-targeted CAR T cells with gene editing in the T-cell receptor α constant locus to avoid graft versus host disease in preclinical models. The authors evaluated more than 50 clones of anti-DLL3 in CAR T cells to later incorporate a rituximab-base, off-switch approach to control the activation of CAR T cells in the case of adverse activity.

Using a subcutaneous tumor model, Zhang *et al.* showed that three CAR T-cell clones (Clone 4, 7, and 9) successfully controlled tumor growth. Although subcutaneous models are useful for proof-of-principle studies, the limitation of subcutaneous models is that the models do not faithfully mimic clinical disease. In contrast, results from clinically relevant models harboring disease at multiple sites could be more relevant, especially in terms of CAR T-cell efficacy in solid tumor models (15,16). The authors investigated the efficacy of DLL3-targeted CAR T cells in a systemic tumor model established by SCLC cell lines treated via tail vein to mimic a metastatic-stage disease commonly seen in the clinical setting. Even though the same three clones of CAR T cells were able to delay tumor growth, only one clone of CAR T cells was associated with tumor-free survival (Clone 4-RSR).

The authors' emphasis on evaluating the safety of DLL3-targeted CAR T cells is commendable. Zhang *et al.* assessed the ability of the soluble binding domains from the three CAR T-cell clones against 36 normal human tissues and found that Clone 7 showed cytoplasmatic binding in

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Table 2 Preclinical and clinical studies for cell-based immunotherapies targeting DLL3 in small cell lung cancer

Status	Cell-based immunotherapy	Model	Reference/NCT#
Preclinical	DLL3 targeting CAR-NK	NSG mice with SCLC lung or flank tumor were treated with intravenous or subcutaneous administration of NK cells, respectively	Liu M *et al*., *Journal of Leukocyte Biology*, 2022 (10)
	DLL3 targeting CAR	NSG mice with lung or orthotopic SCLC tumor were treated via tail vein or intrathoracically	Jaspers J *et al*., *Journal of Clinical Investigation*, 2023 (12)
	DLL3 targeting CAR (in combination with DLL3 bispecific ab and anti-PD1 ab)	NSG mice were treated subcutaneously in a flank tumor model	Chen X *et al*., *Journal for Immunotherapy of Cancer*, 2020 (13)
Clinical	DLL3 targeting CAR-NK	Phase I study of DLL3-CAR-NK cells for relapsed or refractory extensive-stage SCLC	NCT05507593
	DLL3 targeting CAR (AMG 119)	Phase I study of DLL3 targeting CAR T cell (AMG 119) for SCLC	NCT03392064

DLL3, delta-like ligand 3; NCT, National Clinical Trial; CAR, chimeric antigen receptor; NK, natural killer; NSG, non-obese diabetic scid gamma; ab, antibody; PD1, programmed cell death protein 1; SCLC, small cell lung cancer.

pituitary, skin, and ovary tissues, whereas Clone 4 showed binding in the pancreas and placenta. A subsequent study was performed to clarify possible inconsistencies in the tissue cross-reactivity assays. Orthogonal assays revealed that only Clone 7 showed no off-target binding in an array of more than 5,000 membrane proteins. These findings led the authors to investigate the toxicity of DLL3-targeted CAR T cells in an *in vivo* model, in which tumor-bearing mice treated with CAR T cells showed infiltration into the pituitary gland; however, the hormone-secretion function was not affected. By evaluating the toxicity of human DLL3-targeted CAR T cells in mouse tissue, the authors provided information about the cross-reactivity of scFv-DLL3 antibodies in patients and mice; however, the homology of the epitopes in both species is unknown. These results show that the affinity of scFv-DLL3 antibodies is not the same in all clones, and the *in vivo* results show that human DLL3-CAR T cells can kill tumor cells with high antigen expression of murine DLL3; but, as the authors mentioned, the pituitary gland does not have the same antigen density. It is plausible that human DLL3-targeted CAR T cells may not be able to kill cells expressing a low density of murine DLL3 as efficiently as they would cells expressing a high density of DLL3.

Along with the translationally relevant strategy to develop effective DLL3-targeted CAR T-cell constructs, Zhang *et al.* provided important progress in presenting knowledge about safety and efficacy that should be considered before proceeding to a clinical trial.

In summary, CAR therapies that target DLL3 have been shown to be effective *in vitro* and *in vivo* in flank tumors and orthotopic and metastatic models of SCLC. These therapies have also been shown to be safe. Taken together, these findings support the use of DLL3-targeted CAR T-cell therapies to treat patients with extensive-stage SCLC in a clinical setting.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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