Use of Oncolytic Vaccinia Virus Armed with Certain Cytokines (IL-7, IL-12, IL-15, IL-21) in Combination with Checkpoint Inhibitors to Treat Non-Small Cell Lung Cancer

Yuqian Xu[1,*], Yuantong Li[2], Ziyang Yin[3], Zihao Jin[4], Wendao Li[5] and Zhaoyang Che[5]
[1]*School of Pharmaceutical Sciences, Tsinghua University, Beijing, 100084, China*
[2]*Department of Biology, Brandeis University, Waltham, MA, U.S.A.*
[3]*Concordia International School Shanghai, Shanghai, 201206, China*
[4]*Western International School Shanghai, Shanghai, 201206, China*
[5]*Shenzhen Foreign Languages School, Shenzhen, 518083, China*

Keywords: Oncolytic Virus, Checkpoint Inhibitors (Cpis), Cytokines, Non-Small Cell Lung Cancer.

Abstract: A variety of oncolytic viruses (OVs) have been reported in treating different cancers and several have entered clinical trials. This study proposes the combination of cytokines IL-7, IL-12, IL-15, IL-21, and checkpoint inhibitors (CPIs) co-expressed on an oncolytic vaccinia virus JX-594 to treat non-small cell lung cancer (NSCLC) with the aim of tumor regression. The effectiveness of the modified JX-594 vaccinia virus is measured both in H460 cell lines and in LSL-KrasG12D mouse model, including the production of cytokines and CPI, cytotoxicity, tumor growth, overall survival and tumor-infiltrating lymphocytes (TILs). The increased overall survival rate and tumor size regression in mouse model are predicted.

1 INTRODUCTION

1.1 General Overview to Oncolytic Viruses

In 1991, a genetically engineered herpes simplex virus-1 (HSV-I) was found to be a potential therapy in malignant glioma (Martuza, 1991). Since then, increasing attention was paid to the role of virus in treating cancer. Nowadays, oncolytic virus (OV) is a promising cancer immunotherapy. OV could kill cancers in two main ways: direct cell lysis as well as increased anti-tumor immune response (Abd-Aziz N, 2021). OVs are genetically engineered to selectively infect cancer cells via some receptor-mediated pathways, therefore minimizing potential tissue damage (Ferguson, 2012). OVs could also stimulate anti-tumor immune responses, such as presentation of TSAs (tumor specific antigens) and TAAs (tumor-associated antigens) to APCs to activate effector T cells (Ferguson, 2012). As the knowledge of the therapy advanced over years, OVs are genetically modified in diverse ways to enhance antitumor effect. These armed OVs could deliver cytokines, CPIs, tumor suppressors, etc (Marintcheva, 2018). Besides, a wider variety of OVs were tested clinically, including adenovirus, reovirus, measles, HSV-I, vaccinia virus, and Newcastle disease virus (Fukuhara, 2016).

Despite the extraordinary advantages, current oncolytic virus treatments face several challenges, including physical barriers, antiviral immunity, and immunosuppressive tumor microenvironment (TME). Multiple strategies that enhance OV delivery, infiltration, and oncolysis have been developed to increase the efficacy of OV therapy. Besides, recombinant OVs might cause some unexpected toxicities so safety analysis must be considered carefully. Currently, emerging genetic engineering techniques, combined with other therapies, like adoptive T cell therapy (ACT) and CPI are applied in OVs. These will make OV therapy one of the most promising immunotherapies in the future (Zheng, 2019).

* Corresponding author.

Xu, Y., Li, Y., Yin, Z., Jin, Z., Li, W. and Che, Z.
Use of Oncolytic Vaccinia Virus Armed with Certain Cytokines (IL-7, IL-12, IL-15, IL-21) in Combination with Checkpoint Inhibitors to Treat Non-Small Cell Lung Cancer.
DOI: 10.5220/0012032500003633
In *Proceedings of the 4th International Conference on Biotechnology and Biomedicine (ICBB 2022)*, pages 457-466
ISBN: 978-989-758-637-8

1.2 General Functions of Cytokines and CPIs in Regular and Antitumoral Immune Responses

Cytokines are polypeptides or glycoproteins secreted by diverse immune cells including T cells, neutrophils, and macrophages and could regulate immune responses (Cohen, 1996). Some cytokines have been discovered to have potent anti-tumor properties, which makes cytokine a monotherapy or potentiator of other therapies in cancer treatment (Berraondo, 2019). IL-7, IL-12, IL-15, and IL-21 are chosen in this article. IL-7 is needed in B-and T-cells development and could diminish cancer cell growth (Alderson, 1991). IL-7 treatment was also reported to enhance long-term CD8+ T-cell responses in mouse model (Colombetti, 2009). IL-12 can activate effector Th1 response, thus serving as a link between innate and acquired immunity. This also further induces activation of T-cell, NK-cells, and tumor clearance. (Mirlekar, 2021; Zundler, 2015). IL-15 can promote differentiation and expansion of T-cells, B-cells, and NK cells, which leads to enhanced tumor response. Moreover, IL-15 is important in the ontogeny of NK and CD8+ cells (Isvoranu, 2021). IL-21 is involved in co-stimulation of B-cell differentiation and immunoglobulin production, stimulation of NK and CD8+ cytotoxic function and co-mitogen of T-cells (Sondergaard, 2009).

