Combination Therapies Increase the Efficacy of Melanoma Treatment with Reduced Side Effects

Haoming Zhang[1], Jiayi Zong[2], Jingyi Guo[3], Lawrence Ma[4], Yijia Chen[5] and Zhaojun Qiu[6,*]
[1]*College of Animal Sciences and Technology, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong 510025, China*
[2]*Jinling High School, Nanjing, Jiangsu 210029, China*
[3]*Beijing National Day School, Beijing 100039, China*
[4]*Ridge High School, Basking Ridge, New Jersey 07920, U.S.A.*
[5]*School of Life Science, Fudan University, Shanghai 200433, China*
[6]*Shanghai Pinghe School, Shanghai 201206, China*

Keywords: Immune Checkpoint Inhibitor, Kinase Inhibitor, Oncolytic Virus, Combinational Therapy.

Abstract: T Immunotherapy is found to have a promising effect on cancer treatment. It initiates activation of the immune response to fight against cancer. Previous studies have demonstrated that immune checkpoint inhibitors (ICIs), kinase inhibitors, and oncolytic viruses (OV) are possible cancer immunotherapies. Immune checkpoint inhibitors targeting programmed cell death-1 (PD-1) and programmed death-ligand 1 (PD-L1) have achieved great success in cancer immunotherapy. We hypothesized that utilizing anti-PD-1 antibodies, mitogen-activated protein kinase (MAPK) inhibitors, and oncolytic virus (T-VEC) could boost the efficacy of traditional PD-1 therapy towards melanoma, while the side effects can be alleviated with the supplement of anti-cytokine antibodies and anti-inflammatory drugs. A series of experiments were designed to be conducted in melanoma murine models. Expected outcomes of this combination therapy include enhanced tumor regression, extended survival, and mitigated side effects. The success of this study could bring up a new strategy for melanoma therapies.

1 INTRODUCTION

1.1 Melanoma

Melanoma is a major type of skin cancer that arises from genetic mutations in melanocytes, the pigment-producing cell, which can be found throughout the skin, eye, inner ear, and leptomeninges. Although it only takes up a small percentage of all malignant skin cancer, it is the most aggressive and deadliest type. As shown in Figure 1, Once it becomes metastatic (move to other organs from where it originated), the prognosis is very poor (Domingues, 2018), needing better treatments to be studied and applied to.

1.2 Theraputical Mechanism in the Proposal

As shown in Figure 2, T cells, recognizing peptide antigen with the aid of cell surface major histocompatibility complex (MHC) molecules, have two broad classes with very different functions, named by their expression of CD4 or CD8 co-receptor: CD4+ T cells detect the antigen in MHC class II molecules and act as the headmaster in the adaptive immune system by producing cytokine, chemokine, and pro-inflammatory responses. While CD8+ T cells detect antigen with MHC class I molecules and carry out direct toxicity to kill infected or cancerous cells (Darvin, 2018).

Zhang, H., Zong, J., Guo, J., Ma, L., Chen, Y. and Qiu, Z.
Combination Therapies Increase the Efficacy of Melanoma Treatment with Reduced Side Effects.
DOI: 10.5220/0012014600003633
In *Proceedings of the 4th International Conference on Biotechnology and Biomedicine (ICBB 2022)*, pages 104-115
ISBN: 978-989-758-637-8

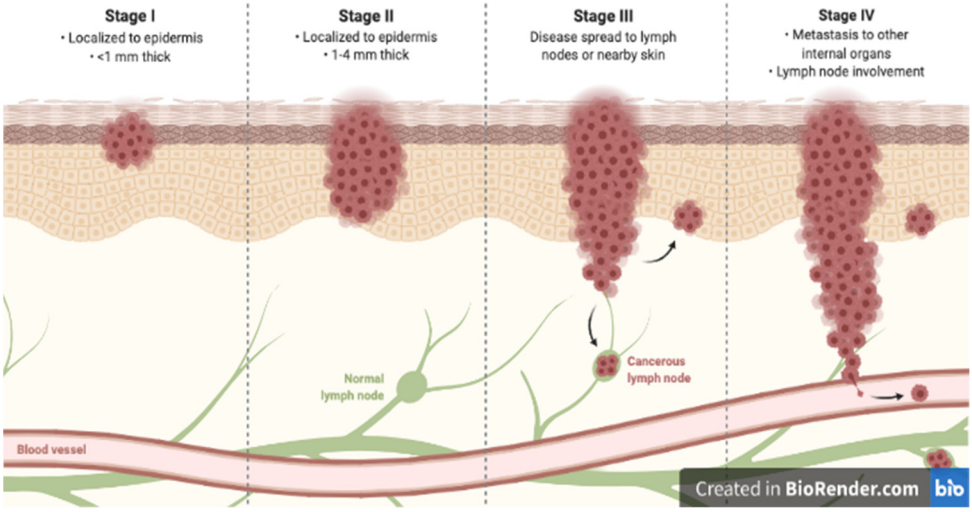

Figure 1: The four stages of melanoma (Biorender. 2021).

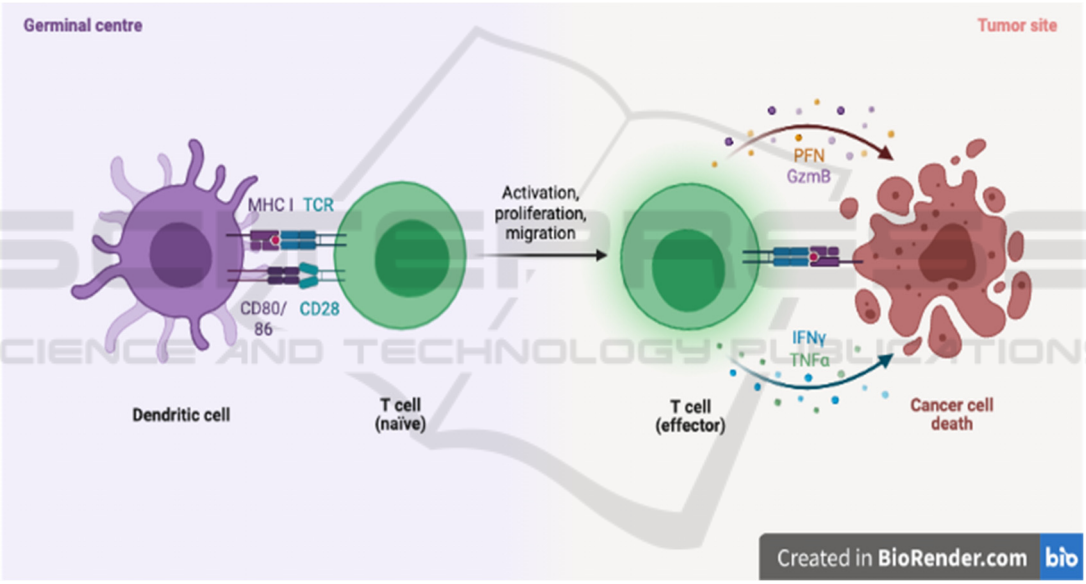

Figure 2: CD8+ T cell activation by Dendritic cell and effects on cancer cell (Biorender. 2021).

Generally, the interaction between multiple checkpoints and regular stimulatory signals regulates T cells in an effective but not autoreactive fashion (Sharpe, 2018). But tumor cells can abnormally utilize this signal system in two ways – reduced stimulatory signals or overexpressed checkpoint signals. Immune checkpoint inhibitors (ICIs) can target the overexpressed checkpoint signals that allow tumor cells to evade immunosurveillance, and function by releasing the natural breaks on immune activation and enhancing the T-cell immune ability to eliminate tumor cells (Darvin, 2018).

The proposal mainly focuses on the druggable inhibitors of the T cell PD-1 (Programmed cell death-1) pathway and its complementary ligand (PD-L1). As shown in Figure 3, PD-1 is a transmembrane inhibitory receptor, and signals through its pathway are mainly responsible for controlling initial T cell activation as well as the effecting function of the cell. On the other hand, PD-L1 is located on cancer cells binds to the PD-1 inhibitor, and it tricks the T cell from functioning (Sharpe, 2018). Antibodies have been engineered to specifically target either PD-1 or PD-L1, the former takes off the brakes from T cells, and the latter prevents cancer cells from "hiding". It is also noteworthy that their feasibility has been further proved by structural analysis (Lee, 2016).

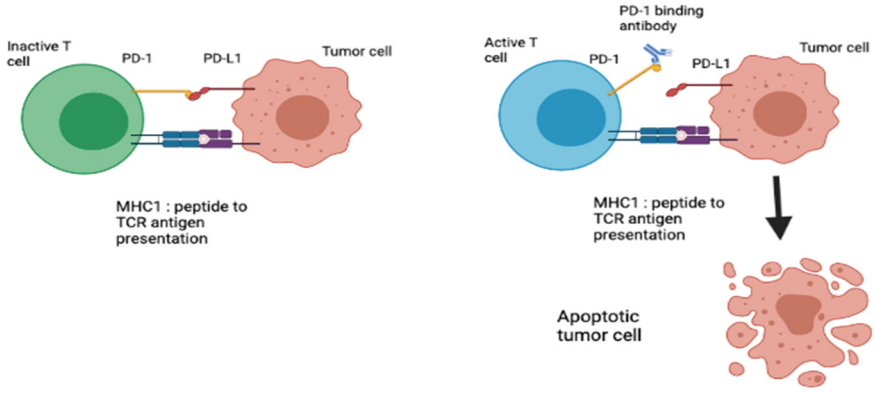

Figure 3: PD-1-PD-L1 interaction and its inhibition strategy.

Left: The schematic model of PD-1-PD-L1 interaction in a tumor cell. The binding suppresses the activation of T cell.Right: The antibody (ICI) binds to PD-1, therefore, inhibits the interaction which leads to T cell activation and induces apoptotic death of tumor cell.

Tumors can evolve to evade both innate and adaptive arms of the immune system, thereby rendering ICI therapy ineffective (Pfirschke, 2016; Mueller, 2015). A subset of patients receiving immune-checkpoint inhibitor therapy develop unconventional response patterns (termed 'pseudo-progression') that can be misinterpreted as disease progression (Nishino, 2017). The MAPK pathway provides significant therapeutic targets due to its aberrant activation in cancer and strong interference with complex molecular pathways, leading to wider use of MAPK inhibitors in melanoma, lung cancer, colorectal cancer, and other types (Smith, 2014; Germann, (2017). Permanent activation of RAS protein caused by mutations accounts for a very high proportion of all human cancers through activating downstream signaling pathways, including the MAPK family (the downstream of RAS GTPase, represented by RAF and its variant), then the MEK family (MAP kinase-ERK kinase), and ERK1/2(Extracellular signal-regulated kinases), specifically shown in Figure 4. Activated Therapies that target RAS-activating pathways or RAS effector pathways could be combined with these direct RAS inhibitors, immune checkpoint inhibitors, or T cell-targeting approaches to treat RAS-mutant tumors (Moore, 2020; Braicu, 2019).

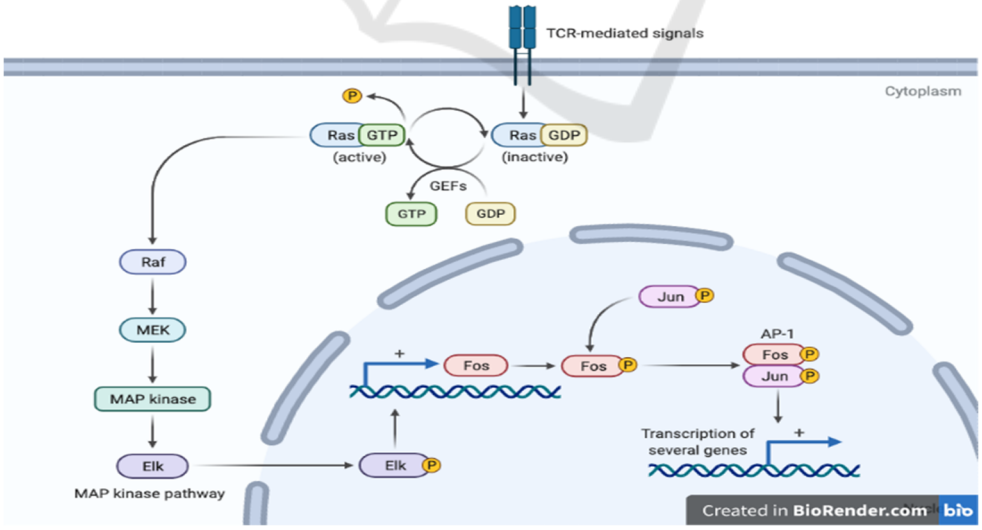

Figure 4: The more detailed and specific schematic demonstration of the RAS/MAPK pathway (Biorender 2021).

Yet some MAPK pathway effectors (e.g., p38α) play a dual role, with suppression in one cancer but inflammation in another cancer, making preclinical studies more considerable (Grossi, 2014). In

melanoma, MAPK inhibitors are mainly resisted by macrophage-derived TNF-α (Smith, 2014), lowering down the efficacy and implying the possibility of adverse effects. To further strengthen the development of MAPK inhibition, complementary combination therapies can be added to raise the efficacy.

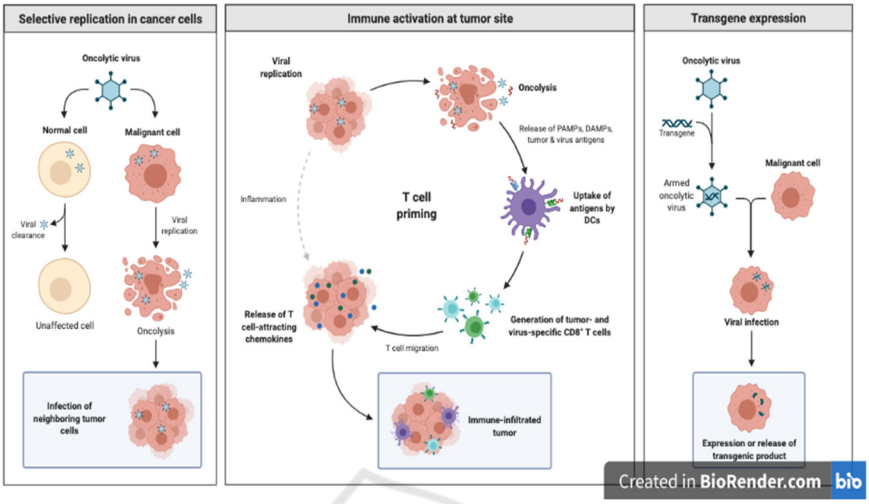

Figure 5: The mechanism of oncolytic virus against tumors (Biorender 2021).

Viruses can be used in attacking tumor cells (Bai, 2019). The left panel illustrates the oncolytic viral response to normal cell vs malignant cell. The middle panel illustrates a general mechanism of the oncolytic virus with T cell activation at the tumor site. The right panel illustrates transgene expression in oncolytic virus.

Oncolytic viruses (OVs) are treated as natural or engineered viruses that replicate specifically in cancer cells and kill them. The mechanism of their kill process is shown in Figure 5. They are harmless to normal organisms (Fukuhara, 2016) and able to be delivered both systemically and locoregionally. Thus, they can act at the primary or metastatic tumor sites (Twumasi-Boateng, 2018).

According to Figure 6, T-VEC is a type of OV for melanoma that consists of a double-mutated oncolytic herpes simplex virus type 1 (HSV-1) armed with granulocyte-macrophage colony-stimulating factor (GM-CSF) (Biorender 2021). It was approved officially as the first oncolytic virus drug by the US Food and Drug Administration (FDA) on 2015, October 27th (Pol, 2016).

Though T-VEC is designed to be injected locally into the tumor, systemic effects are often induced, leaving a body free of cancer, but with flu-like symptoms. Besides, due to the potential for viruses to attack healthy cells, the risk of infection still exists (Marelli, 2018).

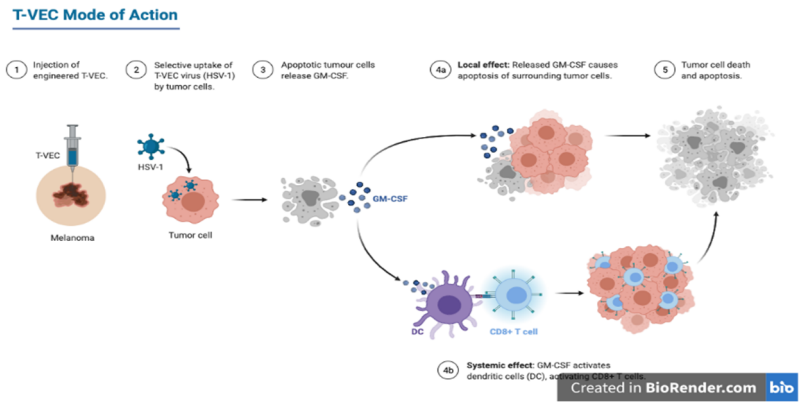

Figure 6: The mechanism of T-VEC oncolytic virus to aid T cell against tumor cell (Biorender 2021).

2 FORMULATION OF HYPOTHESIS

2.1 Combination of Different Orthogonal Therapeutic Approaches

For an optimistic therapeutic effect to be reached, a combinational therapy consisting of checkpoint inhibitors (PD-1, PD-L1), as well as oncolytic viruses (T-VEC) may be adopted. Studies have previously demonstrated the improved efficacy combined therapies are likely to yield, details of which will be introduced later as this article progresses. Recently, therapies of OV combined with ICI have been uncovered. The oncolytic virus can secrete PD-L1-secreting-OVs. It systemically cleaves and inhibits PD-L1 on tumor cells and immune cells, initiating tumor neoantigen-specific T cell responses. Tumor-specific oncolytic immunotherapies for cancer treatment have been made available to patients (Wang, 2020).

Furthermore, a triple combination of OV, ICI, and kinase inhibitors is proposed and compared with a dual combination of OV and ICI or kinase inhibitors and ICI in mice models. The triple combination is consisting of a phosphatidylinositol 3-kinase (PI3K) inhibitor, a PD-L1 inhibitor, and VSVΔ51, which is an engineered OV strain from vesicular stomatitis virus (VSV), commonly is used as a therapy for PTEN-deficient glioblastoma. The result shows that dual combination enhances immune response slightly, while triple combination therapy increases the treatment efficacy significantly. Tumor regression is induced accompanied by complete tumor eradication in most mice that are treated with triple combination therapy. Long-term antitumor immune memory is established in these mice (Xing, 2021).

2.2 Management of Side Effects

ICIs' ability to stimulate the immune system has further contributed to them being appreciated as a desirable treatment for cancer (Simonaggio, 2019). However, they also lead to toxicities and several kinds of inflammation in different organs. Frequently affected organs include the skin, and organs in the digestive, and endocrine systems (Durrechou, 2020). New research has found that the mechanism of action of ICIs reveals a new toxicity profile called immune-related adverse events (irAEs) (Richmond, 2008).

Recurrences (both same types and different types) of irAE after recovery is another difficult problem (Martins, 2019). Currently, the commonly used PD-1/PD-L1 monoclonal antibody has the mechanism of removing immunosuppression and activating T cell function. T cells are also found in normal tissue. While it is killing tumor tissue the side effects may occur at the same time.

The co-stimulation of T-lymphocyte leads to inflammatory cytokines. When the body meets inflammation, the immune system will secrete many inflammatory cytokines, which regulate lots of aspects of cell growth and differentiation and play a key role in the coordination of immune defenses against invading. They are potentially immunogenic which could generate the anti-cytokine autoantibodies (aCA) (Meager, 2014). A significant portion of the anti-inflammatory cells express PD-L1 by inducing target cells, and then T lymphocytes kill the target cells.

With low irAE grade, ICI was discontinued follow-up, and steroids were used for grade 2 or higher adverse events. The core principles of management included continuing treatment with ICI, early detection and adequate control of irAEs may contribute to improved patient prognosis (Matsuoka, 2020). High-risk patients receiving ICIs should be regularly monitored for associated complications by a professional multidisciplinary team, preferably using a personalized monitoring strategy (Martins, 2019).

2.3 Mini Review

The purpose of the proposal is to solve the problem that how to increase the therapeutic effect and range of ICI-associated therapy while decreasing its side effects. Based on previous findings among three kinds of therapies and other related research, we have eventually chosen to focus on melanoma and formed a hypothesis: utilizing the combination between ICI (PD-1 and PD-L1 antibodies), MAPK inhibitor, and oncolytic virus (Talimogene laherparepvec) can effectively increase the therapeutic effect of melanoma treatment, while the side effects can be reduced by anti-cytokine antibodies and anti-inflammatory drugs.

Through the design of experiments in mice models and the estimated results, we expect to shed light on the triple combination immunotherapy in melanoma treatment and provide our views on ideal therapies with high efficacy and low adverse effects.

3 EXPERIMENTAL APPROACHES

In order to test the hypothesis, there will be two major experiments in this study – one is to test the effect of combinational therapy, one is to test the reduction in side effects with anti-cytokine antibodies and anti-inflammatory drugs.

3.1 Experimental Object

3.1.1 A Brief Introduction to Xenograft Tumor on Humanized Mice

In order to examine a patient's tumor's response to a certain therapy, research must examine with human tumor instead of mouse generated tumor (Richmond, 2008). To do so human tumor xenografts can be implanted subcutaneously into immunosuppressed mice, so the mice acquire human tumor cells to establish tumor microenvironment and propagation inside the mice. This is a critical model for various studies on cancer interaction and behavior with the cardiovascular and immune systems and response to the various drugs (Martins, 2019).

3.1.2 Processes of Mouse Model Generation

Hetero transplantation of human cancer cells or tumor biopsies into immunodeficient rodents as patient xenograft models) (PDX)constituted the major preclinical screen for the development of novel cancer. the models have identified clinically efficacious agents and make effort in pharmaceutical industry therapeutics (Morton, 2016). In order to make mouse models, several processes are needed.

SCID is mice with defective combinations of T, B, and NK cells. First, the il2R γ KO mice were backcrossed after the deletion of the IL-2 receptor γ chain gene, which is a common domain of cytokine receptors. Then, NOD/SCID−γ−/−c mice are irradiated (sublethal, 1 Gy, whole-body irradiation) (Gonzalez, 2013), followed by CD34+ stem cells from the same cord blood donor each with a unique UCB donor was performed. In all cases, recipient mice were evaluated for human hematolymphoid engraftment at 12 to 16 weeks post-injection. For new-boring mice, human hematolymphoid cells (HSC) engraftment of newborn mice by intracardiac (IC) injection can be better (Brehm, 2010). Meanwhile, subcutaneous implantation of autologous human thymic tissue (1–2 mm3 fragments) could also work (Shankar, 2020). Followed by the establishment of in vitro human cell lines to be propagated subcutaneously, reconstituting solid tumors (Russell, 2018).

3.1.3 Advantages and Disadvantages of the Processed Mouse Models

As shown in Figure 7, the feature complexity of genetic and epigenetic abnormalities that only present inhuman compared to mice. The results can be obtained relatively rapidly (a few weeks), and multiple therapies can be tested from a single tumor biopsy. Although the humanized mice can partially reconstruct the human immune system, the process to generate humanized mice is tedious and costly (Gong, 2018).

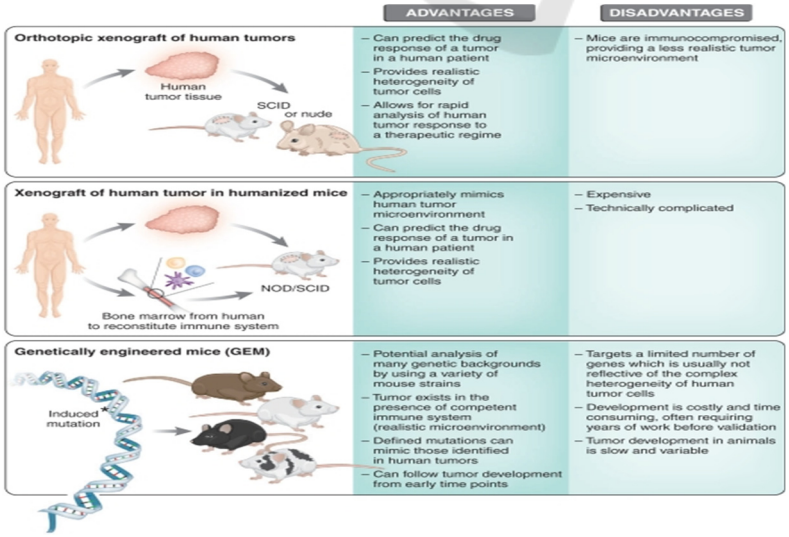

Figure 7: The advantages and disadvantages between three common types of mouse models in cancer research (Russell, 2018).

3.1.4 Control Factors

All the humanized mice are expected to have same age and gender. They are treated with same environmental condition (25 Celsius, same water and food). All the Xenograft tumor is taking from the same human patient. The xenograft human bone marrow is derived from HLA compatible healthy participant. Every kind of drug are produced in same company in similar date.

3.2 Experiment for Combinational Therapy

In this experiment, we will test out the efficacy of ICI and ICI combination therapy by utilizing different combinations of treatment on controlled mice models.

3.2.1 Positive and Negative Control

The positive control in our experiment is humanized mice without tumor xenograft to test out the natural rate of death and normal symptoms as a comparison to the experimental groups. The negative control is tumor xenograft humanized mice without any treatment in order to test out the effect of advanced melanoma on the mice without any outer interference.

3.2.2 General Protocol

Step 1 – humanized mice cultivation (see Figure 8)

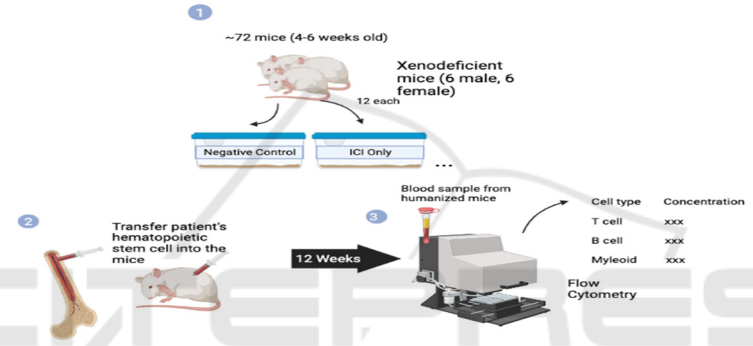

Figure 8: The schematic diagram about the first step in the experiment protocol.

Prepare 72 humanized mice (4-6 all male) and stratified into six sub-groups evenly (n=12) by randomization. Inject healthy individual's (HLA matched with tumor xenografted patient) hematopoietic stem cell into the mice and wait at least 12 weeks to let the immune system become mature. After 12 weeks, examine blood sample from 2-3 mice in each group by flow cytometry to estimate amount of immunological cell in the mice. If it reaches the expected standard, then start Step 2.

Step 2 - