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The oncolytic virus of p53-armed NDV, in combination with GM-CSF and iPD-L1, induces the Immunogenic cell death of melanoma

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Abstract:

Melanoma is a malignant tumor that mutates from melanocytes on the skin and mucous membranes. (Long et al., 2023) Cutaneous melanoma is the most dangerous skin cancer, which causes 80% of causalities (Saeed et al., 2024). The treatment of melanoma includes wide excision and radiotherapy, which makes it hard to perform a systematic impact and could produce side effects (Sakunchotpanit et al.,2024). As immunotherapy, oncolytic virus therapy provides a pathway to combine a direct attack of this cancer, leading to a favorable prognosis. However, oncolytic virus therapy is fraught with challenges as the generally immunosuppressive tumor microenvironment interferes with inducing an effective systemic immune response. To overcome the immunosuppression, activate the innate immune response, and cause an abscopal effect in tumor treatment, we propose building on a previously described recombinant Newcastle disease virus (NDV) encoding GM-CSF, MEDI5395. Oncolytic virotherapy induces immunogenic cell death (ICD) and antitumor immune responses, which can cause the abscopal effect simultaneously. It has previously been shown that p53 expression in OVs can induce more severe ICD and enhance T-cell infiltration, leading to reduced tumor growth and long-lasting systemic anti-tumor immune response in preclinical models by secreting adenosine triphosphate (ATP) and high-mobility group box protein B1(HMGB1). In this way, untreated tumor cells are also suppressed. iPD-L1 combined with GM-CSF effectively reduces tumor viability, enhances immune cell infiltration, and upregulates immune-related genes, promoting systemic anti-tumor immunity and improved survival in mouse melanoma models. We hypothesize that NDV encoding p53 and iPD-L1/GM-CSF effectively induce immunogenic cell death, enhance immune cell infiltration, and reduce tumor growth, demonstrating significant therapeutic potential in

preclinical models. In conclusion, editing such genes in NDV can potentially enhance melanoma's treatment efficacy, resulting in systemic anti-tumor responses.

Keywords: melanoma; GM-CSF; iPD-L1; P53; ICD; NDV

1. Introduction

Melanoma is a malignancy tumor of the cells responsible for producing melanin in the epidermis's basal layer (Saeed et al., 2024).

In Europe, the prevalence of melanoma is 25 new cases per 100,000 individuals; in the USA, it is 30 cases per 100,000; and in Australia and New Zealand, it is 60 cases per 100,000 (Saeed et al., 2024). According to the most recent Surveillance, Epidemiology, and End Results (SEER) data,melanoma is one of the most common types of cancer in the United States, ranking fifth in cancer incidence in the country, excluding other skin cancers (Saeed et al., 2024).

In general, the prognosis for melanoma is usually not very optimistic. Melanoma is the skin cancer type with the highest mortality rate, and 80% of deaths from skin cancer are caused by melanoma (Saeed et al., 2024). Several factors influence the prognosis of the melanoma. Effective immunotherapy and targeted therapy play an important role in improving the treatment outcomes of advanced cancer patients and increasing their survival rates. For instance, ICIs, which provide long-term survival to patients who respond to immunotherapy, are approved to treat the earlier stages of melanoma (Pavlick et al., 2023). In addition, the prognosis of melanoma also depends on the presentation stage (National et al., 2024).

Melanomas' causes include environmental, genetic, and personal factors. UV and ionizing radiation are known risk factors that can cause skin cancer. According to Saeed et al., advanced age contributes to melanoma due to a less effective immune system and a diminished capacity for DNA repair. In addition, a family history of melanoma-related to the mutations of genes such as CDKN2A,CDK4,and BAP1, as well as exposure to ultraviolet radiation, can cause the skin to darken in color and accumulate melanin, are important factors, too (Saeed et al., 2024).

The diagnosis can use methods such as optical coherence tomography (OCT): OCT generates cross-sectional images of skin tissue using low coherence light, allowing for early skin cancer detection. Reflectance Confocal Microscopy (RCM): RCM involves the reflection of near-infrared light to create high-resolution images of the skin. It helps to distinguish benign and malignant tumor lesions better, with high sensitivity and specificity. Other methods include Liquid and Adhesive Patch Biopsy, Raman Spectroscopy, etc. Also, deep learning algorithms help diagnoses much quicker by using past experiencesin AI diagnosis. (Saeed et al., 2024)

The treatments of melanoma include traditional methods like surgery and radiotherapy, such as an Excisional Biopsy, which removes the tumor along with a margin of surrounding healthy tissues, or Mohs Micrographic Surgery, which removes the cancer tissues layer by layer. It also includes advanced therapies such as immunotherapy and targeted therapy. One key method is oncolytic virus therapy. This treatment method infects cancer cells with specific genetically modified viruses, replicates, and reproduces within the cancer cells, ultimately leading to cancer cell death. (Saeed et al., 2024)

In this study, we aimed to develop the concept of a new treatment for melanoma. Our combined new approach is inspired by the following three studies, each of which discussed different orthologous approaches:

2. Literature review

2.1 Study 1. Oncolytic Newcastle disease virus activation of the innate immune response and priming of antitumor adaptive reactions in vitro. (Shannon, et al., 2020)

This article explores the therapeutic potential of an engineered Newcastle disease virus (NDV) to express granulocyte-macrophage colony-stimulating factor (GM-CSF), referred to as MEDI5395, for cancer treatment. The main argument is that MEDI5395, engineered to express GM-CSF, significantly stimulates the immune system, thereby enhancing anti-tumor immunity.

The article's point is that MEDI5395 is a virus modified to carry GM-CSF, which exhibits oncolytic solid activity and can help the body fight tumors more effectively. They used in vitro models of human PBMC, myeloid cells, and DCs to study the virus's mode of action and found that MEDI5395 mainly infects myeloid cells, resulting in

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immune activation, more cytokine production, and better antigen presentation. Not only that, but he also explicitly infected M2-polarized macrophages, which are known for suppressing immune responses in tumors.

In short, the paper shows that MEDI5395 has multiple modes of action, directly destroying tumor cells and activating innate and adaptive immune responses. It is a valuable treatment, especially for turning "cold" tumors that do not respond well to immunotherapy into "hot" tumors that are more easily treated, making MEDI5395 an excellent potential drug candidate.

2.2 Study 2. Telomerase-specific replication-competent oncolytic adenoviruses p53-armed OBP-702 in human OS cells. (Koji et al., 2024)

This article explores a novel treatment method based on osteosarcoma (OS) treatment. At present, the treatment methods for OS include chemotherapy and surgical resection, but these methods have poor therapeutic effects and high recurrence rates. Therefore, this article explores a novel treatment method using oncolytic viruses carrying the p53 gene for treatment. Researchers introduced the p53 gene into telomerase-specific replication-competent oncolytic adenoviruses OBP-702, allowing for the p53 protein expression when oncolytic viruses infect tumor cells, thereby enhancing the killing effect on tumors.

The oncolytic virus carrying the p53 gene induces an observational effect by activating immunogenic cell death (ICD) and inhibiting tumor growth at distant sites. This is generally referred to as an abscopal effect when local treatment-mediated ICD induction subsequently promotes an antitumor immune response against treated and untreated tumors.

This article demonstrates the effect of p53-armed OBP-702 on OS through the following aspects. First, the authors tested the efficacy of several compounds, finding that both doxorubicin and cisplatin (DOX and CDDP) have therapeutic potential and can stimulate the ATP and HMGB1 secretion in OS cells to induce ICD. TOBP-301 and OBP-702 were compared with standard drugs such as DXXP and DOX by observing cell survival and cell death markers. Both OBP-301 and OBP-702 caused more cell death. However, OBP-702 stood out, releasing more markers and having more substantial therapeutic potential. Immunohistochemistry results showed that OBP-702 significantly increased T cell accumulation, especially the number of CD8+ and CD4+ T cells. The researchers then examined the anti-tumor influence of OBP-301 and OBP-702 in the NHOS osteosarcoma-bearing mouse model. OBP-702 affected osteosarcoma and triggered an immune response in untreated areas, which helped stop tumor growth in these sites.

In summary, p53-carrying oncolytic virus therapy can trigger immunogenic cell death and produce distal effects in osteosarcoma, making it a promising treatment option.

2.3 Study 3. An engineered oncolytic virus expressing PD-L1 inhibitors activates tumor neoantigen-specific T-cell responses. (Guan et al., 2020)

Neoantigensmutated self-proteins can induce tumor-specific immune responses. However, neoantigen-specific T-cell responses in most cancers are inefficiently primed due to inadequate antigen expression and presentation combined with immunosuppression molecules.

To overcome these challenges, the authors constructed and built an engineered oncolytic virus (VV-iPDL1/GM) coexpressing a PD-L1 inhibitor and genetically modified granulocyte-macrophage colony-stimulating factor (GM-CSF) that can potently inhibit PD-L1 expression and promote intratumoral T cell infiltration. The administration of VV-iPDL1/GM resulted in a high expression of GM-CSF and iPD-L1. In vitro, secreted iPD-L1 could effectively bind to PD-L1 molecules on tumor and immune cells and reduce the expression of PD-L1 on immune cells. In live animal models, the modified oncolytic virus can enhance anti-tumor immunity, eliciting a strong response even in tumors far from the primary site. The interaction between iPDL1 and PD-L1 was not disrupted by PD-L1 antibodies, meaning it can bypass immunosuppression and produce a robust anti-tumor effect. The virus also helped activate T cells and reduced the number of Treg and MDSC cells (immune cells that usually suppress anti-tumor responses), indicating that it can positively change the tumor environment.

The study showed that the virus can generate a solid and specific T-cell response to new antigen epitopes, releasing cytokine. It also improved antigen presentation, helped dendritic cell (DC) maturation, and produced cytokines such as IL-12, CXCL9, and CXCL10. This enhanced the interaction of dendritic cells and T cells (via IL-12) and directly affected T cell movement and infiltration into tumors (via CXCL).

In addition, the virus also improved the ability of neoantigen-specific T cells to kill tumor,, increased IFN-γ levels, and upregulated genes related to TNF signaling and protein processing. It improved the way T cells recognize tumor cells.

In short, the virus co-expressed PD-L1 inhibitors and GM-CSF, which enhanced neoantigen presentation and activated systemic T-cell responses, resulting in successful targeting and abscopal effects. This ability makes the virus a promising candidate for cancer treatment, especially for

cases that are disturbed by PD-1/PD-L1 blockade therapy.

3. Working model

We propose an engineered oncolytic virus expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) and a PD-L1 inhibitor. This virus activates immune cells to target melanoma cells specifically. This virus also promotes immunogenic cell death (ICD), a form of cell death that enhances immune response. It releases damage-associated molecular patterns (DAMPs) such as ATP and HMGB1. This further elicits an immune response against the tumor, increasing antigen release and activating specific T-cell anti-tumor responses. The anti-tumor immune response triggered by the death of tumor cells prolongs the therapeutic effect. We induce anti-tumor immunity by releasing cell molecules from dying cells in the tumor microenvironment, known as damage-associated molecular patterns (DAMPs). Overall, the immunogenicity conferred by tumor cell death depends on the antigenicity induced by the new antigen epitope and the adjuvant properties produced by specific DAMPs. This cell death method is known as immunogenic cell death (ICD). Therefore, ICD, as a particular form of cell death, can cause cell death and stimulate the body's specific immune response to antigens released by dead cells. Additionally, the combination of IPD-L1 with PD-L1 on tumor and immune cells disrupts immune suppression in the tumor's microenvironment, facilitating the clearance of both primary and distant tumors.

Firstly, we decided to use an engineered oncolytic virus that expresses the p53 gene to trigger cell death through the p53 pathway. It will increase PD-L1 inhibitor production, which blocks PD-L1 interactions, overcoming immune suppression and boosting the host's immune response. This part of our approach, involving the stimulation of the p53 pathway and inhibition of PD-L1, is supported by findings in various studies on immunotherapy and oncolytic virotherapy (Koji et al., 2024; Wang et al., 2020; Demiya et al., 2024).