Checkpoint inhibitors (CPIs), on the other hand, are important in enhancing T cell activation to combat tumors (Zheng, 2019). T cell exhaustion, characterized by loss of effector function and other properties, arises during chronic exposure to antigens, which limits tumor control (Wherry, 2011). Several inhibitory pathways including PD-1 and PD-L1 play important roles in this process. This led to the development of CPIs to recover dysfunctional T cells, including PD-L1 inhibitors, PD-1 inhibitors and CTLA-4 inhibitors (Vaddepally, 2020). Nevertheless, CPIs are ineffective for 'cold' tumors with low infiltration of T cells. OV's infecting and lysing the tumor cells could improve intra-tumoral infiltration and solve the limitations of CPI; thus, co-treatment of OVs and CPI is a natural trend.

1.3 The Non-Small Cell Lung Cancer

Non-small cell lung cancer (NSCLC) is a heterogeneous disease accounting for about 84% of all lung cancer diagnoses in the United States (Molina, 2008). Current treatment of NSCLC includes surgeries, chemotherapy, radiation therapy, and therapies targeting cell cycle control and apoptosis (Molina, 2008). The immune checkpoint inhibitors have been used recently to treat unresectable stage III NSCLC, using anti PD-1/PD-L1 antibodies (Onoi, 2020). Studies have also reported the improved treatment of NSCLC with several cytokines, such as IL-7 and IL-12. The cytokine induced killer cells and chemotherapy can effectively increase the overall survival of patients with advanced stages of NSCLC. IL-7 can aid in the sensitivity of NSCLC towards chemotherapy drug cisplatin. IL-12 is also shown to directly target human lung adenocarcinoma cells as well as adjacent normal bronchial epithelial cells (NBEC) (Airoldi, 2009). However, the primary and acquired resistance to PD-1/PDL1 blockade mechanisms in NSCLC have been reported, which might arise from components in the immunosuppressive tumor microenvironments that leads to inefficient activation and infiltration of T cells (Pathak, 2020).

2 NEW TREATMENT

A new therapy called JX-594alpha is designed, which consists of JX-594 (an oncolytic vaccinia virus) that expresses IL-15, IL-12, IL-7, IL-21 and PD-L1 inhibitor (iPDL1). Oncolytic vaccinia virus (VV), JX-594 is chosen as the delivery platform for several reasons. VV, compared to other types of oncolytic viruses, has a large genome size that allows it to accommodate multiple foreign genes (Breitbach, 2013). This makes it possible to carry a combination of the genes encoding IL-7, IL-12, IL-15, and IL-21. VV also exhibits features such as rapid replication, a wide tropism, and easy recombination for making viral mutants (Hawkins, 2002). Besides, JX-594 with granulocyte-macrophage colony-stimulating-factor (GM-CSF) gene and deletion in thymidine kinase (TK) gene could enhance immune responses and selectively replicate in cancer cells with mutated RAS or p53 genes (Merrick, 2009).

The cytokines (IL-7, IL-12, IL-15, IL-21) could boost T-, B-, and NK cell performance. while CPIs allow tumor recognition by T cells. Therefore, we believe that these two subjects working in tandem could substantially enhance antitumor effect. We hypothesize that the JX-594alpha is able to initiate antitumor immune responses that would eventually lead to tumor regression in NSCLC mouse model.

3 EXPERIMENT

3.1 Generation and Characterization

3.1.1 Generation of 4 Engineered OV

The generation of the JX-594alpha is achieved by inserting genes encoding different ILs（including IL-7, IL-12, IL-15, and IL-21) and iPDL1 into vaccinia virus. During the experiment, five groups will be generated, including VV-iPDL1/IL, VV-IL, VV-iPDL1, VV-empty, and the control group. The VV shuttle vector pSel-DsRed2N1 will be used to deliver iPDL1 into the VV (Wang, 2020). The VV shuttle vector pCMS1-IRES will be used to deliver the ILs into the VV (Ge, 2020).

3.1.2 iPDL1 Expression and Secretion in Infected H460 Cells

24 h after infection, supernatants of H460 cells are collected. iPDL1 concentration will be detected by mice iPDL1 ELISA kit in control, VV-iPDL1/IL, VV-IL, VV-iPDL1, and VV-empty groups.

3.1.3 Characterization of iPDL1

Supernatants of the tumor cells infected with VV-iPDL1/IL are harvested and then purify the supernatant to get iPDL1. Then the binding of purified iPDL1 to PD-L1+/+ or PD-L1 -/- cells is determined. The infected cells are incubated with purified iPDL1 or IgG before they are stained with antibodies against PD-L1 or IgG Fc.

3.1.4 Expression, Secretion and Characterization of IL

To test the expression and secretion of ILs in the H460 cells, harvesting the culture supernatants is planned to measure IL-7, IL-12, IL-15, IL-21 using ELISA. The presence of these ILs will be observed in the control, VV-iPDL1/IL, VV-IL, VV-iPDL1, and VV-empty groups, respectively.

3.1.5 Cytotoxicity

To test cytotoxicity of the immune checkpoint, the H460 cell line with KRAS mutation is used. Tumor cells will be plated in well-plates. Two groups are planned to be used to perform the experiment, with each group containing 5 subgroups with varying gene insertions. The PD-L1 +/+ tumor cells will contain control, VV-iPDL1/IL, VV-IL, VV-iPDL1, and VV-empty cells. The PD-L1 -/- tumor cells will be generated using the CRISP-Cas9 technique and will contain the same subgroups as that in PD-L1 +/+. The cell viability will be determined 48 hours after infection with cell-counting kit or nonradioactive cell proliferation assay (Ge, 2020).

3.2 Antitumor Activity in Mice NSCLC Model

3.2.1 Model Construction

The mouse mutant LSL-KrasG12D is proposed to be used as our mouse model. This mouse model was generated by Xu and others where they crossed mice carrying CC10-CreER allele to Lox-stop-Lox (LSL) K-RasG12D mice. They expressed oncogenic codon 12 mutant K-Ras in CC10- and Sftpc-expressing cells in the adult mice lung using knock-in CreER driver mouse lines. The researchers found that CC10+ type II cells are one of the origins of adenocarcinomas in response to K-Ras activation. The phenotype of the mice shows adenomas and adenocarcinomas with SPC-Cre, which works for our experiment to test the oncolytic viruses in NSCLC (Xu, 2012).

3.2.2 OV Injection

Continuing from the last step, the antitumor activity of the oncolytic virus is evaluated in the established model. The mice are divided into five groups randomly: control group, VV-iPDL1/IL, VV-IL, VV-iPDL1 and VV-empty group. When their tumors reach a volume of 100mm^3 (day 0), they receive intra-tumoral injections of 50μL indicated VV three times on 0,3, 7 days post-transplantation and the control group receive an equal amount of PBS at the same time and site.

3.2.3 Characterization of iPDL1 and IL

After the previous step, the expression of iPDL1 and IL levels in tumor-bearing mice are detected. Serum samples are collected from mice without treatment, injected VV-iPDL1/IL, VV-IL, VV-iPDL1 or VV-empty 3 days after the infusion of indicated VV. The serum levels are determined using mouse PD-1 ELISA kit and mouse IL-7, IL-12, IL-15 and IL-21 ELISA kit. To explore how long the iPDL1 could maintain in tumor-bearing mice, both the serum sample and tumor tissue are collected from the mice 1, 2, 4, 7, 10, 15, 20 …days after VV-iPDL1/IL injection. iPDL1 level is determined using mouse PD-L1 ELISA kit until iPDL1 level is too low to be detected. The kinetics curve of iPDL1 level is made

accordingly. There are three independent samples in a group and each experiment is repeated twice.

3.2.4 Tumor Growth and Survival

The tumor growth is monitored after the injection of VV to observe the progression of NSCLC. At 3, 7, 10, 13, 16, 20, 25, 30 days, each mouse in five groups receives microCT scan, which is a relatively accurate and easy method for tumor volume measurement (Jensen, 2008). MicroCT images are analyzed by certain softwares to get tumor volumes. Kaplan-Meier survival curve is made within 100 days. There are five independent individuals in a group.

3.2.5 Tumor Infiltration and Immune Cell Ativation

Five days after VV injection, tumor tissues are collected from mice without treatment, injected VV-iPDL1/IL, VV-IL, VV-iPDL1 or VV-empty and digested with collagenase type I and DNase. Then the tissues are filtered to prepare single-cell suspensions, which are then subjected to antibodies staining and analyzed by FACS. Antibodies against CD45, CD8, CD4, CD11c, CD11b and Gr-1 are used. Then the plots are drawn based on the percentage of infiltrating CD45+ immune cells, dendritic cell (DC; CD11c+), Myeloid-derived suppressor cells (MDSCs; CD11b+Gr-1+), CD4+ T cells, CD8+ T cells in tumor tissues. Besides, we assume that the virus could also activate the infiltrating effector T cells. The IFN-γ and TNF-α expression of CD8+ T cells can be measured by intracellular staining. There are five independent samples in a group and each experiment is repeated twice.

3.3 Anticipated Results

Below are the idealistic results of the experiments based on conjecture.

3.3.1 Characterization of VV in H460 Cells

In our assumptions, high levels of iPDL1 can be detected in VV-iPDL1/IL and VV-iPDL1 infected H460 cells, while IL-7, IL-12, IL-15 and IL-21 levels of VV-iPDL1/IL and VV-IL infected cells are significantly higher than other groups. The pictures are not shown here. Besides, it is hypothesized that VV-iPDL1/IL-infected cells release a higher level of iPDL1 than VV-iPDL1-infected ones and a higher level of the interleukins than VV-IL-infected ones. This could be explained by the synergy between the interleukins and immune suppressive pathways. Moreover, iPDL1 protein purified from the supernatant of VV-iPDL1/ IL infected H460 cells should bind to PD-L1+/+ tumor cells, but not to PD-L1 -/- tumor cells in vitro. Expected results are shown in figure 2. Flow cytometry is used to characterize PD-L1 expression on different H460 cells first (Figure 1). These could attest to the the successful expression of iPDL1 that can specifically bind to PD-L1+/+ tumor cells.

