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Combined Treatments on Oncolytic Viruses to Improve Efficacy on Pancreatic Ductal Adenocarcinoma

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Abstract. Pancreatic ductal adenocarcinoma has a dense, immunosuppressive stroma that limits therapeutic delivery, viral spread, and T-cell infiltration. Oncolytic virus therapy remains limited by systemic immune clearance, incomplete intratumoral dissemination, and heterogeneous tumor-antigen expression. We propose an engineered herpes simplex virus type 1 coated with CD47-containing nanovesicles and encoding CMV-driven GM-CSF, CD19t, and heat-responsive GSDMDNT. CD19t enables targeting by a CD19×CD3 T-cell engager, while focused ultrasound triggers localized pyroptosis. Delayed ultrasound is expected to allow greater viral replication before pyroptosis, producing higher infectious viral titers, broader viral spread, greater tumor-cell death, stronger immune-cell recruitment, and enhanced T-cell cytotoxicity compared with early or no ultrasound. Further studies should establish an optimal therapeutic window, as premature pyroptosis may restrict viral amplification, whereas excessive delay may compromise treatment control. Viral dose, GM-CSF expression, and T-cell engager scheduling will also require careful calibration. By temporally coordinating viral replication, localized pyroptosis, and targeted T-cell killing, this strategy may improve therapeutic responses in pancreatic ductal adenocarcinoma.

Keywords: pancreatic ductal adenocarcinoma, oncolytic virotherapy, pyroptosis, focused ultrasound, T-cell engager

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent form of pancreatic cancer, constituting more than 90% of pancreatic malignancies. It remains one of the deadliest human cancers; current US statistics report an overall five-year relative survival rate of approximately 13% for pancreatic cancer, and only 8% for exocrine pancreatic tumors, which include most PDAC cases [1, 2].

PDAC develops through the progressive accumulation of genetic alterations, most commonly activating mutations in KRAS, together with silencing of the tumor-suppressor genes TP53, CDKN2A, and SMAD4. These four genes are widely recognized as the principal recurrent driver

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genes in PDAC and contribute to malignant transformation, disease progression, and treatment resistance [3, 4].

The dense desmoplastic stroma, composed largely of extracellular matrix components and cancer-associated fibroblasts, is one of hallmarks of PDAC, composed largely of extracellular matrix components and cancer-associated fibroblasts. This stromal compartment creates a physical and biological barrier that restricts drug delivery, compresses tumor blood vessels, limits immune-cell infiltration, and supports an immunosuppressive tumor microenvironment [5-7].

Because early-stage PDAC is frequently asymptomatic and effective population-level screening remains unavailable, many patients are diagnosed only after the disease has become locally advanced or metastatic. The only potentially curative treatment is offered by surgical resection, but only a small proportion of patients are eligible. Systemic chemotherapy therefore remains the principal treatment for advanced disease. Current first-line regimens include FOLFIRINOX, gemcitabine plus nab-paclitaxel, and NALIRIFOX. Despite improvements in treatment, advanced PDAC continues to have poor clinical outcomes, highlighting the need for new therapeutic approaches.

The objective of this proposal is to develop a next-generation oncolytic virotherapy for pancreatic ductal adenocarcinoma by integrating three complementary engineering strategies recently described in the literature. Specifically, this proposal builds upon three foundational studies that independently demonstrated: virus-mediated artificial-antigen expression for T-cell engagement, GM-CSF-associated enhancement of viral replication, and systemic viral delivery with ultrasound-triggered pyroptosis. By integrating these complementary advances into a single therapeutic platform, we aim to overcome several of the major biological barriers limiting oncolytic virotherapy for PDAC.

2. Literature analysis

2.1. Universal off-the-shelf combination immunotherapy using oncolytic viruses to redirect T-cell engagers to target solid tumors

T-cell engagers such as blinatumomab are effective against B-cell malignancies, where suitable target antigens are well defined. Their application to solid tumors is limited by heterogeneous antigen expression and an immunosuppressive microenvironment. Instead of relying on a naturally occurring tumor antigen, an oncolytic virus could introduce an artificial target selectively into infected tumor cells. Park et al. investigated whether CF33-based vaccinia viruses encoding truncated CD19 or BCMA could make solid tumors recognizable to matching off-the-shelf T-cell engagers [8].

