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Reprogramming Immune-Desert Tumors via Triple-Modality Immunotherapy

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Abstract. Cold tumors lack T cells and therefore can resist most immunotherapies in general, including anti-PD-1 inhibitors. We describe a new strategy to change the phenotype from "cold" tumors to "hot" tumors that can be recognized and attacked by the immune system. We will utilize a spatiotemporal cascade model called "Ignite–Release–Reinforce". Using an intratumoral route of administration, the oncolytic virus will produce pyroptosis in the tumor, along with the release of tumor antigens. Then, the systemic delivery of an anti-PD-1 monoclonal antibody will allow effector T cells to perform their designed function. Finally, the use of human induced pluripotent stem cell-derived chimeric antigen receptor T (iPSC-derived CAR-T) cells will provide lasting cytotoxicity. To validate this innovative concept, we will utilize mouse models of glioblastoma and pancreatic cancer and will have 8 groups to assess the effects of each treatment with mono-, dual-, and triple-therapy administration. Outcomes will include tumor burden, T cell infiltrate and cytokine profiles, and survival. We anticipate that the proposed triple-modality therapy will provide the greatest degree of tumor regression and benefit in terms of survival when compared to the other groups. We expect that the triple-therapy will increase the amount of CD8+ T cell infiltrate by 3-5-fold compared to the control and single-agent treated groups, cause greater elimination of regulatory T cells, and induce higher levels of pro-inflammatory cytokines such as IFN-γ, IL-1β, and CXCL10. Pyroptosis markers and antigen presentation capability are anticipated to be the highest in the triple therapy group, leading to the development of a highly inflammatory tumor microenvironment. We hope that our proposed triple-modality therapy may fundamentally change the treatment of immune-desert tumors by converting their tumor microenvironments to more inflammatory environments and leading to greater efficacy and durability of future immunotherapy treatments for patients.

Keywords: Immune-desert tumors, Oncolytic HSV-1, Pyroptosis, iPSC-CAR-T cells, Combination immunotherapy

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1. Introduction

Immunotherapy has changed the way we treat patients with cancer. Immunotherapy offers an alternative way to treat individuals with cancer compared to traditional chemotherapeutic or radiotherapy regimens. There are many reasons for immune-desert tumors. The tumor may fail to present antigens or to provide immune-activating signals, or the tumor microenvironment (TME) may contain many immune-suppressive factors [1]. Without infiltrating immune cells, the body cannot effectively recognize/attack the tumor and, therefore, standard immunotherapies such as PD-1/PD-L1 inhibitors have low efficacy in immune-desert tumors since the lack of T cells limits the ability to reactivate those cells and use the standard therapy effectively [1, 2]. To overcome the problems with immune-desert tumors, researchers are developing new combination strategies to convert cold tumors to hot tumors. One possible approach involves the use of oncolytic viruses (OVs). An oncolytic virus is a virus that does not express infected cell protein 34.5 and expresses human GM-CSF, so it only infects tumor cells and causes those tumor cells to disintegrate. When the tumor cells lyse, tumor antigens and inflammatory signals are released. In addition to its direct tumor cell toxicity, it is capable of attracting immune cells to the cancerous area. A relationship has been observed between it and anti-PD-1 therapy in creating immune responsive tumors from previously immune desert tumors that lack T cell infiltration [3]. Clinical studies show that the combination approach of it and anti-PD-1 therapy resolves advanced melanoma in about 62% of cases and has a complete response rate of approximately 33%, even for those patients at baseline with low T cell infiltrate into the tumor [4, 5]. However, the immune response generated by viral therapy alone may not be sufficient to overcome immune desert tumors. As a highly promising immune enhancing strategy, T cells engineered to specifically identify and destroy cancer cells can also be utilized. Such T cells may come from induced pluripotent stem cells which serve as an infinite source of customized T cells that specifically recognize tumors [6]. Nevertheless, difficulties still exist for engineered T cells in the tumor microenvironment caused by microenvironment tumor signals that suppresses T cell activity and inhibits their continued survival and persistence.

One exciting area of research is examining the synergistic effect of combining engineered T cell adoptive transfer with oncolytic virotherapy. A study utilizing vesiculovirus as an oncolytic virus demonstrated expanded viability of CAR-T cells after exposure to viral antigens via their native T cell receptor (TCR) recognition [7, 8]. The dual-specific CAR-T cells that formed in these studies recognized both viral and tumor antigens which produced a more effective long-term tumor control [7]. A further finding in that study was using oncolytic viruses by loading the virus directly onto engineered T cells prior to adoptive transfer greatly facilitated access and migration of the T cells to lymph nodes and tumors. This again supports the combined use of viral and direct T cell therapies for enhancing tumor targeting [8]. Further supporting the potential of oncolytic virotherapy in enhancing T cell activity against tumors is the finding that several oncolytic viruses, such as parapox ORFV, can induce a distinct, rapid form of cancer cell death known as pyroptosis [9]. Pyroptosis is unique due to its strong inflammatory nature. During pyroptosis, Gasdermin E (GSDME) is cleaved into fragments that form pores in the cell membrane, resulting in cell rupture and leakage of damage-associated molecular patterns (DAMPs). This inflammatory process that occurs in cell death is highly efficient at calling for activating immune cells, particularly cytotoxic T lymphocytes (CTLs), into the tumor microenvironment (TME). In GSDME low tumors, which are usually less responsive to treatments, ORFV can stabilize GSDME and induce pyroptosis. This can greatly increase the level of infiltration of T cells and improving their overall response to checkpoint inhibition [9, 10].

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The results from these studies, as presented show a strong possibility that a multi-prong approach to targeting immune desert tumors may be an effective method to improve patient outcomes with resistant tumors. We propose to complete a proof of principle study to establish a novel platform using it to initiate tumor inflammation; the use of anti-PD-1 antibodies to facilitate T cell function; and the use of engineered iPSC-derived T cells as a sustained attacking force, as powerful, adaptable immunotherapeutic cancer treatment platform. This work will explore how these treatments, when combined, can work synergistically to modify the tumor microenvironment, enhance immune infiltration, and provide patients with better clinical outcomes with other treatments for tumors resistant to other available therapies.

Three studies have inspired the approaches that will be taken in our proposed research, with each discussing a different combination of orthogonal therapeutic or pharmacological approaches.

2. Summary of foundational papers

2.1. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy

Researchers conducted a Phase Ib clinical trial involving 21 patients with advanced melanoma. The trial employed a sequential combination therapy regimen: first, intratumoural injection of the oncolytic virus T-Vec, with doses escalating from 4 mL × 10^6 pfu/mL to 4 mL × 10^8 pfu/mL; followed six weeks later by intravenous administration of the anti-PD-1 antibody pembrolizumab at a dose of 200 mg per injection. This regimen yielded significant results, increasing the objective response rate to 61.9%, with a complete response rate of 33.3%, far exceeding the efficacy of monotherapy. Mechanistically, T-Vec induces local tumour inflammation by enhancing $CD8^+$ T-cell infiltration within the tumour and upregulating PD-L1 expression, thereby transforming immunologically 'cold' tumours into 'hot' tumours. Notably, in patients with low baseline $CD8^+$ T-cell infiltration, 93.7% of injected lesions and 60% of non-injected lesions regressed, confirming the activation of systemic anti-tumour immunity. Furthermore, safety analyses revealed no dose-limiting toxicities, thereby supporting the clinical translation of this therapy [5].

2.2. Oncolytic parapoxvirus induces Gasdermin E-mediated pyroptosis and activates antitumor immunity

This study utilised oncolytic parainfluenza virus (ORFV) and its recombinant strains, in combination with CRISPR/Cas9-mediated GSDME gene knockout technology and a mouse immunodeficient model, to successfully demonstrate that the virus can trigger protein cleavage by inhibiting the ubiquitination and degradation of the GSDME protein, thereby inducing Gasdermin E-mediated pyroptosis (a form of non-apoptotic cell death) [9].

Flow cytometry and RNA sequencing confirmed that inflammatory mediators released during pyroptosis (such as IL-1β and HMGB1) recruit cytotoxic T lymphocytes to tumour tissues, thereby remodelling the immune microenvironment [9].

In multiple mouse tumour models, ORFV treatment activated 'cold' tumours and enhanced sensitivity to anti-PD-1 therapy. The engineered recombinant virus maintained therapeutic efficacy whilst reducing virulence, offering a new strategy for overcoming resistance to immune checkpoint inhibitors [9].

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2.3. Oncolytic virus–mediated expansion of dual-specific CAR-T cells improves efficacy against solid tumors in mice

This study innovatively engineered bispecific CAR-T cells that simultaneously target the tumour antigen EGFRvIII and viral antigens, and pre-loaded them with oncolytic rotavirus prior to intravenous infusion into mouse models of melanoma and glioma. Dynamic flow cytometric monitoring revealed that the CAR-T cells successfully delivered the virus precisely to the tumour core, triggering cell lysis and the release of viral antigens. Results indicated that a virus-specific CAR-T cell subpopulation, accounting for 25% of the total, expanded 80-fold, exhibited high expression of effector molecules (CD107a, IFN-γ), and reversed T-cell exhaustion. In the treatment of solid tumours, this combination therapy achieved complete tumour regression in over 80% of cases and significantly prolonged survival. Subsequent studies have shown that virus-enhanced stimulation can 'reactivate' CAR-T cells and establish immune memory, thereby overcoming the dual barriers posed by the solid tumour stroma and the immunosuppressive microenvironment [7].

These three studies collectively reveal the key role of oncolytic viruses (OVs) in overcoming the therapeutic barriers posed by 'cold' tumours, with HSV-1 emerging as an ideal vector for this strategy due to its clinical translation potential (e.g. T-VEC) and neurotropic properties [11, 12]. The first clinical trial—using T-VEC (a virus derived from HSV-1) in combination with anti-PD-1 therapy—demonstrated that the HSV-1-based platform, when administered via intratumoural injection, remodels the tumour microenvironment (TME), thereby promoting CD8+ T-cell infiltration and activating the IFN-γ pathway [5, 13]. This produced a synergistic effect with checkpoint inhibitors, achieving an objective response rate of 62%, directly validating the sequential logic we proposed: 'intratumoural HSV-1 injection (Phase 1) → systemic anti-PD-1 administration (Phase 2)' [14]. The core mechanism of the second study (parvovirus-induced pyroptosis)—Gasdermin E-mediated immunogenic cell death—can be replicated using engineered HSV-1: by deleting the ICP34.5 gene to enhance tumour specificity, and inserting Gasdermin E or activating the cGAS-STING pathway (a natural advantage of HSV-1) [15], the virus becomes a potent vector for releasing DAMPs and recruiting T cells [9]. The third study (OV-amplified bispecific CAR-T) directly supports our our design for HSV-1-iPSC-T synergy: systemic phage promotes T-cell expansion by activating the T-cell receptor (TCR) via viral antigens, whilst early HSV-1 antigens (such as ICP27) serve as ideal TCR targets in iPSC-T cells; simultaneously, HSV-1-secreted CXCL9/10/11 (engineered in our proposal to enhance expression) addresses the bottleneck in CAR-T cell infiltration into solid tumours [7, 16].

These studies provide multi-layered validation for the HSV-1-centric triple therapy. T-VEC clinical data confirm the safety and TME-remodeling capacity of the HSV-1 platform [5, 17-19]; the pyroptosis mechanism study guides HSV-1 engineering directions (e.g., GSDME expression modules) [20-22]; and bispecific T cell expansion establishes the biological basis for iPSC-T cells targeting HSV-1-infected sites [16, 23-25]. For recalcitrant tumors like pancreatic cancer and glioblastoma, HSV-1's neurotropism (glioma) and ability to penetrate fibrotic stroma (pancreatic cancer) position it as a key engine to overcome the triple barriers of cold tumors—absent T cell infiltration, insufficient antigen presentation, and immunosuppression [15, 26-28]. The innovation of our proposal lies in translating these findings into an HSV-1-axis-centered spatiotemporal cascade: "Ignite (pyroptosis) → Release (PD-1 blockade) → Reinforce (iPSC-T expansion)". By integrating these advantages through engineering, we provide a clinically translatable solution for next-generation combination immunotherapy.

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3. Overarching hypothesis

As shown in Figure 1, the "Ignite–Release–Reinforce" cascade integrates oncolytic HSV-1–induced pyroptosis and antigen release, anti–PD-1–mediated effector preservation, and iPSC-derived CAR-T–driven cytotoxicity to convert immune-desert tumors into inflamed TMEs.

We hypothesize that combining HSV-1-based oncolytic virotherapy (T-VEC), anti-PD-1 checkpoint blockade, and iPSC-derived CAR-T cell therapy may synergistically convert immune-desert tumors into immune-infiltrated, inflamed environments, thereby enabling robust and durable anti-tumor immunity. This tri-modal strategy leverages spatial and temporal immune activation to overcome key barriers of immune exclusion, T cell exhaustion, and insufficient cytotoxic effector

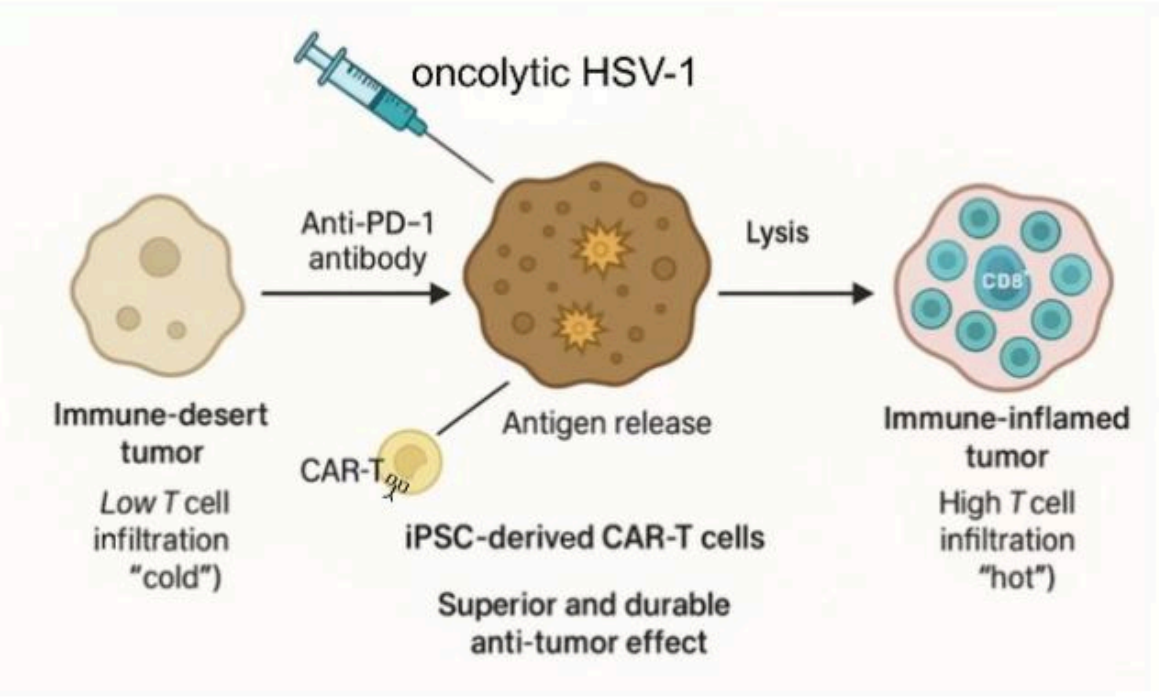

Figure 1. Conceptual overview of oncolytic HSV-1, anti–PD-1, and iPSC-derived CAR-T therapy. Oncolytic HSV-1 induces oncolysis with antigen & DAMP release and PD-L1 upregulation; anti–PD-1 blockade sustains T-cell function; iPSC-derived CAR-T cells provide targeted cytotoxicity. Together these interventions convert an immune-desert ("cold") tumor into an immune-inflamed ("hot") tumor with increased T-cell infiltration

3.1. Working models

We propose a spatiotemporal cascade model ("Ignite–Release–Reinforce"), in which each component addresses a discrete immunological barrier within the suppressive tumor microenvironment (TME). Our working model aims to convert immune-desert TMEs—such as those seen in glioblastoma or pancreatic cancer—into immune-inflamed, cytotoxic milieus capable of sustaining effective anti-tumor responses [29, 30]. This model integrates three components:

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oncolytic Herpes Simplex Virus (HSV), immune checkpoint blockade (anti-PD-1), and iPSC-derived CAR-T cells.

First (Ignite), a genetically engineered HSV-1 is injected intratumorally, designed not only to selectively replicate in tumor cells but also to induce Gasdermin E (GSDME)-mediated pyroptosis —distinct from classical T-VEC. HSV infection releases tumor antigens and damage-associated molecular patterns (DAMPs) such as HMGB1 and ATP [31], establishing a pro-inflammatory context. Our model predicts that intratumoral HSV-1 will increase $CD8^+$ T-cell infiltration by 3–5 fold within 7 days [5]. These changes remodel the TME by elevating IL-1β, IFN-β and chemokines, thereby recruiting dendritic cells and priming naïve T cells [32]. This step establishes an immune-permissive TME and breaks the original immune exclusion.

As illustrated in Figure 1, the "Ignite–Release–Reinforce" cascade integrates oncolytic HSV-1–induced pyroptosis and antigen release, anti-PD-1–mediated effector preservation, and iPSC-derived CAR-T–driven cytotoxicity to transform immune-desert tumors into inflamed, immune-responsive microenvironments.

Second (Release), anti-PD-1 antibody acts on the infiltrating T cells to prevent functional exhaustion and unleash effector function. Importantly, OV infection upregulates PD-L1 expression on tumor and stromal cells, making the remodeled TME more responsive to checkpoint inhibition and amplifying $CD8^+$ cytotoxicity [5, 33]. This phase is expected to double the persistence of effector T cells compared to PD-1 blockade alone, thereby reinforcing the inflammatory loop.

Third (Reinforce), iPSC-derived CAR-T cells are infused systemically. Unlike conventional CAR-T, these are engineered to overexpress CXCR3 for improved chemokine-directed homing and to lack PD-1 for resistance to exhaustion, thereby distinguishing them from standard designs. Guided by OV-induced chemokine gradients such as CXCL10 [7], CAR-T cells infiltrate the inflamed tumor, survive, and exert potent cytotoxic activity. Our model further predicts that iPSC-CAR-T will expand 2–3 fold in vivo when co-stimulated by viral and tumor antigens, providing long-term immunological memory.

As shown in Figures 2 and 3, the expected kinetics of tumor regression and immune-cell activation under this tri-modal therapy are depicted. These schematic data illustrate how HSV-1, anti-PD-1, and iPSC-CAR-T treatments cooperate to progressively enhance tumor control and T-cell infiltration.