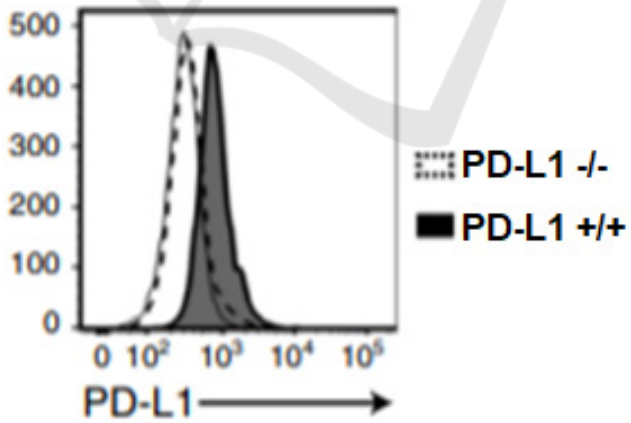

Figure 1: Expression of PD-L1 on PD-L1+/+ H460 cells and PD-L1 -/- H460 cells (Wang, 2020) Flow cytometry is used to show the PD-L1 expression on both wild type H460 cells and PD-L1-knocked out H460 cells.

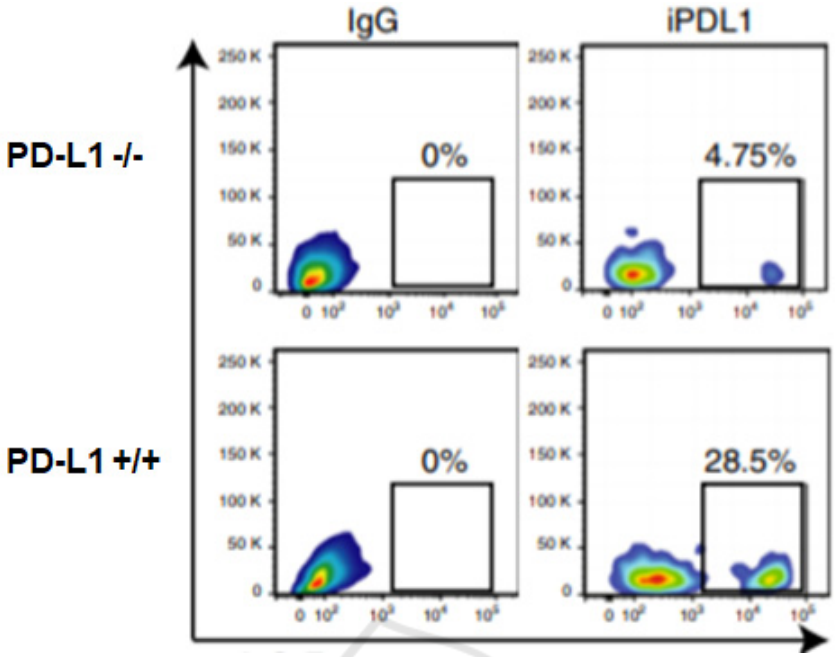

Figure 2: iPDL1 secreted by infected H460 cells could bind to PD-L1 +/+ cells (Wang, 2020) PD-L1 +/+ and PD-L1 -/- H460 cells are incubated with purified iPDL1 or IgG (used as a negative control) before being stained with anti-iPDL1 or anti-IgG antibodies. Flow cytometry shows the percentage of iPDL1 that binds to PD-L1 +/+ H460 cells.

3.3.2 Enhanced Cytotoxicity in H460 Cells

As is shown in figure 3, for the PD-L1 +/+ H460 cells, the viability of the cells infected with VV-iPDL1/IL should be lower than that of those infected with VV-iPDL1, VV-IL and VV-empty (the cell viability of control group is 1). As is shown in figure 4, for the PD-L1 -/- H460 cells, although we hypothesize that the overall cytotoxicity is higher due to the lack of immune checkpoint pathway, there should be so significant difference between VV-iPDL1/IL and VV-IL, VV-iPDL1 and VV-empty. In conclusion, the secretion of the iPDL1 from JX-594 alpha is assumed to lead to cell killing, in a PD-L1 dependent manner.

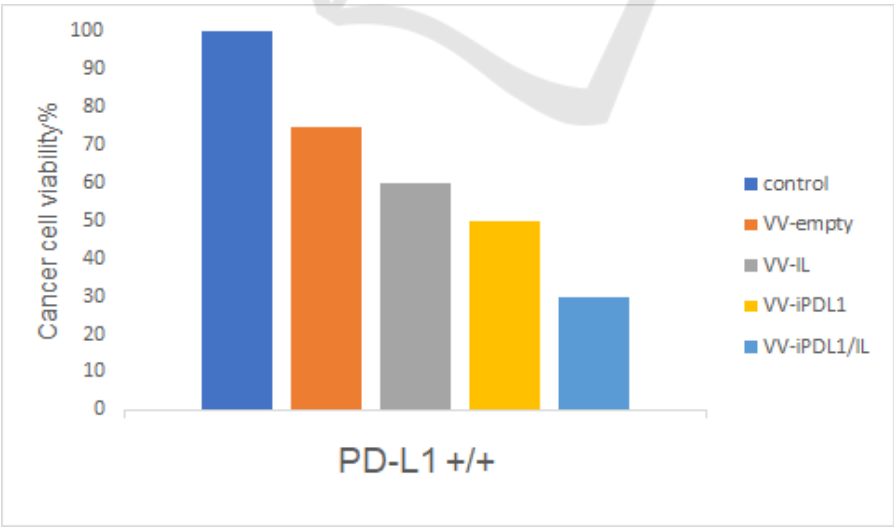

Figure 3: Cell viability of wild type H460 cells infected with indicated VVs H460 cells are infected with VV-iPDL1/IL, VV-IL, VV-iPDL1 and VV-empty respectively. Control group is incubated with the same amount of PBS. The cell viability is determined 48 hours after infection using nonradioactive cell proliferation assay.