OV19t and OVBCMAt induced surface expression of their respective antigens across several solid-tumor models. When combined with CD19- or BCMA-targeting engagers, the infected cells stimulated T-cell activation, cytokine release and tumor-cell killing. The combinations produced stronger tumor regression than either treatment alone in xenograft models. In bilateral-tumor experiments, injecting OV19t into only one tumor followed by systemic CD19-TCE treatment generated responses at both treated and untreated sites [8].

This approach separates tumor recognition from native antigen expression and avoids patient-specific CAR-T manufacturing. However, its efficacy depends on successful viral infection, and the study did not use immunocompetent models. Future work should therefore assess safety, infection variability and immune responses in clinically relevant settings [8].

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2.2. A novel GM-CSF-encoding oncolytic adenovirus induces profound autophagy and promotes viral replication to enhance antitumor efficacy

Granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates macrophage and granulocyte proliferation and enhances antigen presentation by innate immune cells. For this reason, GM-CSF is commonly used to arm oncolytic viruses. However, excessive GM-CSF expression can promote immune cell exhaustion and, in some settings, support tumor progression. Therefore, controlling the timing and level of GM-CSF expression may improve the therapeutic efficacy of oncolytic viruses. The authors investigated whether regulating GM-CSF expression using either the endogenous adenoviral E3 promoter or an exogenous CMV promoter would influence viral replication and antitumor activity [9].

The authors generated two GM-CSF-expressing oncolytic adenoviruses based on the conditionally replicative adenovirus vector Adv5/dE1A. In both immunodeficient and immunocompetent mouse tumor models, the virus expressing GM-CSF under the CMV promoter (oAd-CMV-GM-CSF) exhibited greater antitumor efficacy than the virus using the endogenous E3 promoter. Mechanistic studies demonstrated that oAd-CMV-GM-CSF induced stronger autophagy, promoted viral replication, and consequently enhanced tumor cell killing [9].

This study demonstrates that promoter selection can substantially influence the therapeutic performance of cytokine-armed oncolytic viruses. Future work should optimize cytokine expression to maximize antitumor immunity while minimizing potential immune exhaustion and evaluate this strategy in additional tumor models and combination immunotherapies [9].

2.3. Genetic engineering of systemically injectable oncolytic viruses for pyroptosis-accelerated cancer virotherapy

Gasdermin D (GSDMD) and gasdermin E (GSDME) are key executioner proteins that mediate pyroptosis, a highly inflammatory form of programmed cell death. Although combining pyroptosis-inducing proteins with oncolytic viruses has the potential to enhance tumor killing, systemic delivery remains inefficient, and nonspecific expression of GSDMD could damage healthy tissues. The authors sought to improve the safety and efficacy of systemic oncolytic virotherapy by developing strategies to enhance intravenous delivery, increase intratumoral viral infection, and restrict GSDMD expression specifically to tumor tissues [10].

The researchers engineered an HSV-1-based oncolytic virus by coating it with CD47-containing immunoengineered nanovesicles to generate iNV-GOV, thereby improving systemic delivery and reducing immune clearance. In addition, the GSDMD gene was inserted into the viral genome and designed to be activated by focused ultrasound, allowing spatial control of pyroptosis. These modifications significantly improved intravenous delivery, enhanced tumor-specific viral infection, and increased pyroptosis-mediated immunogenic cell death while minimizing damage to normal tissues [10].

This study demonstrates several complementary engineering strategies that improve the delivery, specificity, and therapeutic efficacy of systemically administered oncolytic viruses. These findings provide a promising framework for developing next-generation oncolytic virotherapies capable of treating metastatic disease. Future studies should evaluate long-term safety, optimize ultrasound-triggered gene activation, and investigate combinations with immune checkpoint inhibitors or other immunotherapies [10].

