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Enhancing Solid Tumor Immunotherapy: A Multi-Model Strategy Integrating Synapse-Tuned CAR Cells with Oncolytic Virotherapy and Immune Checkpoint Modulation

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Abstract:

Although past successes have been achieved in chimeric antigen receptor (CAR) T cell therapies in the past decade, especially in the field of hematology, they are not without challenges. CAR T cell therapies are fraught with problems for treating solid tumors such as melanoma, a frequently fatal skin cancer with increasing incidence in multiple regions. In 2020, there were a total of 325,000 new cases and around 57,000 deaths associated with the disease being reported (Saginala et al., 2021). The ineffectiveness of CAR T therapy for treating melanoma and other solid tumors can be attributed in part to the tumor's immunosuppressive microenvironment, physical barriers, antigen heterogeneity, poor T cell infiltration and dynamic tumor biology. Therefore, in this study we propose an approach aimed at increasing the activation of CAR T cells and help CAR T cells to eliminate melanoma more effectively. Building on recently published work we propose to add a post-synaptic density-95, discs large and zona occuldens (PDZ) binding motif to the C-terminus of the CAR and CAR T cells are preloaded with oncolytic poliovirus. In our proposed system poliovirus would be engineered to express a Cas9 system to target PD-L1 gene and inhibit the expression of PD-L1, a well-known immune checkpoint protein. Overall, with the precise targeted killing of melanoma by poliovirus and enhanced activation of CAR T cells, this combined therapy holds promise to significantly improve the efficacy of solid tumor immunotherapy. This comprehensive approach offers promising directions for the development of more effective treatment strategies and their clinical translation.

Keywords: melanoma, CAR T cells, poliovirus, PD-L1, combined therapy

1. Introduction

Solid tumor is one of the most significant challenges in tumor therapies and chimeric antigen receptor (CAR) T cell therapies are not successful when facing solid tumors due to the poor T cell infiltration, immunosuppressive tumor microenvironment and the resilience of solid tumors to conventional CAR therapies. Although CAR T cell therapies have achieved success in treating blood cancer, their efficacy against solid tumors has been hindered by these intrinsic barriers (Chockley et al., 2023).

With the development of oncolytic virus (OV) therapy and CAR cell engineering, new approaches for overcoming challenges mentioned above emerge (Evgin et al., 2022). OVs can infect and kill tumour cells selectively, in that case, OVs have shown effectiveness to modulate the tumor microenvironment, which can promote the activity of CAR T cells. Recent studies have shown that by recognizing viral antigens, the native T cell receptor (TCR) of CAR T cells can be stimulated, which can promote CAR-directed antitumour function, enhance the proliferation of CAR T cells and produce long-lasting memory phenotypes in immunocompetent mouse models, indicating that OVs can promote the ability of CAT T cells. This approach has shown potential in prolonging survival in models of melanoma and glioma (Evgin et al., 2022).

Melanoma is a type of skin cancer that mostly appears in the skin. In 2020, the reported incidence of melanoma was 3.4 out of 100,000 with a mortality rate of 0.55 of 100,000 (Huang et al., 2023). Case number has increased greatly in European countries, but overall mortality has decreased (Huang et al., 2023). Several lifestyle choices can increase the risk of melanoma, including excessive sun exposure, heavy smoking, alcohol abuse, and dietary unbalance (Huang et al., 2023). Besides, genetic factors can also increase the risk of melanoma. For example, the pathogenic variants of gene named cyclin-dependent kinase inhibitor 2A (CDKN2A) can cause the development of melanoma (NIH, 2024).

OVs and CAR T cells are potential and innovative therapies to treat Melanoma as OVs can only lyse tumor cells without hurting normal cells and CAR T cells can be activated by tumor antigens and recognize and kill tumor cells precisely.

In this study we aimed to develop new tumor therapy by combining OVs and CAR T cells together and our combined new therapy is inspired by the following three studies.

2. Study 1: An oncolytic virus–T cell chimera for cancer immunotherapy (Chen et al., 2024)

This study describes a new approach to strengthen oncolytic adenoviruses (OAs) in cancer treatment. Early generation OAs have limitations in effectively delivering therapeutic agents to tumors and they increase the expression of immune inhibitory ligands, such as Programmed Death Ligand 1 (PD-L1), which can hinder immune responses against tumor.

To overcome these challenges, the researchers developed a new therapeutic system — ONCOTECH, in which OAs are combined with T cells by cloaking OAs with T cell membranes, therefore allowing OAs to be physically attached to T cells. This design allows T cells to "find" tumors naturally, achieving efficient delivery of the OAs. Also, for solving problem related to immune checkpoints (PD-L1) the OAs were engineered with CRISPR-Cas9 system and sgRNA targeting the PD-L1 gene. By inhibiting this gene, the therapy lowers the level of PD-L1, and therefore weakens the tumor's immune system escape mechanisms. The combination therapy overall allow OAs selectively lyses tumor cells without affecting normal cells.