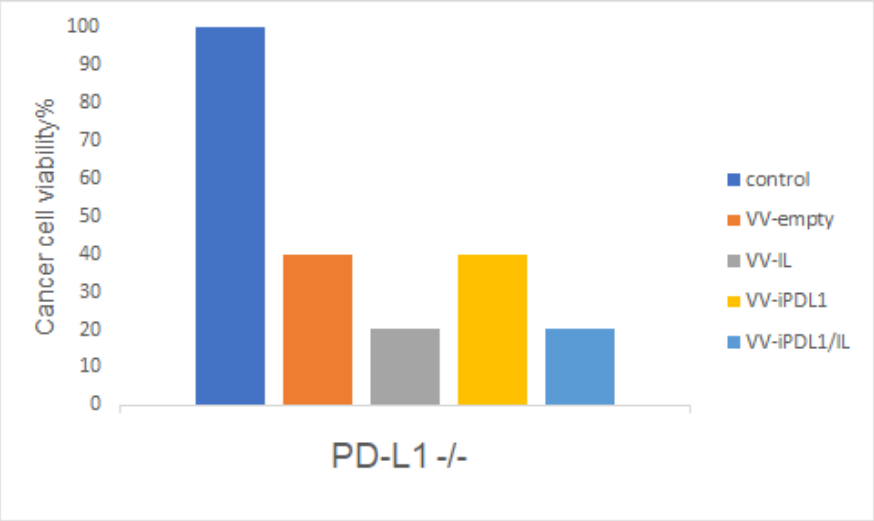

Figure 4: Cell viability of PD-L1-knocked out H460 cells infected with indicated VVs CRISPR/Cas9 is used to generate PD-L1 knocked out H460 cell lines. The following procedures are parallel to those in wild type H460 cells.

3.3.3 Characterization of VV in LSL-KrasG12D Mouse Model

High levels of iPDL1 should be detected in mice infected with VV-iPDL1/IL and VV-iPDL1, while IL-7, IL-12, IL-15 and IL-21 levels in mice infected with VV-iPDL1/IL and VV-IL group should be significantly higher than other groups. In a word, the anticipated results are similar to that of in vitro studies and the figures are omitted here. Additionally, iPDL1 levels in both the tumor and serum of mice injected with VV-iPDL1/IL are assumed to reach their peak several days after injection and could last for about 20 days (Figure 5), which is of great clinical importance.

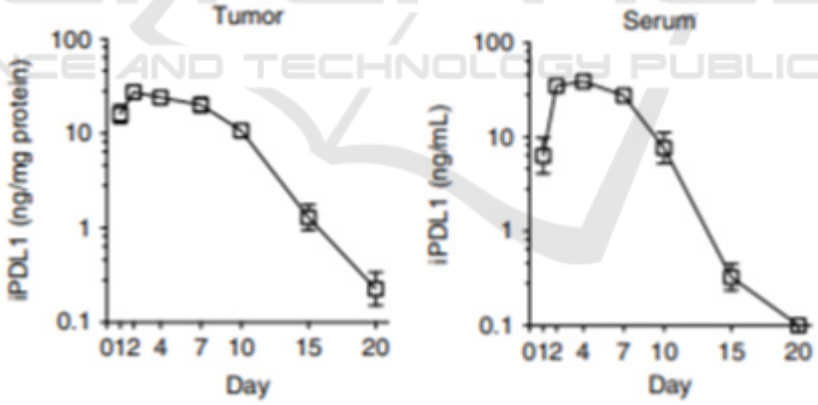

Figure 5: Kinetics of iPDL1 levels in both tumors and sera of the VV-iPDL1/IL-treated mice (Wang, 2020). iPDL1 concentrations are determined using PD-L1 ELISA kit 1, 2, 4, 7, 10, 15, 20 days after intra-tumoral injection.

3.3.4 Enhanced Antitumor Activities in LSL-KrasG12D Mouse Model

As is shown in figure 6, we assume that tumors in mice treated with VV grow slightly before shrinking and the tumors all disappear finally. But the tumor volume of mice treated with PBS should increase substantially. Among the mice treated with VV, the tumors disappear earliest in VV-iPDL1/IL group within 30 days, the second is VV-iPDL1 group and following are the VV-IL and VV-empty groups. As is the survival curve in figure 7, we hypothesize that mice in VV-iPDL1 group have the highest survival rate 100 days after injection. These all demonstrate that intra-tumoral injection of VV-iPDL1/IL could significantly inhibit tumor growth in KrasG12D mouse model although other VV are also potent tumor inhibitors.

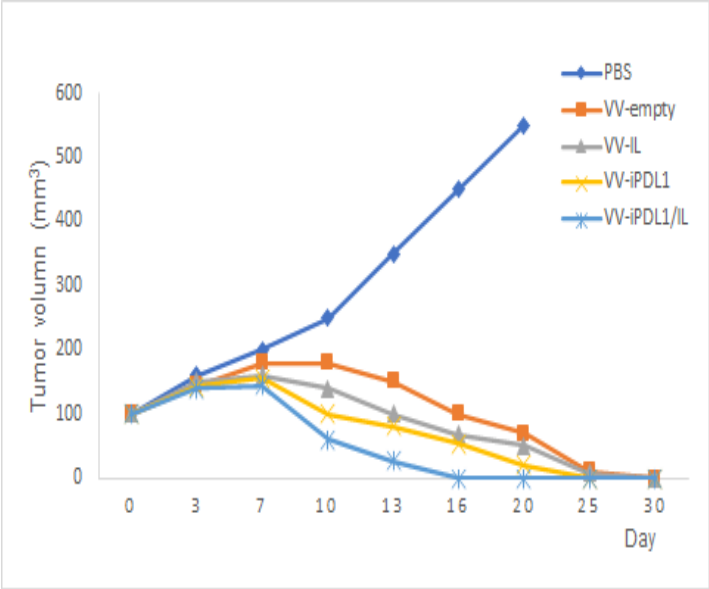

Figure 6: Tumor volume of mice injected with indicated VVs within 30 days LSL-KrasG12D mice are intratumorally injected with indicated VVs when their tumors reach a certain size. At 3, 7, 10, 13, 16, 20, 25, 30 days, each mouse receives microCT scan to determine tumor volume.

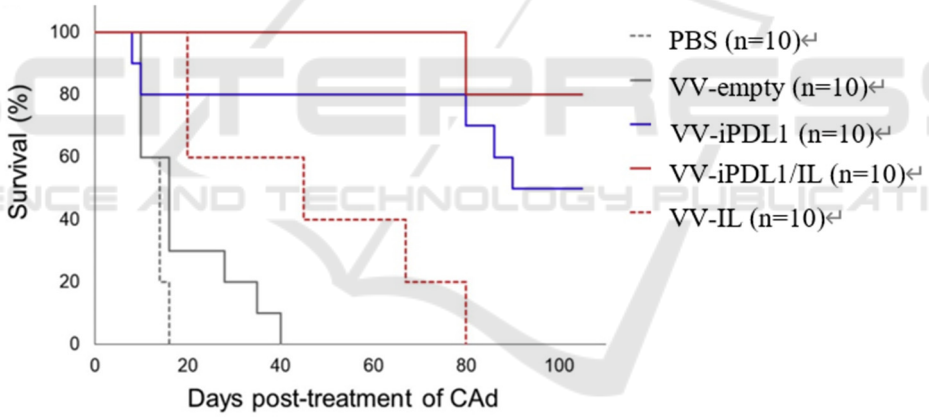

Figure 7: Kaplan-Meier survival curve of tumor-bearing mice injected with indicated VVs within 100 days (Caroline, 2020) LSL-KrasG12D mice are intratumorally injected with indicated VVs and monitored in 100 days. Each group has five independent samples.

3.3.5 Enhanced Tumor Infiltration and Immune Cell Activation

We hypothesize that FACS analysis (Figure 8) could indicate a higher level of overall lymphocytes marked by CD45+ and a higher percentage of CD4+, CD8+ T cells and dendritic cells in VV-iPDL1/IL group compared to VV-IL and VV-empty group. For MDSC cell, VV-IL and VV-iPDL1/IL should both enhance its composition compared to PBS group. However, we assume the MDSC cells in VV-iPDL1/IL group doesn't increase as much as the other two groups, indicating the ability of VV-iPDL1/IL to inhibit immune suppressive cells. Besides, VV-iPDL1/IL should activate effector T cells by enhancing the expression of IFN-γ, CD107a and TNF-α in CD8+ T cells (Figure 9). In a word, it's speculated that VV-iPDL1/IL could enhance the infiltration of lymphocytes, inhibit the suppressive cells and activate CD8+ effector T cells.

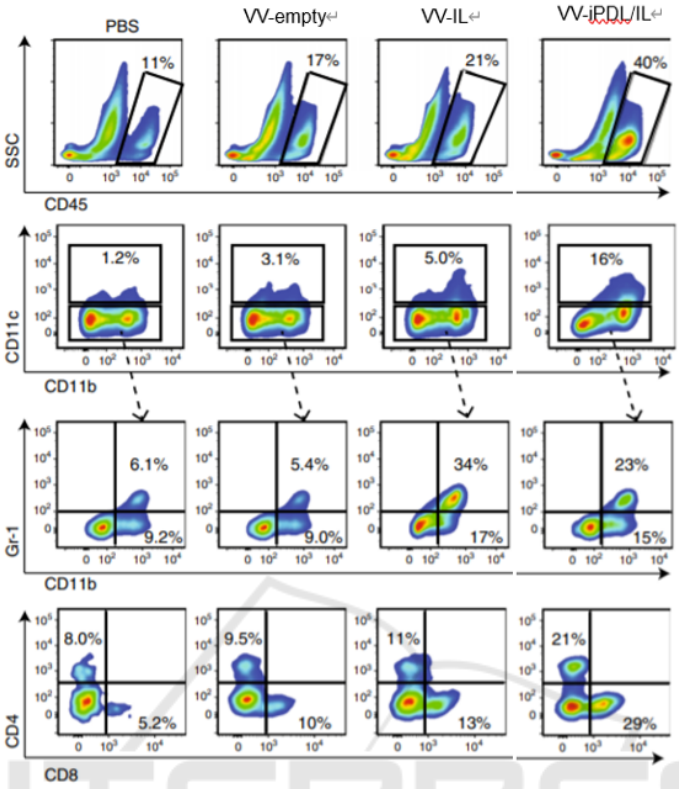

Figure 8: FACS analysis of infiltrating CD45+ immune cells, CD4+ T cells, CD8+ T cells, dendritic cells and MDSCs in tumors (Wang, 2020) 5 days after VV injection, tumors are harvested and digested to prepare single-cell suspensions, which are stained by antibodies against CD45, CD8, CD4, CD11c, CD11b and Gr-1.

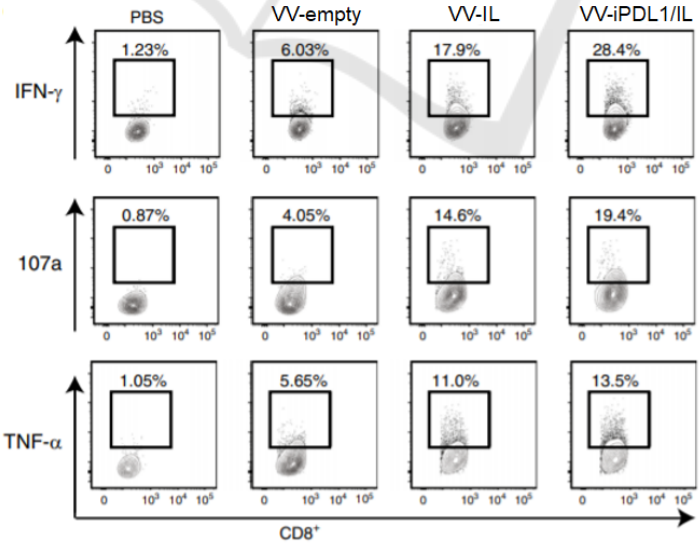

Figure 9: Expression of IFN-γ, TNF-α, and CD 107a in infiltrating CD8+ T cells (Wang, 2020) Intracellular staining is used to measure IFN-γ and TNF-α expression of CD8+ T cells.

4 DISCUSSION

The JX-594 alpha has large genome size which enables it to express multiple cytokines and checkpoint inhibitors (CPI) and thus induce T-cell proliferation and antibody production. With the help of several cytokines with different functions, our modified virus can increase immune response and target tumor cells more efficiently. In our expectations, the overall benefits of our virus are to induce a regression of tumor size and increase survival rates.

Despite the benefits of combination stated above, our experiment has some drawbacks. First, five subgroups are designed here: VV-iPDL1/IL, VV-IL, VV-iPDL1, VV-empty, and the control group. We would explore how the addition of iPDL1, cytokines and the combination of both iPDL1 and cytokines could affect the properties of VV. However, we regard the four cytokines as a whole rather than explore their functions separately. This might bring about a question of whether or not the final increase in immune responses arises from the synergy of cytokines. It's possible that one or two cytokines don't act as immune activating agent in this condition. Thus, more detailed studies on the function of each cytokine in VVs are needed. Besides, oncolytic virus therapy could be combined with other therapies in further studies. For instance, a study has investigated the combination effect of ReoT3D and some chemotherapeutic agents in NSCLC cells and demonstrated that ReoT3D and taxane could achieve synergy through apoptosis (Sei, 2009). To make the virus more aggressive to cancer cells, we may also start to look for other proteins besides cytokines and CPIs that also increase immune responses. Finally, genetic modifications of VVs could enhance their antitumor effect. However, some properties of viruses might be impaired at the same time like their stability and safety, which can cause some unexpected side effects.

5 CONCLUSION

Based on previous studies of OV therapy, a new remedy, JX-594 alpha is conceived and its effectiveness in NSCLC is tested in both H460 cell lines and KrasG12D mouse model. We hypothesize that both VV-iPDL1 /IL and VV-iPDL1 infected H460 cells could produce iPDL1 and the levels of IL-7, IL-12, IL-15 and IL-21 in VV-iPDL1 /IL infected cells were higher than those in other groups. Besides, iPDL1 produced in vitro should be able to bind PD-L1 +/+ tumor cells, which is tested by flow cytometry. VV-iPDL1 /IL is also assumed to cause cell killing in a PD-L1-dependent way. As to the in vivo experiment, it's also hypothesized that constructed VVs could produce iPDL1 or the cytokines successfully in mouse mode l. The long-term level of iPDL1 in both the serum and tumor is also measured. Tumor growth and overall survival of the mice are monitored within 100 days. It is expected that the tumor disappeared earliest in VV-iPDL1 /IL group, and the survival rate of VV-iPDL1 /IL group is also the highest. Other VVs should also be potent tumor suppressors, but weaker than VV-iPDL1 /IL. FACS should be used to characterize tumor infiltration and immune cell activation. We assume that the VV-iPDL1 /IL group has a higher overall CD45+ lymphocyte level and a higher percentage of DC, CD4+ and CD8+ T cells. In addition, VV-iPDL1 /IL should activate effector T cells by enhancing IFN-γ and TNF-α expression. In conclusion, JX-594 alpha is assumed to enhance lymphocyte infiltration, activate CD8+ effector T cells and finally achieve the goal of tumor elimination in NSCLC mouse model. Admittedly, the experiment design has some drawbacks discussed above. But we believe that emerging studies on OV therapy will definitely bring it to clinical application.