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3. Working model

Our proposed therapeutic strategy integrates the complementary strengths of the three foundational studies into a single engineered oncolytic HSV-1 platform (Figure 1). First, the virus will be coated with immunoengineered nanovesicles containing CD47 to reduce immune clearance by phagocytic cells following intravenous administration and thereby improve systemic delivery to pancreatic tumors. Once localized within the tumor, focused ultrasound will activate virally encoded GSDMD to induce localized pyroptosis, generating inflammatory cell death while minimizing damage to healthy tissues.

To maximize viral amplification within the tumor, the virus will additionally express GM-CSF under the control of the constitutively active CMV promoter, promoting autophagy-mediated viral replication and enhancing direct tumor cell lysis. Increased viral replication should facilitate more efficient spread throughout the dense PDAC stroma while simultaneously amplifying local immune activation.

Finally, the engineered virus will express the artificial surface antigen CD19t on infected tumor cells. This modification converts heterogeneous pancreatic tumor cells into uniform targets for a clinically established CD19×CD3 T-cell engager. Between cDC1 and PD-1^{+} T cells, Bispecific DC-T Cell Engager is a bispecific antibody which plays the promoting role in physical interactions in the tumor and tumor-draining lymph nodes [11]. Since we suppose in CD19 and CD3 cells, the CD19×CD3 T-cell engager has the same effect as the Bispecific DC-T Cell Engager in cDC1 and PD-1^{+} T cells. thereby redirecting endogenous T cells toward infected tumor cells regardless of their native antigen expression. Together, localized pyroptosis, enhanced viral replication, and installation of an artificial immune target are expected to function synergistically to increase viral spread, promote inflammatory remodeling of the tumor microenvironment, and improve T-cell-mediated tumor eradication.

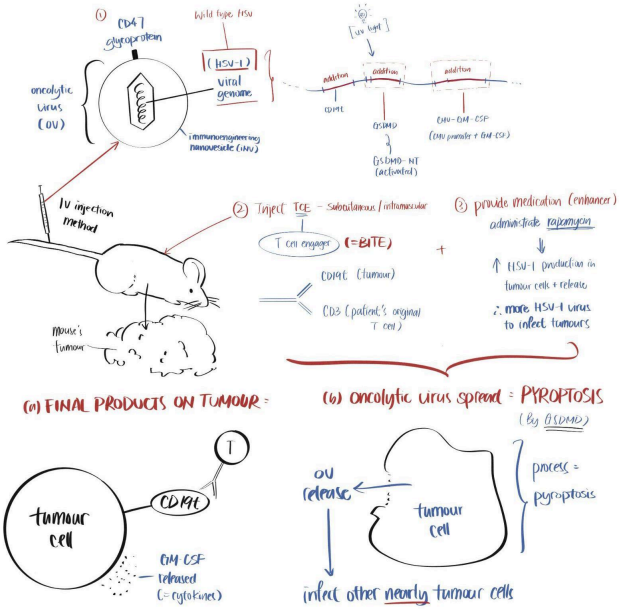


Figure 1. Hypothesised model to enhance the efficacy of the current OV treatment

Accordingly, we hypothesize that combining immune-cloaked systemic viral delivery, ultrasound-triggered pyroptosis, GM-CSF-enhanced viral replication, and virus-mediated CD19t

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expression will substantially improve the efficacy of oncolytic virotherapy against pancreatic ductal adenocarcinoma compared with current oncolytic virus approaches alone.

4. Methodology

The experiment is designed to investigate the efficacy of OV after combining systemic delivery, CD19t-directed T cell targeting and CMV-GM-CSF techniques, to seek potential enhancement on treatments encountering pancreatic cancers.

4.1. Mouse model

We propose to use immunocompetent mouse PDAC cell line C57BL/6 in experiments for our treatment model. It involves syngeneic orthotopic implants Pan02 and KPC models, and is the one of the most commonly used syngeneic mouse models in immunology as well as in antitumour activity research [12]. The paper has also suggested that C57BL/6 mice is a reliable model that the mice are genetically identical and are immunocompetent. This ensures consistency and positive experimental outcomes.

4.2. Experimental design and treatment group

To improve immune efficiency, we insert GM-CSF gene and GSDMD gene into HSV separately, then coat HSV with iNV, which CD19t is incorporated on its surface. We inject the OVs and T-cell engagers respectively into the mouse in different combinations twice a week. The ultrasound is applied at different time points simultaneously to promote tumor cell lysis and to release more OVs. The combinations are shown in Table 1, and the ultrasound time points are shown in Table 2.

Table 1. Two types of oncolytic viruses are injected intravenously into mice, T-cell engager is injected subcutaneously. PBS is used as a solvent in both oncolytic virus and T-cell engager injections. Our assumption is that the efficacy in group7 is the best

Treatment Group			
Group	GSDMD (IV)	GM-CSF (IV)	T-cell engager (SC)
1	P	O	O
2	O	P	O
3	O	O	P
4	P	P	O
5	O	P	P
6	P	O	P
7	P	P	P
8 blank control group inject with PBS only	O	O	O

Table 2. Ultrasound treatment was applied to the 7th group at different time points. Our assumption is that the efficacy in d is the best

Ultrasound treatment was applied to the 7th group at different time points	
Group	time points
a	O
b	normal
c	early
d	delay

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4.3. Tumour size measurement

The growth of tumours will be monitored throughout the course of the experiment by bioluminescence imaging (BLI). Since the technique is non-invasive to mouse models, growth rate and real-time progression of tumour in size can be recorded in each individual mouse set-up. D-luciferin substrate is injected to mice model that is responsible for emitting light in relation to the quantity of living cancer cells. The emission of light is then detected by Cooled Charge-coupled Device (CCD) camera system and total flux in photons per second will be recorded and compared between various combinations of set-ups. It is expected that in group 7, as the treatments combine, the efficacy increases, thus tumour suppression and regression are more significant in the combined treatment models.

Bioluminescence imaging:

Photon flux may not scale linearly at later stages of tumours. This is because of hypoxia and necrosis that lead to a reduction of activity in ATP-dependent luciferase [13], hence a potential underestimation of measurement to the true cell counts.

Alternative approaches:

Using real-time MRI enables a more accurate measurement of absolute tumour volume and structural changes at a later stage of mouse tumour development. However, the method is generally not recommended to measure at an early stage due to the high cost and the risk of performing repeated anesthesia on mice, since real-time MRI requires at least 15 minutes to scan.

