Editorial Commentary

Small cell, big promises: targeting small cell lung cancer with CAR T cells

Estela Noguera-Ortega[1,2], Steven M. Albelda[1,2]

[1]Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [2]Pulmonary and Critical Care Division, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Correspondence to: Steven M. Albelda, MD. Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, 3400 Civic Center Blvd, Philadelphia, PA 19104, USA; Pulmonary and Critical Care Division, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, 3450 Hamilton Walk, Philadelphia, PA, USA. Email: albelda@pennmedicine.upenn.edu.

Comment on: Zhang Y, Tacheva-Grigorova SK, Sutton J, *et al.* Allogeneic CAR T Cells Targeting DLL3 Are Efficacious and Safe in Preclinical Models of Small Cell Lung Cancer. Clin Cancer Res 2023;29:971-85.

Keywords: Small cell lung cancer (SCLC); chimeric antigen receptor T cells (CAR T cells); delta-like ligand-3 (DLL3)

Submitted Jan 03, 2024. Accepted for publication Feb 19, 2024. Published online Apr 24, 2024.
doi: 10.21037/tlcr-24-10
View this article at: https://dx.doi.org/10.21037/tlcr-24-10

Introduction

Although great successes have been achieved treating leukemias, lymphoma, and multiple myeloma using chimeric antigen receptor T (CAR T) cells, resulting in multiple regulatory agency approvals, reports showing efficacy in solid tumors are much fewer. To date, some promising results have been seen in neuroblastoma (1) and gastro intestinal (GI) tumors (2), but lung cancer, and specifically small cell lung cancer (SCLC) has not been successfully treated. This is unfortunate, as successful treatments for this deathly cancer remain very limited.

There are many postulated reasons for this lack of success in solid tumor. Key limitations include: (I) lack of highly specific targets for the CAR T cells, (II) poorly functioning T cells that produce suboptimal CAR T cells, (III) poor trafficking of the CAR T cells into tumors, (IV) tumor heterogeneity, (V) poor persistence of the cells, and (VI) rapid development of CAR T cell hypofunction. SCLC presents some additional challenges as the disease is often extensive and rapidly progressive requiring rapid preparation of the CAR T cells [reviewed in (3)].

A recent paper by Zhang *et al.* entitled "Allogeneic CAR T Cells Targeting DLL3 Are Efficacious and Safe in Preclinical Models of Small Cell Lung Cancer" (4) describes a sophisticated, multipronged approach for SCLC that can serve as an example for the development of CAR T cells.

Finding the right T cells

The authors have elected to address some of these important solid tumor CAR T cell challenges by using allogeneic T cells. Off-the-shelf CAR T cell approaches provide a ready-to-use clinical product that can be derived from cells from young healthy donors in large scale, avoiding delays, cutting costs (by eliminating the need for a personalized product for each patient) and generating a consistent, highly effective cell therapy product (5,6).

However, allogenic CAR T cell products have a number of potential issues. By using T cells from a different donor to generate CAR T cells and infusing them into the cancer patient, foreign cells are being transplanted into the donor. This results in the host immune system potentially rejecting the infused product—via host versus graft (HvG) rejection, but also, since the transplanted cells are T cells, these cells can also mount an immune response against the host, resulting in graft versus host disease (GvHD) (7).

Zhang and collaborators approach these two potential limitations by using a previously described gene-editing methodology—Transcriptional Activator-Like Effector Nuclease (TALEN) to knockout genes to avoid each problem. To limit GvHD, they "knock out" the T-cell receptor α constant (TRAC) locus that encodes for the T cell receptor of the infused CAR T cells making them non-reactive against the host cells (4). Of note, other

investigators have successfully used this strategy, knocking out the TRAC loci and at the same time inserting the CAR into the same locus, where it results in more uniform expression and prevents tonic signaling (8). Similar approaches are currently being evaluated in clinical trials (4,7).

To enhance engraftment, CAR T cell persistence, and diminish HvG rejection, this group used lymphodepletion/immunosuppression using a pan-T cell anti-CD52 antibody. However, to prevent the CAR T cells from also being depleted by the antibody, they genetically modified the T cells using TALENs to remove CD52. This strategy was found to be effective in clinical trials (9). Both of these approaches are being developed by Allogene Therapeutics for use in other CAR T cells targeting CD19, BCMA, and CD70. An alternative approach, used by others to prevent HvG rejection, has been to knock down the beta2 microglobulin gene which prevents the surface expression of human leukocyte antigen (HLA) molecules on the surface of the T cells (10). Although this prevents them from being recognized by endogenous CD8 T cells, it does make more susceptible to NK cell mediated attack (11).