In addition to GM-CSF, molecular signals released by host cells enhance dendritic cell maturation, which in turn presents antigens to activate T-cells (Burke et al., 2020; Guan et al., 2020). As tumor cells die, the neoantigens they release are presented to T-cells by these DCs, stimulating a strong immune response (Burke et al., 2020).

Third, tumor-specific T cells, particularly CD8+ cytotoxic T cells, proliferate to attack the primary tumor and migrate to distant metastases. GM-CSF directly stimulates the proliferation of APCs and encourages them to release cytokines that support T-cell activity. (Burke et al., 2020; Wang et al., 2020).

Fourth, the PD-L1 inhibitor will also be expressed to reduce the suppression of T cells. It binds to PD-L1 on tumor cells and immune cells, blocking the PD-1/PD-L1 interaction. The iPD-L1 inhibits Treg cells, upregulates the T cell /Treg cell ratio, and overcomes immunosuppression.

Through the combination of enhanced immunogenic cell death (ICD) and the use of PD-L1 inhibitors, our model not only targets primary melanoma tumors but also activates systemic immune responses (Burke et al., 2020; Wang et al., 2020). This approach leads to the abscopal effect, ensuring the immune system can also eliminate metastatic tumors (Guan et al., 2020).

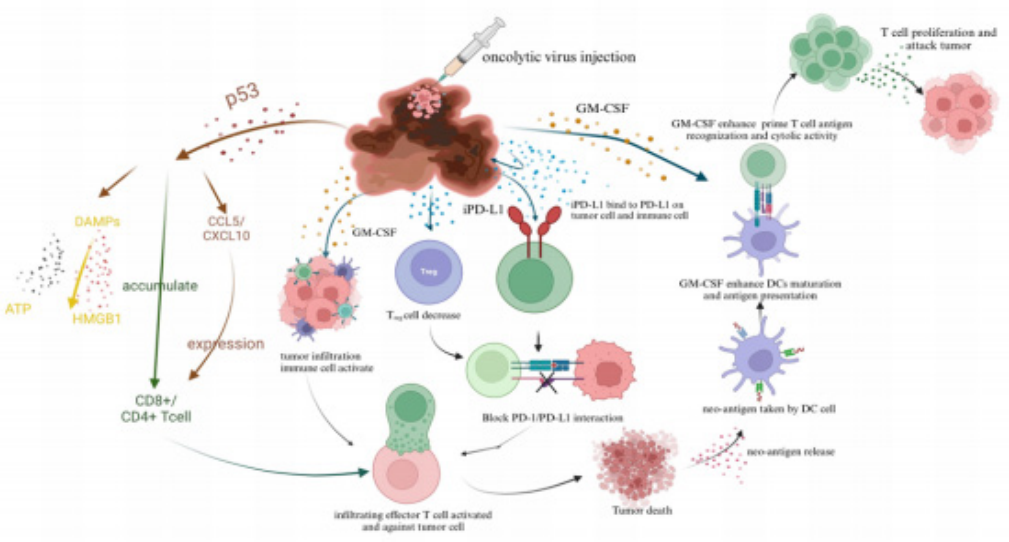

figure1:Overall model diagram

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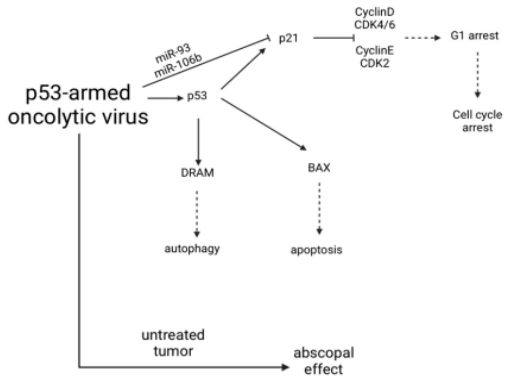

figure2: Regulation and abscopal effect of OBP-702 and P53 pathway
Figure2:Introduction to the process of p53 armed NDV inducing cell arrest, apoptosis, and death by inhibiting the p53 pathway.