4.4. Experimental strategy

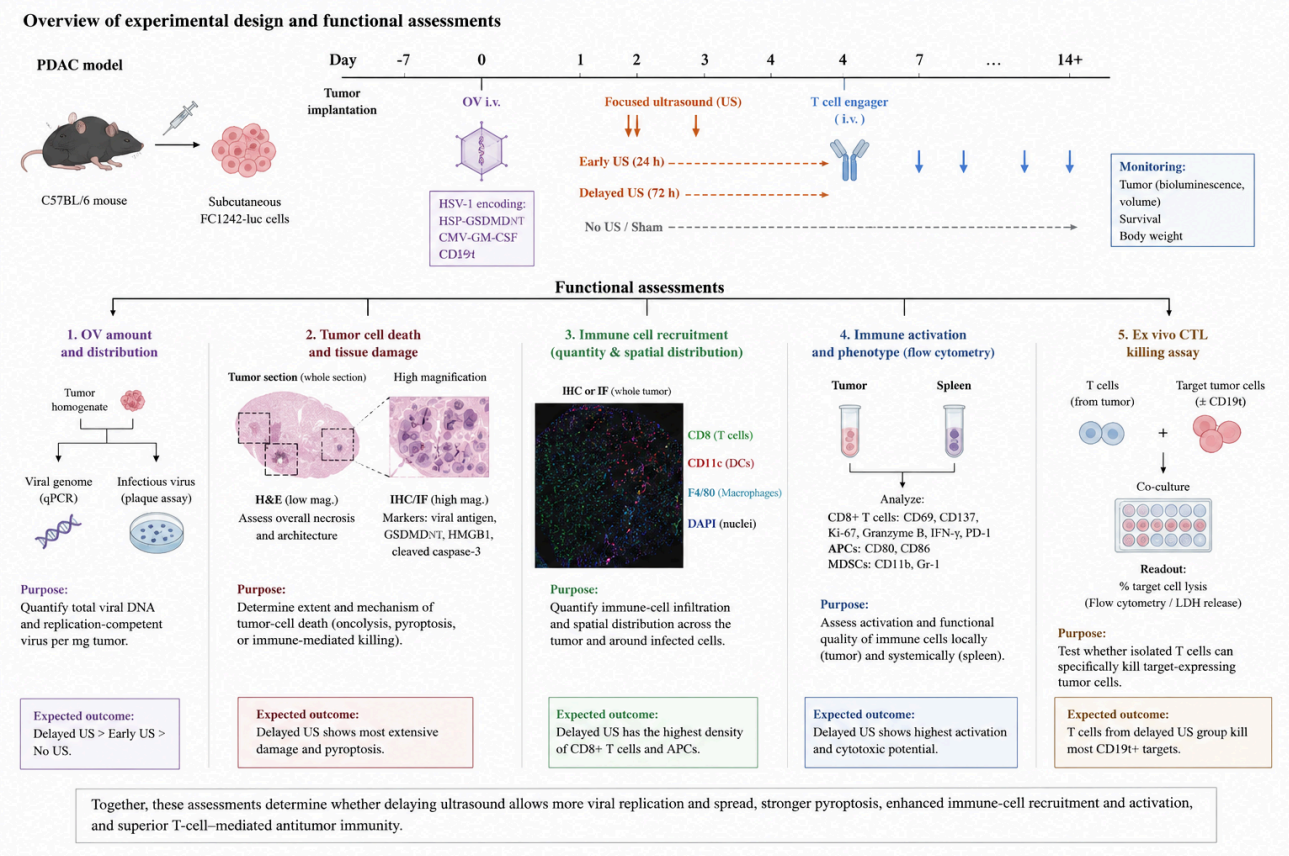


Figure 2. Hypothesised model to enhance the efficacy of the current OV treatment

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Our experimental strategy follows the biological sequence of the therapy: viral replication and spread, tumor-cell killing, immune-cell recruitment, immune activation, and finally T-cell-mediated cytotoxicity. The experimental timeline and assessments are shown in Figure 2.

4.4.1. Intratumoral OV amount and distribution

Tumors will be collected at matched time points after OV administration and ultrasound treatment. Total intratumoral viral burden will be quantified by qPCR for HSV-1 genome copies, while viral titration by plaque assay will measure replication-competent virus. (Figure 2, assessment 1) Both measurements will be normalized to tumor tissue mass for direct comparison among groups. In parallel, viral antigen staining of whole-tumor sections will determine whether the virus remains localized or spreads throughout a larger proportion of the tumor.

In the engineered OV system, focused ultrasound generates mild local hyperthermia that activates the heat-responsive GSDMDNT payload. This triggers tumor-specific pyroptosis, accelerates oncolysis, and promotes rapid release of progeny virus from lysed tumor cells, thereby amplifying infection of neighboring tumor populations [10].

The timing of ultrasound is therefore expected to be critical. Early ultrasound may trigger pyroptosis before sufficient viral replication and progeny production have occurred. In contrast, delayed ultrasound should allow greater intracellular viral amplification before promoting tumor-cell lysis and viral release. We therefore expect the delayed-ultrasound group to show the highest infectious viral titre and the broadest intratumoral viral distribution.

Total viral genome copies may not follow exactly the same pattern as infectious viral titre because qPCR can also detect DNA from non-infectious or damaged viral particles. Therefore, qPCR, plaque assay, and spatial viral staining should be interpreted together.

These measurements establish whether differences in downstream tumor-cell death and immune activation are associated with successful viral amplification and spread.