ONCOTECH was examined in multiple mouse cancer, such as pancreatic adenocarcinoma, melanoma and glioblastoma. The results were positive, showing the therapy had significantly accumulated in tumor cells, reduced PD-L1 expression, and improved survival rates. In the melanoma model the treatment with ONCOTECH prolonged the life-span of the mice as 80% of the mice were alive over 70 days after the treatment.

3. Study 2: Synapse-tuned CARs enhance immune cell anti-tumor activity (Chockley et al., 2023)

CAR: antigen complexes form disordered synapses and do not consist of *bona fide* central, peripheral or distal supramolecular activation complexes (SMACs). In order to improve CAR synapse formation, the authors focused on post-synaptic density-95, discs large and zona occuldens (PDZ) binding moieties as approximately 400 proteins contain PDZ domains and some PDZbms aid in synapse formation and polarity of immune cells. Thus, the authors selected the PDZbm of cytotoxic and regulatory T-cell-associated molecule (CRTAM) and then added this PDZbm to the C-terminus of CAR NK cells, thereby testing whether PDZbms can improve the function of CAR NK cell or not.

First, by doping recombinant human EphA2 protein to poly-lysine-coated glass slides and allowing CAR NK cells to incubate and interact for 30 minutes, researchers found that including a PDZbm domain results in improved formation of immune synapse. Then the researchers co-cultured the CAR NK cells and tumor cells, researchers found that PDZbms enable the CAR NK cells have smaller synapse area, enhanced calcium flux and improved

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congregation of lysosome. These phenomena all indicate a more efficient synapse formation.

With the enhanced formation of the immune synapse, researchers want to test the functional consequences. In particular, the authors found that CAR NK cells that express the PDZbms have an distinct increase in the frequency of perforin-secreting and IFNγ-secreting cells, showing that cytokine production of the NK cells are improved.

Next, researchers explored the cytolytic activity in CAR NK cells in three-dimensional (3D) culture systems. Researchers found that CAR NK cells that have PDZbms reduced tumor size to a greater degree than CAR NK cells. What's more, CAR.PDZ cells also showed an improved ability of migration and invasion.

Finally, team of investigators compared the anti-tumor efficacy of CAR NK cells and CAR.PDZ NK cells in three solid tumor models. researchers found that only mice treated with CAR NK cells with the PDZbms had an increase in survival. Also, researchers found that adding PDZbms to the C-terminus of CAR enhances the effector function of both CAR NK cells and CAR T cells.

Collectively, this study reveals distinct advantages by adding a domain to the C-terminus of the CAR cells to improve synapse formation.

4. Study 3: Oncolytic virus-mediated expansion of dual-specific CAR T cells improves efficacy against solid tumors in mice (Evgin et al., 2022)

This study explores the enhancement of CAR T cell therapy for solid tumors by combining it with OVs such as vesicular stomatitis virus (VSV) encoding interferon beta (VSV-IFNβ) and reovirus. The researchers developed a novel strategy in which CAR T cells are preloaded with OVs and then activated through the native TCR in vivo. This method significantly improved therapeutic outcomes compared to the administration of either CAR T cells or OVs alone, achieving over 80% cure rates in mouse models, including both subcutaneous and intracranial tumors.

The combination therapy led to the selective expansion of CAR T cells with specificity for viral antigens, resulting in a distinct differentiation program that included the development of both effector and long-lived memory populations. Notably, the expression of memory markers such as killer cell lectin like receptor G1 (KLRG1) indicated enhanced functionality and persistence of these CAR T cells.

Additionally, the study found that this combination approach bypassed the need for lymphodepletion, a common preconditioning step in CAR T cell therapy, reducing toxicity of the treatment. The researchers also observed that virus-loaded CAR T cells trafficked more efficiently to lymph nodes, further boosting their therapeutic efficacy.

The findings suggest that stimulating the native TCR in CAR T cells with OVs not only enhances the expansion and function of CAR T cells but also promotes endogenous immune responses through epitope spreading. This approach presents a promising strategy for overcoming the limitations of CAR T cell therapy in solid tumors, warranting further investigation and potential clinical translation.