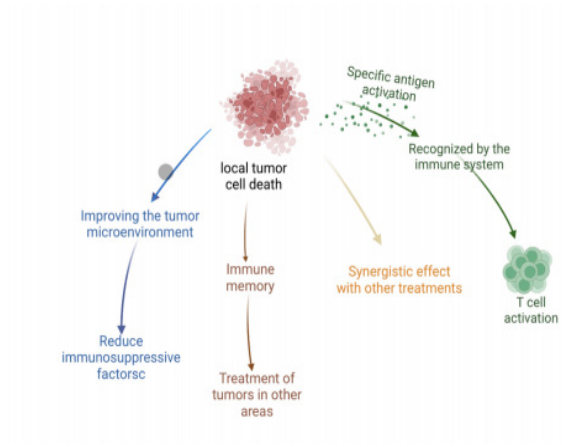

Figure: Abscopal effect

Figure pathways leading to remote effects.

4. Hypothesis

For our working model, we hypothesize that after an engineered oncolytic virus is administered, many molecules (DAMPs, GMCSF) will be released, inducing a potent immune response and altering the tumor microenvironment, overcoming
immunosuppression. This, in turn, can conceivably lead to a systematic anti-tumor response, minimizing tumor evasion and creating a complete and long-lasting treatment.

5. Experiments and results

In experiment 1, we observe the impact of MEDI5395 on different cell lines, such as monocytes, macrophages, and dendritic cells, by infecting them. Moreover, the infected dendritic cells are co-cultured with allogeneic T cells to evaluate the antigen-specific response of T cells.

To measure the cell viability, the cell viability after the infection will be measured. Moreover, an enzyme-linked immunosorbent assay (ELISA) will be used to calculate the concentration levels of pro-inflammatory cytokines in the cell culture supernatant after viral infection, such as the levels of IFN-a2a, IL-6, IL-8, and TNF-a. If the virus can successfully activate immune cells, there will be a significant increase in the level of these cytokines. We also propose using ELISPOT and flow cytometry to analyze the proliferation of T cells in co-culture and the release of functional cytokines. The ability of the DC that MEDI5395 infects to take up and present tumor antigens is proposed to determine the activation status of DC.

We expect that the MEDI5395 can induce tumor cell immunogenic death and antigen-specific T-cell responses.

The primary goal of experiment 2 is to evaluate the therapeutic potential of NDV; we designed NDV by inserting the human wild-type p53 gene into OBP-301.

Based on our design for the vitro experiment, in vitro, experiments aim to assess NDV's ability to induce ICD through the secretion of ATP and HMGB1 in tumor cells, particularly in combination with chemotherapy. We plan to treat the tumor cells with chemotherapy drugs (DOX-,CDDP) followed by NDV infection. Cell viability will be assessed using 30-{1-[(phenylamino)-carbonyl]3,4-tetrazolium}-bis (4-methoxy-6-nitro) benzenesulfonic acid hydrate (XTT) assay. We will test OBP-702 for its ability to induce ICD, which can measured by analyzing ATP and HMGB1 secretion in human and mouse tumor cells. At the same time, western blot will detect key protein expressions like E1A, p53, and cleaved PARP.

Based on previous fundamental research studies(Koji,2024), we expect NDV to significantly induce immunogenic cell death (ICD) in tumor cells, as demonstrated by increased secretion of ATP and HMGB1 compared to control or chemotherapy alone.

We will mainly divide the in vivo experiment into three parts, namely the tumor immune infiltration effect of recombinant NDV infection on T cells, the abscopal effect, and the systemic anti-tumor immune effect,

First, in the experiment of enhancing T-cell immune infiltration through recombinant NDV infection. We will first divide the mice with good immune function into two groups. One group was injected with PBS and the other with recombinant NDV to treat mice once a week for three weeks. After treatment, immunohistochemical experiments were conducted on mouse tumor cells to analyze the number of CD8+ and CD4+ cells. The expected result is that compared to the PBS group, the number of T cells in tumor cells of mice infected with recombinant NDV is significantly higher, indicating that recombinant

NDV can enhance T cell immune infiltration and has better therapeutic potential.