4.4.2. Tumor-cell death and tissue damage

Whole-tumor histology will be used to evaluate the overall extent and distribution of tumor-cell death. (Figure 2, assessment 2) Low-magnification images will show the proportion of damaged or viable tissue, while higher-magnification imaging will examine cellular morphology and the relationship between damaged cells and virus-infected regions.

Because ordinary histology cannot independently distinguish direct viral cytolysis, pyroptosis, and immune-mediated killing, additional staining for viral infection and pyroptosis will be used to support the proposed mechanism. The delayed-ultrasound group is expected to show the greatest overall tissue destruction because it should combine viral replication, ultrasound-triggered pyroptosis, and immune-mediated killing.

4.4.3. Immune-cell recruitment

Immunohistochemistry (IHC) or immunofluorescence (IF) microscopy will be used to measure the number and spatial distribution of immune cells within the tumor using lineage-specific antibodies. (Figure 2, assessment 3) Whole-section or low-magnification analysis will determine the overall quantity of recruited immune cells, while higher-magnification imaging will identify specific cell populations and determine whether they are located near infected or dying tumor cells.

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Because the BiTE redirects T cells toward OV-infected tumor cells expressing the introduced target antigen, the combination treatment is expected to increase intratumoral T-cell recruitment. The delayed-ultrasound group should show the greatest T-cell infiltration because enhanced viral spread should increase the number of target-expressing tumor cells.

4.4.4. Immune-cell activation and cytotoxic function

Flow cytometry will be performed on immune cells isolated from the tumor and from a systemic immune organ, such as the spleen or tumor-draining lymph node. (Figure 2, assessment 4) Tumor analysis will determine whether infiltrating T cells are activated and cytotoxic, while analysis of the spleen will indicate whether the treatment also generates a systemic immune response.

A CTL functional assay (Figure 2, assessment 5) will then test whether isolated T cells can directly kill target tumor cells outside the animal. This is important because an increased number of T cells does not necessarily mean that those cells are functional. We expect T cells from the delayed-ultrasound group to show the strongest activation and tumor-cell killing.

5. Expected functional results

The expected differences among the untreated, no-ultrasound, early-ultrasound, and delayed-ultrasound groups are summarized in Figure 3.

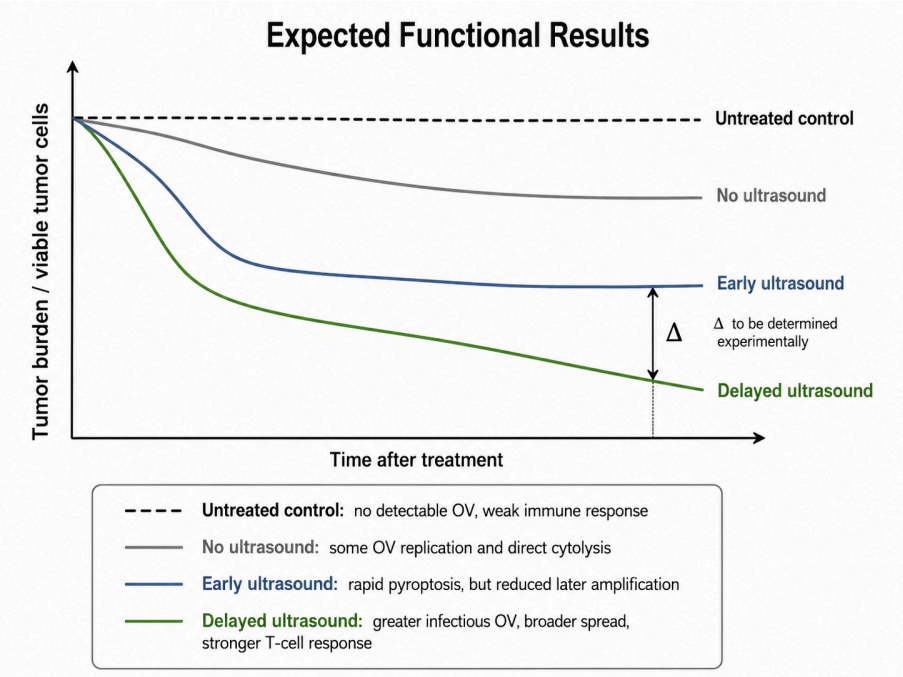


Figure 3. Expected functional results of different ultrasound schedules in the engineered oncolytic-virus treatment model