5. Working Model

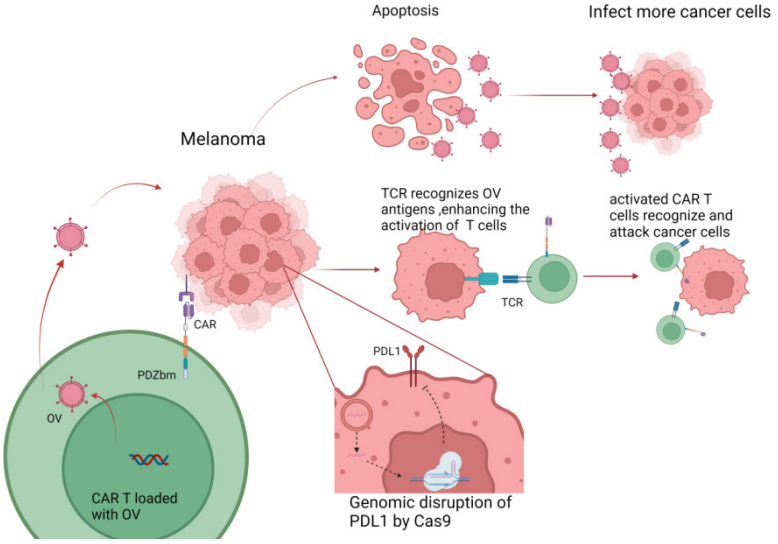

Figure 1. Working Model. Human CD19 anti-epidermal growth factor receptor variant III (EGFRvIII) CAR T cells is engineered with PDZbm. Meanwhile, CAR T cells are also loaded with an engineered oncolytic virus that contain a CRISPR - Cas9 system targeting PD-L1 gene.

6. Summary of how the model works

Engineered with PDZbm Human CD19 anti-epidermal growth factor receptor variant III (EGFRvIII) CAR T cells, which are capable of enhancing immune synapse efficiency through increasing calcium flux and decreasing synaptic cleft, thus accelerating lysosome production. This will also increase the production of cytokines, which further increases the effect of T cells on melanoma cells. At the same time, CAR T cells are also loaded with an engineered oncolytic virus that contain a CRISPR - Cas9 system targeting PD-L1 gene, antagonizing T cell suppression. Collectively, these features enhance activation of tumor T cells both resulting conceivably in improve killing of melanoma cells. Since the eOVs are replication competent they can amplify in initially infect tumor cells and can spread to cells within the tumor. We hypothesize that this combination will enhance the overall efficacy and specificity of CAR T cells against melanomas and potentially other (solid tumors).

7. Hypothesis

Overall, the hypothesis is that if we combine all these treatments, it will help melanoma immunotherapy to enhance better ability in terms of targeting tumor cells, weakening the tumor's immune bypass mechanism and increasing immune synaptic efficiency. Overall, the multiple model strategy will be more effective than the original individual immunotherapy in terms of reducing and eliminating mass of tumor and increasing survival rates.

8. Mouse model

We propose to inject subcutaneously B16EGFRvIII (epidermal growth factor receptor variant III) mouse melanoma cells into syngeneic C57Bl/6 adult mice. 8 days following tumor cells transfer we propose to intravenously inject CAR T cells preloaded with poliovirus.
Treatment A: Adding a PDZ binding motif to the C-terminus of CAR
Treatment B: Loading human CD19 anti-epidermal growth factor receptor variant III (EGFRvIII) CAR T cells with Poliovirus.
Treatment C: Polioviruses are designed to produce the CRISPR-Cas9 system targeting the PD-L1 gene in tumor cells.

9. Key experiments

1. Survival: After tumor implantation, we can count the survival rate for both the experimental and control groups.
2. Tumor size: After tumor implantation, we can test the tumor volume for both the experimental and control groups.
3. The binding force between CAR T cells and melanoma, quantification of single-cell calcium flux levels, the secretion of cytokine: To test the function of PDZ binding motif, we can co-culture CAR T cells and melanoma in vitro. With the technology of a single-cell avidity measurement technology named z-MOVI, live cell image analysis and multiplex cytokine assessment, we can expect the amplified binding capabilities, enhanced calcium flux and increased secretion of IFN-γ after CAR.PDZ T cells co-cultured with melanoma compared with CAR T cells without PDZ binding moieties.
4. The activation of CAR T cells: CAR T cells were isolated from spleens of mice in the experiment at the end of the experiment. Then Cells were cocultured with live B16 cells or B16EGFRvIII cells pretreated for 24 hours with IFN or with murine in vitro matured dendritic cells preloaded for 24 hours with VP3 or OVA-derived SIINFEKL peptide .Forty-six hours later, we can measure the secretion of IFN by ELISA.
5. Southern blot and western blot: we can use Southern blot to analyze PD-L1 indel frequency in melanoma tumor tissue and Western blot to analysis PD-L1 expression after treatment
6. Flowcytometry: The number of CAR T cells per gram of melanoma to indicate the infiltration of CAR-T cells.