Finding the right antigen

The main focus of the manuscript is to validate a SCLC target called delta-like ligand-3 (DLL3) and to optimize CAR T cells expressing DLL3 single chain antibodies.

The three key factors to consider for CAR target antigen selection are specificity, expression levels, and stability of the antigen (12). In terms of specificity, DLL3 expression in normal tissues (e.g., neurons, pituitary cells, testis cells) is sparse and exclusively intracellular, particularly on membranes of the Golgi apparatus and cytoplasmic vesicles (13,14). It should be noted that unlike endogenous T cells, CAR T cells can only react with antigens that are expressed on the cell surface. However, in high-grade neuroendocrine tumors, like SCLC, DLL3 is upregulated and expressed on the cell membrane. In SCLC, this cell surface expression is robust across different stages of disease and lines of therapy as DLL3 has demonstrated a stable expression along time by repeated biopsies (15) making DLL3 a suitable CAR T cell target. This stability may be because DLL3 has been demonstrated to promote cell proliferation and mediate migration and invasion—although its prognosis value is under debate with studies with opposing conclusions (14,16).

However, despite good specificity, one limitation of this new target protein is lack of uniform expression resulting in tumor heterogeneity. Rojo *et al.* evaluated DLL3 expression by immunohistochemistry in more than 1,000 SCLC patient samples. The criterion to determine DLL3 positivity was ≥25% of tumor cells which was met by 85% of the patients. However, only 68% of the patients had expression on over 75% of tumor cells (13). Lack of uniform expression is worrisome because unless this new DLL3-CAR T cell therapy induces antigen spreading that mounts a robust immunological bystander effect response, patients will likely experience antigen escape and relapse with DLL3 negative tumors (17,18). An immunological bystander effect may be particularly difficult to generate in their proposed protocol because it uses an anti-CD52 antibody which may result in prolonged lymphodepletion. Although this may enhance engraftment and persistence, it may limit the generation of new anti-tumor antibodies and T cells.

Finding the right CAR with regard to efficacy

Once a suitable target was identified, the authors needed an effective single-chain variable fragment (scFv) antibody against DLL3. Despite a decade of work, there are no clear rules of selection, so antibody identification remains empiric. For example, the affinity of the antibody towards its antigen does not necessarily predict the avidity of the CAR molecule (3). Accordingly, the authors generated a large panel of anti-DLL3 monoclonal antibodies, using transgenic mice so that the antibodies created would be human rather than murine. They selected antibodies with species cross-reactivity (allowing animal efficacy and toxicology studies), high specificity, and that had a wide range of affinities. Over 50 scFv fragments were cloned into a standard second-generation CAR plasmid (with the CD3zeta and 41BB cytoplasmic domains) and lentiviruses produced. Clones that showed >30% T cell surface expression were selected to be cultured with target cell lines expressing a range of DLL3 expression densities for short term and long term (rechallenge) assays. The six best performing clones were chosen for testing in murine SCLC tumor models that had subcutaneous and metastatic tumors and the three most effective candidates chosen.

Finding the right CAR with regard to safety

For additional safety considerations, each CAR had rituximab mimotopes inserted allowing potential removal of the CAR T cells using the antibody rituximab, if needed. The addition of the mimotopes in the scFv sequence

significantly changed the behavior of the CAR T cells, so the lead constructs were reevaluated for efficacy.

To further explore safety issues, the authors not only addressed concerns about on-target off-tumor toxicities but also off-target toxicities (19). For the latter, soluble scFv from the top three clones were screened for binding against a panel of 36 normal tissues by immunohistochemistry. Two of the three clones appeared specific. They then studied the antibodies from these two clones using a commercially available assay in which 5,000 human plasma membrane proteins are expressed in HEK293 cells (Retrogenix). Both were deemed to show acceptably specific binding characteristics. Performing these kind of studies using the scFv from the lead construct instead of using the original antibody clone is critical since, as mentioned before, changes in the sequence might have significant impact on the affinity to the antigen (20).