The untreated control should show no detectable OV, limited tissue damage, low immune-cell infiltration, and weak CTL activity. The no-ultrasound group should show some OV replication and direct viral cytolysis, but less viral release, immune recruitment, and T-cell activation.

The early-ultrasound group may show rapid pyroptosis and early tissue damage. However, premature destruction of infected cells may reduce later viral amplification, viral spread, and

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expression of the BiTE target.

The delayed-ultrasound group is expected to show the strongest overall response: greater infectious OV production, broader viral distribution, more extensive tumor-cell death, increased T-cell infiltration, stronger T-cell activation, and the highest CTL killing activity. This result would support the hypothesis that allowing the OV to replicate before ultrasound-triggered pyroptosis improves both virotherapy and BiTE-mediated antitumor immunity. As illustrated by the Δ in Figure 3, the magnitude of the difference between early and delayed ultrasound remains uncertain and must be determined experimentally.

6. Discussion

Three connected mechanisms in the treatment model

1. Direct tumor killing:

The OV infects and lyses PDAC cells, while ultrasound-activated GSDMDNT enhances localized pyroptosis and tumor-cell destruction.

2. BiTE-mediated killing:

Infected tumor cells express CD19t, allowing the CD19×CD3 T-cell engager to redirect T cells toward them. This provides rapid and target-specific killing, but the effect may decrease when the engager is cleared or CD19t-expressing cells are eliminated. The use of OV-induced artificial antigens to redirect T-cell engagers has been demonstrated in solid-tumor models [8].

3. Priming of PDAC-specific T cells:

OV-mediated lysis and pyroptosis may release native PDAC antigens together with inflammatory signals. Dendritic cells may take up these antigens and activate endogenous tumor-specific T cells. This could create a broader and potentially longer-lasting response than BiTE-mediated killing alone. OVs can act as an in-situ vaccination strategy by promoting tumor-antigen release and adaptive antitumor immunity, although this effect must still be demonstrated in our model [14].

6.1. Targeting tumor with ultrasound as an essential additional parameter

To expose targeted tumors at internal organs like pancreas to ultrasound, specialised high-frequency ultrasound (HFUS) is performed. Fast-scan HFUS has been performed without anesthesia in mice bearing breast tumor grafts, with an average scanning duration of 47 seconds per mouse [15]. Whether this approach can be applied without anesthesia to orthotopic pancreatic tumors requires further assessment. High-frequency acoustic ultrasound waves are emitted to generate cross-sectional tumor images by echogenicity of different tissues and acoustic impedance of the waves [16]. Thus, real-time orthotopic organ tumor can be located with limited potential harm to the mice.

6.2. Additional benefit in a "cold" tumor

PDAC is considered an immunologically cold tumor because it usually has limited functional T-cell infiltration, dense desmoplastic stroma, weak antigen presentation, and strong myeloid-mediated immune suppression.

The proposed treatment may make the tumor environment more inflamed. OV infection and GSDMDNT-mediated pyroptosis release tumor antigens and inflammatory signals, while GM-CSF may support antigen-presenting-cell recruitment and activation. The T-cell engager can then redirect T cells toward CD19t-expressing infected cells.

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Beyond immediate tumor lysis, pyroptosis and GM-CSF-mediated immune recruitment may enhance the presentation of native PDAC antigens, allowing endogenous T cells to recognize uninfected or metastatic lesions. Whether this response can generate durable immune memory remains to be tested directly.

A cautious conclusion is that the proposed OV–BiTE treatment may partially convert cold PDAC into a more inflamed and immune-responsive tumor environment, but it may not completely overcome stromal and myeloid suppression.