Altogether, this cascade—Ignite by HSV-1, Release by PD-1 blockade, reinforce by engineered iPSC-CAR-T—transforms the TME from "cold" to "hot." By coordinating innate and adaptive immunity with mechanistic predictiveness and quantifiable expectations, our model seeks not only to reverse immune exclusion but also to establish durable tumor surveillance. Figures 2 and 3 depict idealized data, illustrating the expected kinetics of immune activation under this tri-modal therapy.

BLI mirrors tumor-volume readouts: triple and HSV-1+CAR-T exhibit true post-CAR-T regression or stable low plateaus, while other regimens show slowed growth or modest declines; control increases exponentially. Dashed lines mark treatment timing; curves are expected trends. (Plotted on a log scale.)

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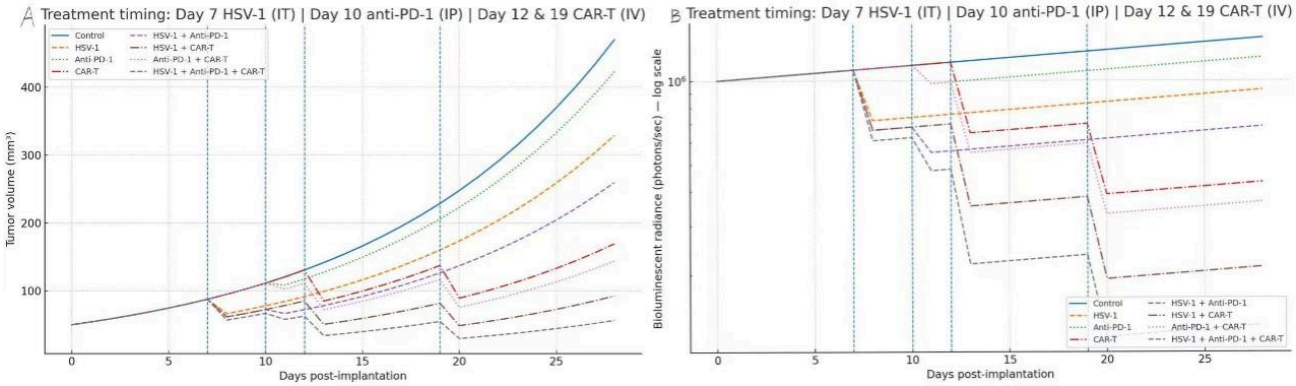

Figure 2. Tumor burden under different treatments. (a) Tumor volume measured by caliper (mm³). (b) Tumor bioluminescent radiance (log scale). Dashed vertical lines indicate treatment time points (Day 7: HSV-1; Day 10: anti-PD-1; Days 12 and 19: CAR-T)

Tumor volume and bioluminescence indicate that the triple therapy leads to sustained regression, whereas other groups show limited growth control. Treatment timings are indicated for reference.

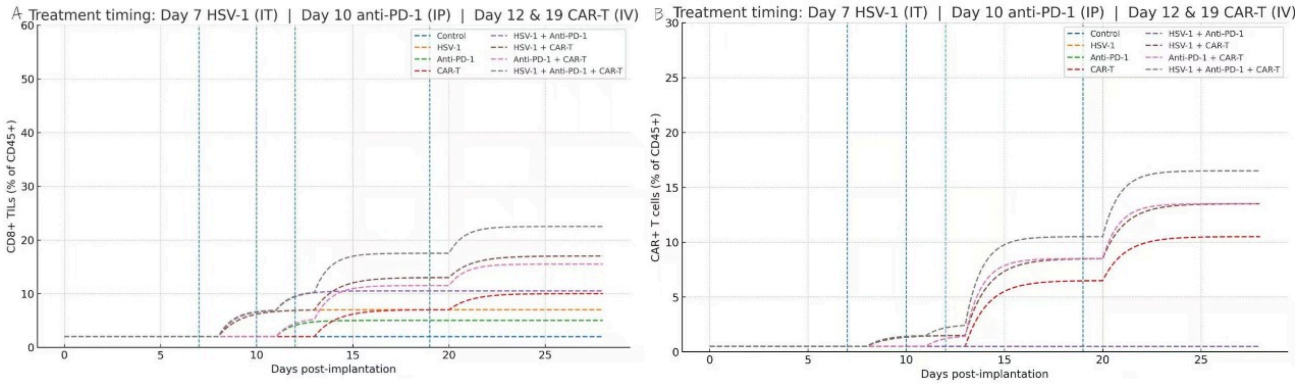

Figure 3. T-cell dynamics in the tumor microenvironment

CD8⁺ and CAR-T cell proportions increase following treatment, reflecting enhanced immune infiltration and activation.

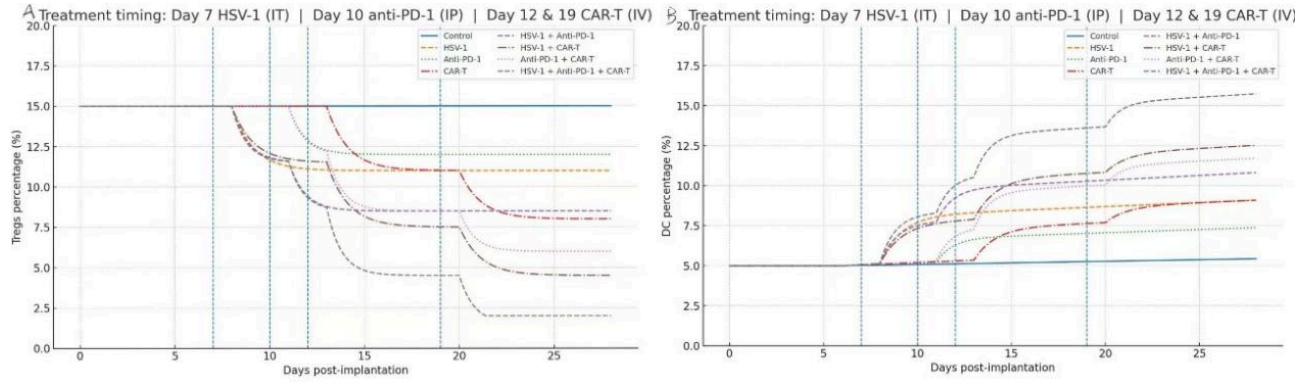

Figure 4. Changes in Tregs and dendritic cells. (a) Tregs (% of CD45⁺). (b) Dendritic cells (% of CD45⁺). Dashed vertical lines indicate treatment time points (Day 7: HSV-1; Day 10: anti-PD-1; Days 12 and 19: CAR-T)

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Reduced Tregs and increased dendritic cells suggest decreased immunosuppression and improved antigen presentation.

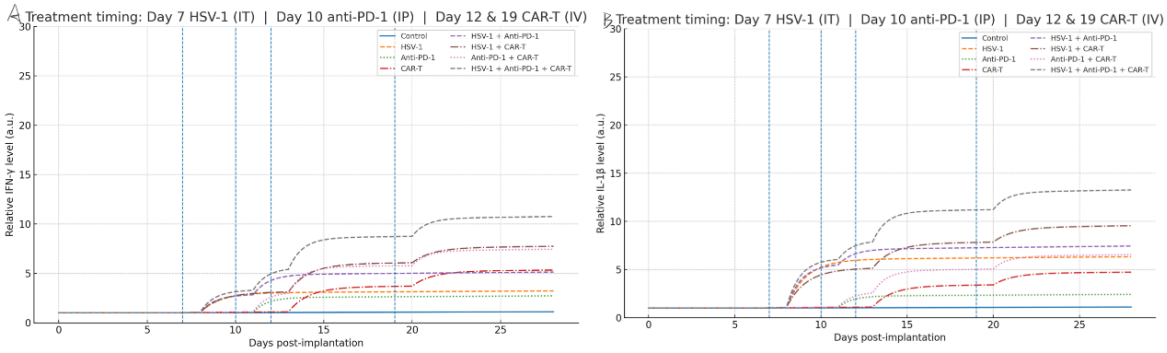

Figure 5. Expected cytokine dynamics. (a) IFN-γ (arbitrary units). (b) IL-1β (arbitrary units). Dashed vertical lines indicate treatment time points (Day 7: HSV-1; Day 10: anti-PD-1; Days 12 and 19: CAR-T)

IFN-γ and IL-1β increase, indicating activation of inflammatory and cytotoxic immune responses.

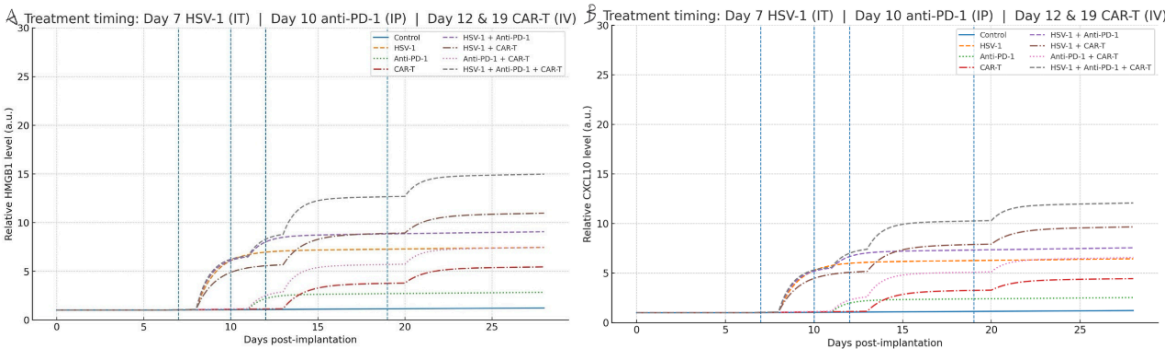

Figure 6. Expected damage-associated molecular patterns and chemokine release. (a) HMGB1 (arbitrary units). (b) CXCL10 (arbitrary units). Dashed vertical lines indicate treatment time points (Day 7: HSV-1; Day 10: anti-PD-1; Days 12 and 19: CAR-T)

HMGB1 and CXCL10 levels increase, supporting immune activation and T-cell recruitment.

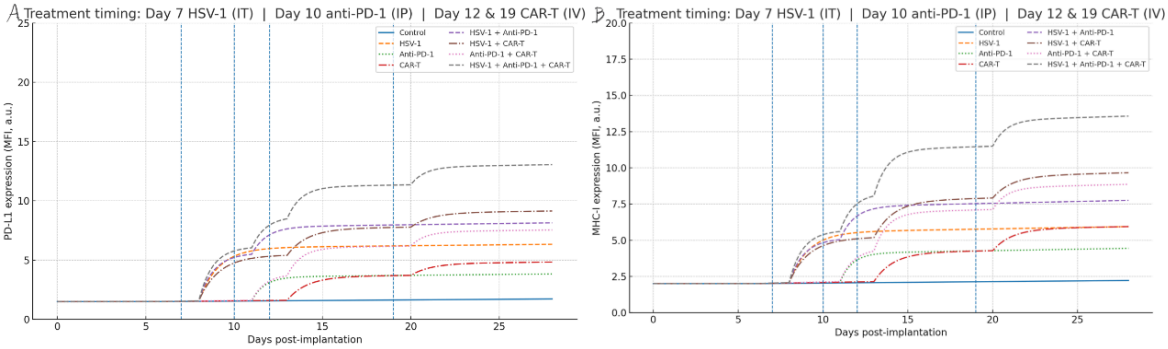

Figure 7. Expected changes in immune checkpoint and antigen presentation markers. (a) PD-L1 expression (mean fluorescence intensity). (b) MHC-I expression (mean fluorescence intensity). Dashed vertical lines indicate treatment time points (Day 7: HSV-1; Day 10: anti-PD-1; Days 12 and 19: CAR-T)

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PD-L1 and MHC-I expression changes reflect immune activation and enhanced antigen presentation.