Finally, it is also imperative that the CAR T cells that are going to be potentially used in the clinical trials can be used in immunodeficient preclinical models in order to predict any on-target off-tumor toxicities (21). Since the clones chosen were cross reactive with mice, formal murine toxicology studies were performed by injecting CAR T cells from their two optimal clones intravenously at high doses into naïve immunodeficient mice. DLL3 mRNA is expressed in normal pituitary and brain tissue, so special attention was paid to these organs. No toxicity was observed, however, toxicity may be underestimated in the absence of CAR activation or target-driven expansion, so the study was repeated in tumor-bearing mice. Again, no toxicity was noted. Although some human T cells were observed in the pituitary, no tissue damage was observed, and pituitary hormone levels were normal. They concluded that their top candidates were efficacious and safe and should be moved into clinical development.

Limitations

Although, this is a compelling and well-done preclinical study, the ultimate value of this approach will need to be confirmed in clinical trials. The allogeneic CAR T cell platform remains unproven so far in terms of engraftment and persistence, as does the use of an anti-CD52 antibody to induce lymphodepletion compared to the standard use of flucytosine/cyclophosphamide. The acquisition of CAR T cells hypofunction was not addressed, and the important issue of tumor heterogeneity remains, since a large percentage of SCLC cells do not appear to express DLL3. Finally, there has been mixed success in a number of DLL3-based clinical trials. A DLL3-targeted antibody drug conjugate (rovalpituzumab) that showed promising results in a phase I trial, had an unfavorable analysis due to side effects related to the drug conjugate interaction with DNA. A DLL3-targeted bispecific T-cell engager through the CD3 molecule showed some clinical response and acceptable tolerability in early phase trials (22-25). Most interestingly, a phase 1 study of AMG119, a DLL3-targeted CAR T cell, was initiated by Amgen, but then suspended in 2021 after enrolling just five patients for unclear reasons, despite a recently published report showing safety, good T cell proliferation, and one partial response in the higher dose cohort (23).

Conclusions

This paper is significant for a number of reasons. First, it additionally validates DLL3 as a promising target for the treatment of SCLC. More importantly, it provides a textbook example of the optimal way to develop CAR T cells. Key positive features were: (I) testing a large number of humanized antibodies (to prevent immune responses against the CAR) that had cross reactivity with mouse and nonhuman primate (NHP) allowing efficacy and toxicity studies, (II) selection of clones for *in vitro* efficacy via both acute and repetitive killing assays, as well as *in vivo* models, and (III) extensive safety testing tailored to the specific antigen including antibody binding studies to tissues and cells, as well as murine toxicology studies in naïve and tumor bearing animals. Given this careful and comprehensive analysis, and the encouraging preliminary results reported from the Amgen AM119 trial, we agree that this DLL3 CAR should be moved to clinical trials, and we look forward to seeing the results.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Translational Lung Cancer Research*. The article has undergone external peer review.

Peer Review File: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-10/prf

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-10/coif). The authors have no conflicts of interest to declare.

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.