10. Experimental & Control group

Treatment A: Adding a PDZ binding motif to the C-terminus of CAR
Treatment B: Loading human CD19 anti-epidermal growth factor receptor variant III (EGFRvIII) CAR T cells with Poliovirus.
Treatment C: Polioviruses are designed to produce the CRISPR-Cas9 system targeting the PD-L1 gene in tumor cells.

Table 1. Experimental & Control Group

Experimental Group	Control Group
Treatment A+B+C	No treatment (control group 1)
Treatment A+B+C	Treatment A+B (control group 2)
Treatment A+B+C	Treatment B+C (control group 3)
Treatment A+B+C	Treatment A (control group 4)

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11. Results

All three therapies exhibited enhanced therapeutic effects in our melanoma model: CAR T cells that were augmented with PDZ binding motif (Treatment A), poliovirus loading (Treatment B), and CRISPR-Cas9 based PDL1 targeting therapy (Treatment C). This was achieved through the treatment that boosted the rates of survival and significantly minimized the size of the tumours from the control groups. In vitro, CAR T cells with the PDZ motif achieved increased binding, time to peak calcium flux, and IFNγ secretion, signifying better synapse formation and T cell activation. Furthermore, the engineered poliovirus interrupted PDL1 expression in tumours, which reduced the immunosuppressive effect and improved tumour elimination. The flow cytometry results also indicated that the infiltration of CAR T cells in tumours was higher in the Treatment A and C groups.

The challenges observed were fluctuations in tumour progression rates and some indication of CAR T cell depletion over time. Nevertheless, possible modifications like localized virus delivery or inducible CAR T cell activation can be discussed for future developments. In conclusion, the experiment showed improved CAR T cell efficiency, more effective tumour targeting, and on increased CAR T cell durability, which, all point to the utility of this kind of multi-modal strategy in the treatment of solid tumour cancers.

12. Discussion

Based on our study's findings, the co-administration of a PDZ binding motif-enhanced CAR T cell therapy with an oncolytic virus that targets PD-L1 could enhance the treatment of melanoma. This strategy makes CAR T cell activation more effective, raises tumour destructiveness, and tackles issues connected to the suppression of the tumour microenvironment. These results suggest that further development of this strategy may promote solid tumour immunotherapy.

The current study aimed to examine a complex model for enhancing CAR T cell therapy for melanoma. More concretely, we plan to create CAR T cells with added PDZ binding motifs and load them with an oncolytic poliovirus that would target and eliminate PD-L1 expression, respectively, through the help of CRISPR-Cas9. This combination presents several advantages. One major advantage of the study is the enhanced specificity of the therapy.

Here, the integration of the PDZ binding motif and the oncolytic virus means that the direct cytotoxic impact of the oncolytic virus and the CAR T cell-mediated immune response is targeted towards melanoma cells and not normal cells, thereby minimizing on-target toxicity.

However, the proposed method has some weak points. The first is the durability of the resultant CAR T cells. Enhanced activation triggered by the PDZ binding motif combined with an oncolytic virus could cause rapid exhaustion of CAR T cells, which may compromise their therapeutic potential. The second risk is associated with side effects. Despite the oncolytic virus's specificity toward cancer cells, the possibility exists that it may harm healthy cells or provoke an over-aggressive immune response, leading to toxicity.

Additionally, the implication of the findings of the present study has some limitations. Although it might work well when tested on preclinical models, it may not be very effective when applied in a clinical setting. Tumour heterogeneity and immune system differences could affect the efficiency of the therapy and toxicity of the preparation. Additionally, this multi-modal therapy might be challenging to perform and might lead to a high cost of production and implementation; hence, it might not be easily available to many practitioners.

Based on these issues, future work could enhance CAR T cell durability, potentially through the application of inducible expression systems to avoid beginning-of-life decay. Localized delivery of the oncolytic virus has reduced off-target effects and low systemic toxicity. Also, the feasibility and efficiency of this approach should be enhanced by cost reduction measures such as cutting the manufacturing processes.

Finally, potential ethical issues are to be discussed, especially the possible risks of viruses and immune cells modified genetically. The positive aspects of genome editing have to be weighed against the unknown risks, including secondary effects or long-term impacts. Consent procedures have to be well executed to the extent that the patient is fully aware of the likely complications. Also important is the need to focus on the cost of such novel treatments and ensure that no one is locked out from the therapeutic options due to lack of funds.

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Qi Yan, Xiaxi Tian, Zihan Mo amd Xinjun Wen contributed equally to this work and should be considered co-first authors

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