Second, in experiments on abscopal effects. To investigate the role of recombinant NDV therapy in the untreated portion of internal local treatment. We first seeded tumor cells on both sides of mice with good immune function, treating one side with PBS/NDV and the other without treatment once a week for three weeks. After treatment, analyze the T cell count and tumor growth of the untreated portion of the tumor. Our expected outcome is that the number of T cells in the untreated portion of mice treated with NDV is much higher than that in the PBS-treated portion. Similarly, the number of T cells in the tumor-treated portion of mice treated with NDV is significantly lower than in the PBS group. This means that mice treated with NDV exhibited better abscopal effects.

Third, the role of systemic anti-tumor immunity. Firstly, a two-week pretreatment experiment was conducted on mice treated with PBS and NDV. First, treat the right side of the mouse with PBS/NDV for three weeks. After treatment, the original tumor was removed, the same tumor cells were injected into the left side of the mouse, and the growth of the mouse tumor cells was observed. Our expected outcome is that mice treated with NDV will have smaller tumors when reinfected compared to untreated mice, demonstrating the potential of NDV-treated mice to stimulate systemic tumor immunity when reinfected.

For the vivo experiment, we determine whether NDV can enhance immune cell infiltration into tumors, induce systemic immune responses in untreated tumors (abscopal effect), and establish long-lasting systemic anti-tumor immunity through a tumor rechallenge model. We propose to use NDV to treat C57BL/6 mice infected with B16-F10 tumors and treat one side of the double-sided tumor to check the systemic immune responses in untreated tumors. A follow-up re-challenge experiment will test NDV's ability to induce long-lasting systemic anti-tumor immunity by comparing tumor growth in treated and control mice.

Based on previous fundamental research studies, we predict that NDV will significantly increase CD8+ and CD4+ T cell infiltration in treated tumors, induce an abscopal effect with reduced growth in untreated tumors, and establish durable systemic immunity, leading to reduced tumor growth upon rechallenge.

In experiment 3, we will evaluate the therapeutic potential of iPD-L1; we plan to use Western blot and ELISA to confirm that IPD-L1 and GM-CSF were efficiently produced and released. Flow cytometry demonstrated that iPD-L1 effectively bound to PD-L1 on tumor cells, resulting in reduced PD-L1 expression and inhibited PD-1/PD-L1 interaction. In our prediction, this binding will also decrease Treg cell levels, reducing immune suppression within the tumor microenvironment. iPD-L1 will enhance the infiltration of immune cells, particularly CD8+ T cells, into tumors and mediate antibody-dependent cellular cytotoxicity (ADCC). At the same time, the T cell activation will be increased, with higher IFN-γ production levels and neoantigen-specific T cell proliferation.

Based on the experimental design involving the mouse melanoma B16-F10 model, we predict that treatment with iPD-L1/GM-CSF will significantly reduce tumor viability and size and improve survival rates in treated mice. We anticipate that RNA sequencing will reveal an upregulation of immune-related genes, indicative of enhanced activation of systemic anti-tumor immunity facilitated by iPD-L1/GM-CSF.

Table 1 Expected experiment

Experiment	Project	Expected results
Western blot	iPDL1 and GM-CSF express	produced and efficiently released
Flow Cytometry	Infection of tumor cells by viruses in vitro	Recombinant NDV-infected tumor cells are inhibited
ELISA	ATP	significant increase
ENLITEN	HMGB1	significant increase
ELISPOT	IFN-γ	significant increase
Bioluminescence	The growth status of tumor cells	Tumor cell growth inhibited by recombinant NDV infection
immunohistochemical	CD8+ and CD4+	Increased number of fine T cells after infection with recombinant NDV