REFERENCES

Abd-Aziz N, Poh CL. (2021) "Development of oncolytic viruses for cancer therapy". Transl Res: 237:98-123.

Alderson, M. R., et al. (1991). "Interleukin-7 induces cytokine secretion and tumoricidal activity by human peripheral-blood monocytes." Journal of Experimental Medicine 173(4): 923-930.

Airoldi, I., et al. (2009). "IL-12 Can Target Human Lung Adenocarcinoma Cells and Normal Bronchial Epithelial Cells Surrounding Tumor Lesions." Plos One 4(7).

Berraondo, P., et al. (2019). "Cytokines in clinical cancer immunotherapy." British Journal of Cancer 120(1): 6-15.

Breitbach, C. J., et al. (2013). "Oncolytic Vaccinia Virus Disrupts Tumor-Associated Vasculature in Humans." Cancer Research 73(4): 1265-1275.

Caroline E, P. (2020). "Oncolytic Adenovirus Armed with BiTE, Cytokine, and Checkpoint Inhibitor Enables CAR T Cells to Control the Growth of Heterogeneous Tumors. " Molecular therapy: the journal of the American Society of Gene Therapy. 28(5): 1251-1262.

Cohen, M. C. and S. Cohen (1996). "Cytokine Function: A Study in Biologic Diversity." American Journal of Clinical Pathology 105(5): 589-598.

Colombetti, S., et al. (2009). "IL-7 adjuvant treatment enhances long-term tumor antigen-specific CD8(+) T-cell responses after immunization with recombinant lentivector." Blood 113(26): 6629-6637.

Ferguson, M. S., et al. (2012). "Systemic delivery of oncolytic viruses hopes and hurdles." Advances in virology 2012: 805629-805629.

Fukuhara, H., et al. (2016). "Oncolytic virus therapy: A new era of cancer treatment at dawn." Cancer Science 107(10): 1373-1379.

Ge, Y., et al. (2020). "Oncolytic vaccinia virus delivering tethered IL-12 enhances antitumor effects with improved safety." Journal for Immunotherapy of Cancer 8(1).

Hawkins, L. K., et al. (2002). "Oncolytic biotherapy: a novel therapeutic platform." Lancet Oncology 3(1): 17-26.

Isvoranu, G., et al. (2021). "Therapeutic potential of interleukin-15 in cancer (Review)." Experimental and Therapeutic Medicine 22(1).

Jensen, M. M., et al. (2008). "Tumor volume in subcutaneous mouse xenografts measured by microCT is more accurate and reproducible than determined by 18F-FDG-microPET or external caliper." BMC medical imaging 8: 16-16.

Martuza, R. L., et al. (1991) "Experimental therapy of human glioma by means of a genetically engineered virus mutant." Science 252.5007:854-856.

Marintcheva, B. (2018). Chapter 9 - Virus-Based Therapeutic Approaches. Harnessing the Power of Viruses. B. Marintcheva, Academic Press: 243-276

Merrick AE, Ilett EJ, Melcher AA (2009). "JX-594, a targeted oncolytic poxvirus for the treatment of cancer. Curr Opin Investig Drugs."10(12):1372-82.

Mirlekar, B. and Y. Pylayeva-Gupta (2021). "IL-12 Family Cytokines in Cancer and Immunotherapy." Cancers 13(2).

Molina, J. R., et al. (2008). "Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship." Mayo Clin Proc 83(5): 584-594.

Onoi, K., et al. (2020). "Immune Checkpoint Inhibitors for Lung Cancer Treatment: A Review." Journal of Clinical Medicine 9(5).

Pathak, R., et al. (2020). "Acquired Resistance to PD-1/PD-L1 Blockade in Lung Cancer: Mechanisms and Patterns of Failure." Cancers 12(12).

Sei, S., et al. (2009). "Synergistic antitumor activity of oncolytic reovirus and chemotherapeutic agents in non-small cell lung cancer cells." Molecular Cancer 8.

Sondergaard, H. and K. Skak (2009). "IL-21: roles in immunopathology and cancer therapy." Tissue Antigens 74(6): 467-479.

Vaddepally, R. K., et al. (2020). "Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence." Cancers 12(3).

Wang, G., et al. (2020). "An engineered oncolytic virus expressing PD-L1 inhibitors activates tumor neoantigen-specific T cell responses." Nature Communications 11(1).

Wherry, E. J. (2011). T cell exhaustion. Nature Immunology, 12(6), 492.

Xu, X., et al. (2012). "Evidence for type II cells as cells of origin of K-Ras-induced distal lung adenocarcinoma." Proceedings of the National Academy of Sciences of the United States of America 109(13): 4910-4915.

Zheng, M., et al. (2019). "Oncolytic Viruses for Cancer Therapy: Barriers and Recent Advances." Molecular Therapy-Oncolytics 15: 234-247.

Zundler, S. and M. F. Neurath (2015). "Interleukin-12: Functional activities and implications for disease." Cytokine & Growth Factor Reviews 26(5): 559-568.