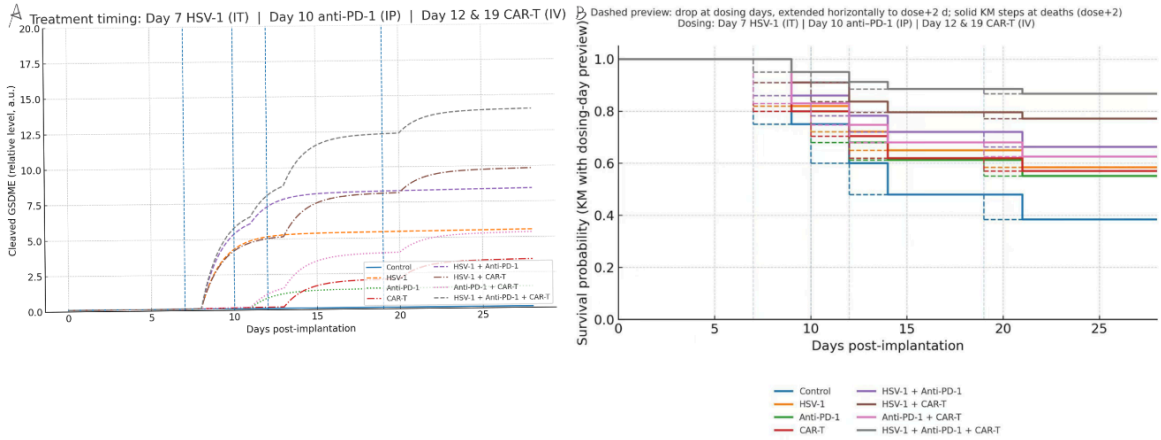

Figure 8. Expected pyroptosis marker and survival outcomes. (a) Cleaved GSDME (arbitrary units). (b) Kaplan–Meier survival curve. Dashed vertical lines in (a) indicate treatment time points (Day 7: HSV-1; Day 10: anti-PD-1; Days 12 and 19: CAR-T); survival steps correspond to actual death events

GSDME cleavage indicates pyroptosis, while survival curves show improved outcomes in combination therapy.

3.2. Experimental outline

To test the efficacy of our proposed triple-combination strategy in immune-desert tumors, we designed a preclinical in vivo study utilizing well-established syngeneic murine models. The experimental goal is to evaluate whether the integration of oncolytic virotherapy, anti-PD-1 checkpoint blockade, and iPSC-derived CAR-T cell therapy can effectively remodel the tumor microenvironment (TME) and elicit robust anti-tumor immunity.

3.3. Tumor models

We will employ two immune-excluded tumor models known for poor T cell infiltration:

- GL261 orthotopic glioma model in C57BL/6 mice.
- Panc02 orthotopic pancreatic cancer model in C57BL/6 mice.Optional validation can be performed using humanized NSG mice bearing patient-derived xenografts (PDX) for testing human iPSC-derived CAR-T cells.

3.4. Treatment arms

Eight treatment groups (n = 8–10 per group) will be established:

a. Untreated control
b. HSV-1 alone
c. Anti-PD-1 alone
d. iPSC-derived CAR-T cells alone
e. HSV-1+ anti-PD-1
f. HSV-1 + CAR-T

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g. anti-PD-1 + CAR-T

h. Triple combination: HSV-1 + anti-PD-1 + CAR-T

Sample size justification: Group size (n=8–10) is based on prior power analyses in syngeneic tumor models, targeting 80% power to detect at least a 50% reduction in tumor volume relative to control (α=0.05).

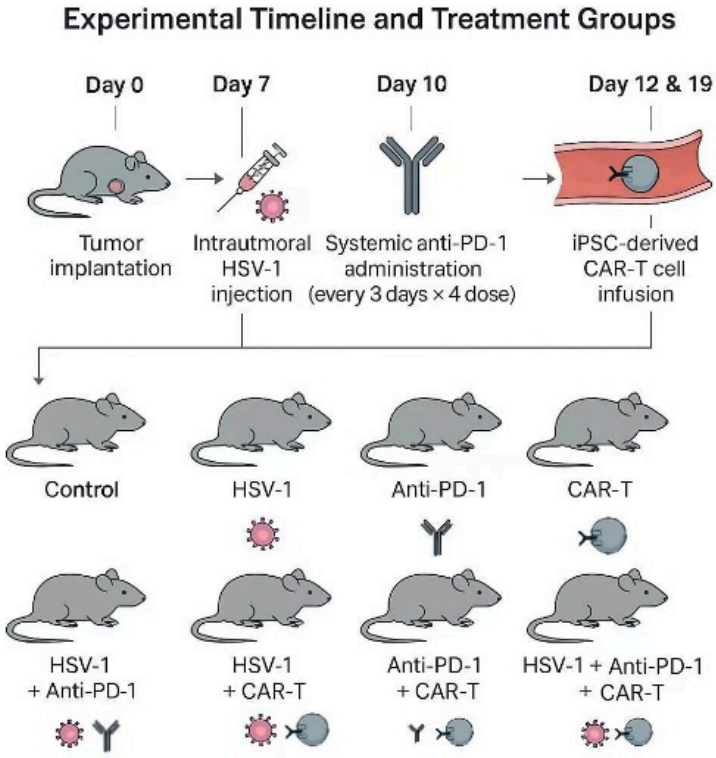

Figure 9. Experimental timeline and treatment groups

The overall experimental timeline and treatment allocations are summarized in Figure 9, which outlines the sequence and timing of HSV-1, anti-PD-1, and iPSC-CAR-T administrations.

3.5. Treatment timeline

• Day 0: Tumor implantation

• Day 7: Intratumoral injection of HSV-1. This time point is selected because tumors typically reach 50–100 mm³, ensuring sufficient OV uptake.

• Day 10: Systemic administration of anti-PD-1 antibody (e.g., clone RMP1-14, every 3 days × 4 doses). This coincides with the expected upregulation of PD-L1 induced by HSV-1 infection [5, 33].

• Day 12 and 19: Intravenous infusion of iPSC-derived CAR-T cells targeting a tumor-associated antigen (e.g., mesothelin) [24, 34]. These dual infusions are intended to maximize persistence and expansion within the OV-inflamed TME.

3.6. CAR-T cell engineering

iPSCs will be reprogrammed and differentiated into $CD8^+$ effector T cells [8], followed by lentiviral or CRISPR-mediated integration of a CAR construct. To enhance TME compatibility:

• CXCR3 overexpression will facilitate chemokine-directed trafficking into inflamed TMEs [7].

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• PD-1 knockout and IL-15 co-expression may be incorporated to prevent exhaustion and improve persistence in suppressive niches.

3.7. Endpoints and readouts

• Tumor burden: Measured by bioluminescent imaging and caliper size twice weekly. We anticipate a significant reduction (>50%) in tumor volume relative to control, consistent with prior OV+checkpoint blockade studies [5, 33], with triple therapy outperforming mono- and dual-therapy groups.

• Immune infiltration: Flow cytometry and IHC for $CD8^+$, CAR^+ T cells, Tregs, and dendritic cells. Triple therapy is expected to yield the highest $CD8^+$ infiltration (estimated 3–5× baseline) and the lowest Treg/Teff ratio (<0.5× baseline).

• TME remodeling: Cytokine/chemokine profiling and transcriptional signatures (RNA-seq).

• Cell death model: Pyroptosis markers (cleaved GSDME).

• Additional readouts: multiplex cytokine analysis and in vivo NK depletion experiments to evaluate CAR-T persistence under inflammatory conditions.

3.8. Statistical analysis

Tumor growth will be analyzed by ANOVA with multiple comparison correction (Tukey post-hoc test), and survival differences assessed by log-rank (Mantel–Cox) test.

4. Discussion

Our study explores a triple-modality immunotherapy integrating oncolytic HSV-1, anti–PD-1 blockade, and iPSC-derived CAR-T cells to remodel the tumor microenvironment (TME) in immune-excluded cancers. Preclinical data indicate enhanced antigen presentation, pyroptotic tumor cell death, and improved $CD8^+$ T-cell infiltration, suggesting potential synergy between viral immunogenicity, checkpoint release, and engineered T cell therapy [5, 17, 22, 33].

Several challenges remain. Safety concerns are paramount: oncolytic HSV-1 can disseminate in immunocompromised hosts, and iPSC-derived T cells carry risks of genomic instability, mandating stringent quality control and dose-escalation safeguards [12]. Mechanistically, NK-mediated clearance of HLA-edited "universal" CAR-T cells and tumor antigen heterogeneity may limit durability of responses [35]. While murine syngeneic models provide proof-of-concept, they fail to fully replicate human stromal and immune complexity, underscoring the need for validation in humanized NSG-PDX systems [36].

Alternative strategies could further enhance efficacy. If pyroptosis induction by oncolytic virotherapy is insufficient, incorporating cGAS–STING agonists may strengthen innate sensing and dendritic cell activation [17]. If CAR-T trafficking remains suboptimal, chemokine receptor engineering (e.g., CCR5, CXCR3) offers a means to improve homing and overcome stromal barriers. These refinements aim to optimize T cell persistence and infiltration in otherwise poorly penetrable tumor contexts. Clinically, glioblastoma and pancreatic cancer are highly relevant testbeds. Glioblastoma within the immune-privileged CNS and pancreatic cancer through a dense desmoplastic stroma both exemplify immune exclusion [37]. Demonstrating efficacy in these settings would represent a meaningful advance in immunotherapy for tumors resistant to current modalities.

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In summary, this work provides a framework to test whether combining viral, checkpoint, and cellular immunotherapies can overcome immune exclusion. Translation to the clinic will require balancing efficacy with biosafety and addressing human-specific barriers, but this multifaceted strategy highlights a promising path forward for otherwise refractory solid tumors.

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Junyue Ji, Shanshan Huang, Shenbing Zhang, Tianyu Zhou and Yumin Min contributed equally to this work and should be considered co-first authors.

References

[1] Clifton, G. T., et al. (2023). Developing a definition of immune exclusion in cancer: results of a modified Delphi workshop. *Journal for ImmunoTherapy of Cancer, 11*, e006773. https://doi.org/10.1136/jitc-2023-006773.
[2] Liu, Y. T., & Sun, Z. J. (2021). Turning cold tumors into hot tumors by improving T-cell infiltration. *Theranostics, 11*(11), 5365–5386. https://doi.org/10.7150/thno.58390.
[3] Hughes, T., & Kaufman, H. L. (2014). The use of oncolytic herpesvirus for the treatment of cancer. In: Lukashevich, I., & Shirwan, H. (eds.) *Novel Technologies for Vaccine Development*. Springer, Vienna. https://doi.org/10.1007/978-3-7091-1818-4_11.
[4] Ferrucci, P. F., Pala, L., Conforti, F., & Cocorocchio, E. (2021). Talimogene laherparepvec (T-VEC): An intralesional cancer immunotherapy for advanced melanoma. *Cancers, 13*(6), 1383. https://doi.org/10.3390/cancers13061383.
[5] Ribas, A., et al. (2017). Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. *Cell, 170*(6), 1109-1119.e10. https://doi.org/10.1016/j.cell.2017.08.027.
[6] Soldner, F., & Jaenisch, R. (2012). iPSC disease modeling. *Science, 338*, 1155–1156. https://doi.org/10.1126/science.1227682.
[7] Evgin, L., et al. (2022). Oncolytic virus–mediated expansion of dual-specific CAR T cells improves efficacy against solid tumors in mice. *Science Translational Medicine, 14*(640), eabn2231. https://doi.org/10.1126/scitranslmed.abn2231.
[8] Qiao, J., Kottke, T., Willmon, C., et al. (2008). Purging metastases in lymphoid organs using a combination of antigen-nonspecific adoptive T cell therapy, oncolytic virotherapy and immunotherapy. *Nature Medicine, 14*, 37–44. https://doi.org/10.1038/nm1681.
[9] Lin, J., et al. (2023). Oncolytic Parapoxvirus induces Gasdermin E-mediated pyroptosis and activates antitumor immunity. *Nature Communications, 14*(1), 224. https://doi.org/10.1038/s41467-023-35917-2.
[10] Zamarin, D., et al. (2025). Oncolytic virotherapy converts immunologically inert tumors into in situ vaccines via immunogenic cell death. *Immunity, 58*(4), 1–16. https://doi.org/10.1016/j.immuni.2025.03.014.
[11] Fukuhara, H., Ino, Y., Kuroda, T., Martuza, R. L., & Todo, T. (2023). Engineering IL-12 and IL-15 into oncolytic HSV enhances systemic antitumor immunity in murine models. *Molecular Therapy Oncology, 5*(3), 102–115. https://doi.org/10.1016/j.omton.2023.100267.
[12] Fang, Y., Chen, Y., & Li, Y.-R. (2025). Engineering the next generation of allogeneic CAR cells: iPSCs as a scalable and editable platform. *Stem Cell Reports, 20*(7), 102515. https://doi.org/10.1016/j.stemcr.2025.102515.
[13] Chesney, J., et al. (2022). Tumor microenvironment remodeling by talimogene laherparepvec potentiates PD-1 blockade efficacy. *Nature Communications, 13*(1), 4625. https://doi.org/10.1038/s41467-022-32246-8.
[14] Zamarin, D., et al. (2018). Timing of PD-1 blockade is critical to effective combination immunotherapy with oncolytic viruses. *Molecular Therapy, 25*(6), 1416–1426. https://doi.org/10.1016/j.ymthe.2018.03.016.
[15] Chen, J., et al. (2024). Oncolytic HSV-1 remodels the glioblastoma immunosuppressive landscape via cGAS-STING activation and neutrophil reprogramming. *Cancer Cell, 42*(5), 789–803. https://doi.org/10.1016/j.ccell.2024.03.009.
[16] Wang, X., et al. (2023). HSV-1 virotherapy remodels the tumor microenvironment to enhance homing of iPSC-derived CAR-T cells. *Molecular Therapy, 31*(8), 2410–2424. https://doi.org/10.1016/j.ymthe.2023.05.015.
[17] Zhong, H., et al. (2023). Nanodrug augmenting antitumor immunity for enhanced TNBC therapy via pyroptosis and cGAS-STING activation. *Nano Letters, 23*(11), 5083–5091. https://doi.org/10.1021/acs.nanolett.3c01008.
[18] Ribas, A., et al. (2019). Talimogene laherparepvec (T-VEC) as a paradigm for oncolytic immunotherapy. *Nature Reviews Drug Discovery, 18*(11), 689–706. https://doi.org/10.1038/s41573-019-0029-0.

DOI: 10.54254/2753-8818/2025.AU36289

[19] Andtbacka, R. H. I., et al. (2015). Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *Journal of Clinical Oncology, 33*(25), 2780–2788. https://doi.org/10.1200/JCO.2014.58.3377.
[20] Wang, Q., et al. (2020). Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of GSDME. *Science Advances, 6*(47), eabb4917. https://doi.org/10.1126/sciadv.abb4917.
[21] Zhang, Z., et al. (2021). Gasdermin E suppresses tumor growth by activating anti-tumor immunity. *Nature, 579*(7799), 415–420. https://doi.org/10.1038/s41586-020-2071-9.
[22] Chen, J., et al. (2024). GSDME-expressing oncolytic HSV-1 overcomes therapeutic resistance in glioblastoma by pyroptosis-mediated immune activation. *Science Translational Medicine, 16*(736), eadf3646. https://doi.org/10.1126/scitranslmed.adf3646.
[23] Baeuerle, P. A., et al. (2023). Bispecific T-cell engagers: Mechanisms driving efficacy and strategies to extend duration of response. *Cancer Immunology Research, 11*(5), 545–560. https://doi.org/10.1158/2326-6066.CIR-22-0925.
[24] Themeli, M., et al. (2024). Off-the-shelf antigen-specific iPSC-derived T cells for targeted cancer immunotherapy. *Science Translational Medicine, 16*(734), eadg7254. https://doi.org/10.1126/scitranslmed.adg7254.
[25] Choi, B. D., et al. (2025). Oncolytic HSV-1 primes the glioblastoma microenvironment for bispecific antibody-enhanced iPSC-T cell therapy. *Nature Cancer, 6*(3), 321–335. https://doi.org/10.1038/s43018-024-00754-7.
[26] Cloughesy, T. F., et al. (2023). Retrograde axonal transport enables oncolytic HSV-1 to bypass the blood-brain barrier and target invasive glioblastoma. *Nature Communications, 14*(1), 4123. https://doi.org/10.1038/s41467-023-39855-x.
[27] Stromnes, I. M., et al. (2022). Oncolytic herpes simplex virus degrades pancreatic cancer extracellular matrix to enhance T cell infiltration. *Science Translational Medicine, 14*(670), eabn7381. https://doi.org/10.1126/scitranslmed.abn7381.
[28] Rojas, L. A., et al. (2023). Systemic immune activation by matrix-depleting oncolytic HSV overcomes resistance in pancreatic ductal adenocarcinoma. *Cell, 186*(15), 3241–3256. https://doi.org/10.1016/j.cell.2023.06.031.
[29] Binnewies, M., et al. (2018). Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nature Medicine, 24*(5), 541–550. https://doi.org/10.1038/s41591-018-0014-x.
[30] Mariathasan, S., et al. (2018). TGF-β attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature, 554*(7693), 544–548. https://doi.org/10.1038/nature25501.
[31] Krysko, D. V., Garg, A. D., Kaczmarek, A., Krysko, O., Agostinis, P., & Vandenabeele, P. (2012). Immunogenic cell death and DAMPs in cancer therapy. *Nature Reviews Cancer, 12*(12), 860–875. https://doi.org/10.1038/nrc3380.
[32] Melcher, A., Parato, K., Rooney, C. M., & Bell, J. C. (2011). Thunder and lightning: Immunotherapy and oncolytic viruses collide. *Molecular Therapy, 19*(6), 1008–1016. https://doi.org/10.1038/mt.2011.65.
[33] Ribas, A., et al. (2023). Phase Ib trial of talimogene laherparepvec (T-VEC) plus pembrolizumab sequential therapy for advanced melanoma. *Journal of Clinical Oncology, 41*(15), 2789–2801. https://doi.org/10.1200/JCO.22.01903.
[34] Vizcardo, R., Masuda, K., Yamada, D., Ikawa, T., Shimizu, K., Fujii, S.-I., Koseki, H., & Kawamoto, H. (2013). Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature CD8(+) T cells. *Cell Stem Cell, 12*(1), 31–36. https://doi.org/10.1016/j.stem.2012.12.008.
[35] Jo, S., et al. (2022). Endowing universal CAR T-cell with immune-evasive properties using TALEN-gene editing. *Nature Communications, 13*(1), 3453. https://doi.org/10.1038/s41467-022-30896-2.
[36] Olson, B., Li, Y., Lin, Y., Liu, E. T., & Patnaik, A. (2018). Mouse models for cancer immunotherapy research. *Cancer Discovery, 8*(11), 1358–1365. https://doi.org/10.1158/2159-8290.CD-18-0044.
[37] Watt, J., & Kocher, H. M. (2013). The desmoplastic stroma of pancreatic cancer is a barrier to immune cell infiltration. *Oncoimmunology, 2*(12), e26788. https://doi.org/10.4161/onci.26788.