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6. Discussion

The PD-1/PD-L1 pathway is an essential immune checkpoint that regulates the T cell response threshold (Xu et al., 2020). The interaction of PD-1 and PD-L1 can inhibit the proliferation of intracellular activities in CTLs, eventually leading to the death of activated T cells (Ghosh et al., 2021). This pathway is used to alter the tolerance of T cells and prevent autoimmunity. However, tumor cells hijack this pathway to escape T cell-mediated immunity by over-expression of PD-L1(Xu et al., 2020). Which also causes tumor resistance to many immunotherapies. To bypass immune checkpoints, researchers used immune check-point blockade drugs (ICBs), but ICBs can only affect a few kinds of cancer and can not alter tumor molecular environment (TME) (Vesely et al., 2022). Our armed oncolytic virus, through the release of iPD-L1 from tumor cells, can efficiently bind to PD-1 on both tumor and immune cells. Besides, it can also inhibit Treg cells, combine with GM-CSF, potently alter the TME, overcome the immunosuppression, and avoid the suspension of treatment. Therefore, the persistence of therapy can be guaranteed.

The recombinant NDV in our model is engineered to express the p53 gene to stimulate the p53 pathway, produce a PD-L1 inhibitor, and express GM-CSF. Compared with only expressing one pathway, this virus holds the potential to kill tumors more effectively and cause an abscopal effect. However, it still needs to combine with other methods, such as CAR T cell immunotherapy, to direct the virus specifically to tumor cells.

We could combine our recombinant NDV with the polypeptide vaccine to improve the virus. The principle of the peptide vaccine includes the pieces of specific antigens. These antigens can elicit a robust immune response to the particular tumor. The antigen-presenting cells (APCs) will present these peptides to T cells and help recognize tumor cells infected by the oncolytic virus (Liu et al., 2024). However, peptide vaccines have a less impressive effect on tumors than other immunotherapies (Liu et al., 2024). Combining the peptide vaccine with our recombinant NDV can enhance the immune response. In addition, using these two kinds of immunotherapies and traditional methods like chemotherapy and radiotherapy can also significantly improve the effect on the tumor.

In conclusion, our research discussed the potential melanoma treatment virus by combining the oncolytic virus with GM-CSF and PD-L1 inhibitors. The results in the original p53-armed oncolytic virus and the inhibitors would harm the tumor cells and activate a systemic immune response to control metastases. Further experimental research is needed to validate its efficacy in mice tests in the future to understand if the combination of inhibitors works with the p53-armed oncolytic virus.

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Ziyu Chen, Lexi Qi, Xiaoxuan Tan, Shuning Xie, and Xiaoran Yu contributed equally to this work and should be considered co-first authors.

References

Ai, L., Xu, A., & Xu, J. (2020). Roles of PD-1/PD-L1 pathway: Signaling, cancer, and beyond. In J. Xu (Ed.), *Regulation of cancer immune checkpoints*. Advances in Experimental Medicine and Biology, vol. 1248. Springer, Singapore. https://doi.org/10.1007/978-981-15-3266-5_3

Burke, S., Shergold, A., Elder, M. J., Whitworth, J., Cheng, X., Jin, H., Wilkinson, R. W., Harper, J., & Carroll, D. K. (2020). Oncolytic Newcastle disease virus activation of the innate immune response and priming of antitumor adaptive responses in vitro. Cancer Immunology, Immunotherapy, 69(6), 1015–1027. https://doi.org/10.1007/s00262-020-02495-x

Demiya, K., Tazawa, H., Kondo, H., Kure, M., Mochizuki, Y., Komatsubara, T., Yoshida, A., Uotani, K., Hasei, J., Fujiwara, T., Kunisada, T., Urata, Y., Kagawa, S., Ozaki, T., & Fujiwara, T. (2024). p53-armed oncolytic virotherapy induces the abscopal effect in osteosarcoma by promoting immunogenic cell death. *Molecular Therapy: Oncolytics, 32*(3), 200845. https://doi.org/10.1016/j.omton.2024.200845