References

1. Del Bufalo F, De Angelis B, Caruana I, et al. GD2-CART01 for Relapsed or Refractory High-Risk Neuroblastoma. N Engl J Med 2023;388:1284-95.
2. Mackensen A, Haanen JBAG, Koenecke C, et al. CLDN6-specific CAR-T cells plus amplifying RNA vaccine in relapsed or refractory solid tumors: the phase 1 BNT211-01 trial. Nat Med 2023;29:2844-53.
3. Albelda SM. CAR T cell therapy for patients with solid tumours: key lessons to learn and unlearn. Nat Rev Clin Oncol 2024;21:47-66.
4. Zhang Y, Tacheva-Grigorova SK, Sutton J, et al. Allogeneic CAR T Cells Targeting DLL3 Are Efficacious and Safe in Preclinical Models of Small Cell Lung Cancer. Clin Cancer Res 2023;29:971-85.
5. Depil S, Duchateau P, Grupp SA, et al. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. Nat Rev Drug Discov 2020;19:185-99.
6. Metelo AM, Jozwik A, Luong LA, et al. Allogeneic Anti-BCMA CAR T Cells Are Superior to Multiple Myeloma-derived CAR T Cells in Preclinical Studies and May Be Combined with Gamma Secretase Inhibitors. Cancer Res Commun 2022;2:158-71.
7. Cutmore LC, Marshall JF. Current Perspectives on the Use of off the Shelf CAR-T/NK Cells for the Treatment of Cancer. Cancers (Basel) 2021;13:1926.
8. Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature 2017;543:113-7.
9. Benjamin R, Jain N, Maus MV, et al. UCART19, a first-in-class allogeneic anti-CD19 chimeric antigen receptor T-cell therapy for adults with relapsed or refractory B-cell acute lymphoblastic leukaemia (CALM): a phase 1, dose-escalation trial. Lancet Haematol 2022;9:e833-43.
10. Das S, Valton J, Duchateau P, et al. Stromal depletion by TALEN-edited universal hypoimmunogenic FAP-CAR T cells enables infiltration and anti-tumor cytotoxicity of tumor antigen-targeted CAR-T immunotherapy. Front Immunol 2023;14:1172681.
11. Liao NS, Bix M, Zijlstra M, et al. MHC class I deficiency: susceptibility to natural killer (NK) cells and impaired NK activity. Science 1991;253:199-202.
12. Wei J, Han X, Bo J, et al. Target selection for CAR-T therapy. J Hematol Oncol 2019;12:62.
13. Rojo F, Corassa M, Mavroudis D, et al. International real-world study of DLL3 expression in patients with small cell lung cancer. Lung Cancer 2020;147:237-43.
14. Tanaka K, Isse K, Fujihira T, et al. Prevalence of Delta-like protein 3 expression in patients with small cell lung cancer. Lung Cancer 2018;115:116-20.
15. Farago A, Isse K, Drapkin B, et al. P3.12-02 Dynamics of DLL3 and ASCL1 Expression in SCLC Over Disease Course. J Thorac Oncol 2018;13:S970-1.
16. Furuta M, Kikuchi H, Shoji T, et al. DLL3 regulates the migration and invasion of small cell lung cancer by modulating Snail. Cancer Sci 2019;110:1599-608.
17. Gulley JL, Madan RA, Pachynski R, et al. Role of Antigen Spread and Distinctive Characteristics of Immunotherapy in Cancer Treatment. J Natl Cancer Inst 2017;109:djw261.
18. Klampatsa A, Leibowitz MS, Sun J, et al. Analysis and Augmentation of the Immunologic Bystander Effects of CAR T Cell Therapy in a Syngeneic Mouse Cancer Model. Mol Ther Oncolytics 2020;18:360-71.
19. Flugel CL, Majzner RG, Krenciute G, et al. Overcoming on-target, off-tumour toxicity of CAR T cell therapy for solid tumours. Nat Rev Clin Oncol 2023;20:49-62.
20. Greenman R, Pizem Y, Haus-Cohen M, et al. Shaping Functional Avidity of CAR T Cells: Affinity, Avidity, and Antigen Density That Regulate Response. Mol Cancer Ther 2021;20:872-84.
21. Mhaidly R, Verhoeyen E. Humanized Mice Are Precious Tools for Preclinical Evaluation of CAR T and CAR NK Cell Therapies. Cancers (Basel) 2020;12:1915.
22. Owen DH, Giffin MJ, Bailis JM, et al. DLL3: an emerging target in small cell lung cancer. J Hematol Oncol

2019;12:61.
23. Zhou D, Byers LA, Sable B, et al. Clinical Pharmacology Profile of AMG 119, the First Chimeric Antigen Receptor T (CAR-T) Cell Therapy Targeting Delta-Like Ligand 3 (DLL3), in Patients with Relapsed/Refractory Small Cell Lung Cancer (SCLC). J Clin Pharmacol 2024;64:362-70.
24. Leonetti A, Facchinetti F, Minari R, et al. Notch pathway in small-cell lung cancer: from preclinical evidence to therapeutic challenges. Cell Oncol (Dordr) 2019;42:261-73.
25. Paz-Ares L, Champiat S, Lai WV, et al. Tarlatamab, a First-in-Class DLL3-Targeted Bispecific T-Cell Engager, in Recurrent Small-Cell Lung Cancer: An Open-Label, Phase I Study. J Clin Oncol 2023;41:2893-903.

Cite this article as: Noguera-Ortega E, Albelda SM. Small cell, big promises: targeting small cell lung cancer with CAR T cells. Transl Lung Cancer Res 2024;13(4):956-960. doi: 10.21037/tlcr-24-10