6.3. Potential challenges

When taking MRI as an alternative method to monitor tumor growth in mice, anaesthesia will be performed to avoid models' movements. However, it is not only difficult to manage the correct dosage, repeat anaesthesia is also harmful to the mice. MRI is a relatively more complicated and expensive method to measure later stage tumor size, therefore a challenge to overcome if the procedure is needed. Besides, viral genome editing and antiviral coating require a certain level of knowledge to engineer the relatively complicated OV model.

Apart from that, the proposed model involves potential uncertainty on the effect when combining cytokine-producing GM-CSF gene and pyroptosis-inducing GSDMD gene. In the GM-CSF part, we aimed to prolong the length of time viruses stay in the living tumor cell to maximise the amount of monocytes, granulocytes and dendritic cell-attracting cytokines released. However, in the GSDMD part of the model, we aimed to induce pyroptosis on the host tumour cell with the aid of ultrasound. It is important and may be challenging to control the balance of the time between cytokine release and programmed cell death, to maximise both the function of GM-CSF and GSDMD.

A major challenge is balancing viral replication with GSDMDNT-mediated pyroptosis. If ultrasound is applied too early, infected cells may die before enough progeny virus and CD19t are produced. If ultrasound is applied too late, viral replication may already have peaked or the virus may have been partly cleared. Viral genome copies, infectious viral titre, CD19t expression, and pyroptosis should therefore be compared at different time points.

The dense PDAC stroma may still limit both viral spread and T-cell penetration. Desmoplastic stroma can restrict T-cell movement into tumors and contribute to immune exclusion and immunosuppression [7]. Therefore, strong viral replication in one region may not produce uniform infection or immune killing throughout the whole tumor.

In addition, strong BiTE-mediated killing does not prove that lasting PDAC-specific immunity has formed. Further experiments would be required, such as testing whether T cells can kill CD19t-negative parental PDAC cells, treating a distant untreated tumor, or performing tumor rechallenge after the original tumor has been cleared.

6.4. Imaging and ultrasound guidance

High-frequency ultrasound may be used to locate orthotopic pancreatic tumors and guide focused-ultrasound treatment. This imaging function should be distinguished from the therapeutic focused ultrasound used to generate mild hyperthermia and activate GSDMD-NT.

Bioluminescence imaging can remain the main method for repeated tumor monitoring, while ultrasound or MRI may provide additional structural information. MRI can measure tumor volume more directly, but repeated scanning is costly and may require longer anesthesia.

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6.5. Important next steps clinical trial / testing other models

If the model has progressed to the next step, some elements have to be considered when moving from syngeneic mouse model to human.

Dosage:

The dosage tested in mice may not be a safe and suitable scaled-up amount to put in humans and oncolytic immunotherapy may trigger dosage-independent inflammation. It is important to test the dosage toxicity and alter the amount in the in vitro tissues before applying in clinical trials.

Immune states:

C57BL/6 has been used as our mouse model in the experiment. The type is tested to be immunocompetent by Song and Hwang [12], but when the oncolytic immunotherapy is tested on a different model, it is advised to ensure the immune of the new model is similar to the model or is strong enough to carry out the treatment.

7. Conclusion

Our proposal combines an engineered oncolytic virus with a CD19×CD3 T-cell engager to improve the treatment of pancreatic ductal adenocarcinoma. We expect the complete treatment containing GM-CSF, GSDMDNT, CD19t, the T-cell engager, and focused ultrasound to produce the strongest antitumor effect.

The timing of ultrasound is expected to be important. Delayed ultrasound gives the OV more time to replicate and express CD19t before GSDMDNT-mediated pyroptosis is triggered. Ultrasound-induced mild hyperthermia can then accelerate oncolysis and promote rapid release of progeny virus from lysed tumor cells, allowing further infection of neighboring tumor cells [10].

Therefore, the BiTE mainly provides rapid killing against CD19t-expressing cells, whereas OV-induced immune priming may support longer-term immunity against native PDAC antigens.

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