Ghosh, C., Luong, G., & Sun, Y. (2021). A snapshot of the PD-1/PD-L1 pathway. *Journal of Cancer, 12*(9), 2735-2746.https://doi.org/10.7150/jca.57334

Hasei, J., Sasaki, T., Tazawa, H., Osaki, S., Yamakawa, Y., Kunisada, T., Yoshida, A., Hashimoto, Y., Onishi, T., Uno, F., Kagawa, S., Urata, Y., Ozaki, T., & Fujiwara, T. (2013). Dual programmed cell death pathways induced by p53 transactivation overcome resistance to oncolytic adenovirus in human osteosarcoma cells. *Molecular Cancer Therapeutics, 12*(3), 314-325. https://doi.org/10.1158/1535-7163.MCT-12-0869

Liu, D., Liu, L., Li, X., Wang, S., Wu, G., & Che, X. (2024). Advancements and challenges in peptide-based cancer vaccination: A multidisciplinary perspective. Vaccines, 12(8), 950. https://doi.org/10.3390/vaccines12080950 Long, G. V., Swetter, S. M., Menzies, A. M., Gershenwald, J. E., & Scolyer, R. A. (2023). Cutaneous melanoma. *Lancet, 402*(10400), 485-502. https://doi.org/10.1016/S0140-6736(23)00821-8

National Comprehensive Cancer Network. (2024). Melanoma: Cutaneous, Version 2.2024. Journal of the National Comprehensive Cancer Network, 22(5), 290-298.https://doi.org/10.6004/jnccn.2024.0036

Palanivelu, L., Liu, C. H., & Lin, L. T. (2023). Immunogenic

cell death: The cornerstone of oncolytic viro-immunotherapy. Frontiers in Immunology, 13,
1038226. https://doi.org/10.3389/fimmu.2022.1038226

Pavlick, A. C., Ariyan, C. E., Buchbinder, E. I., Davar, D., Gibney, G. T., Hamid, O., Hieken, T. J., Izar, B., Johnson, D. B., Kulkarni, R. P., Luke, J. J., Mitchell, T.
C., Mooradian, M. J., Rubin, K. M., Salama, A. K., Shirai, K., Taube, J. M., Tawbi, H. A., Tolley, J. K., Valdueza, C., … Sullivan, R. J. (2023). Society for Immunotherapy of Cancer (SITC) clinical practice guideline on immunotherapy for the treatment of melanoma, version 3.0. *Journal for immunotherapy of cancer*, 11(10), e006947.
https://doi.org/10.1136/jitc-2023-006947

Saeed, W., Shahbaz, E., Maqsood, Q., Ali, S. W., & Mahnoor, M. (2024). Cutaneous oncology: Strategies for melanoma prevention, diagnosis, and therapy. *Cancer Control, 31*, 1–24.
https://doi.org/10.1177/10732748241274978

Sakunchotpanit, G., Patil, M. K., Venkatesh, K., Rohan, T. Z., Cheng, D., & Nambudiri, V. E. (2024). Treatment of malignant melanoma with coxsackievirus A21 (V937): An emerging oncolytic virotherapy. *Experimental Dermatology, 33*, e15169.
https://doi.org/10.1111/exd.15169

Tazawa, H., Hasei, J., Yano, S., Kagawa, S., Ozaki, T., & Fujiwara, T. (2020). Bone and soft-tissue sarcoma: A new target for telomerase-specific oncolytic virotherapy. Cancers (Basel), 12(2), 478.
https://doi.org/10.3390/cancers12020478

Vesely, M. D., Zhang, T., & Chen, L. (2022). Resistance mechanisms to anti-PD cancer immunotherapy. *Annual Review of Immunology, 40*, 45-74.
https://doi.org/10.1146/annurev-immunol-070621-030155

Wang, G., Kang, X., Chen, K. S., Jehng, T., Jones, L., Chen, J., Huang, X. F., & Chen, S. Y. (2020). An engineered oncolytic virus expressing PD-L1 inhibitors activates tumor neoantigen-specific T-cell responses. Nature Communications, 11(1), 1395.
https://doi.org/10.1038/s41467-020-15229-5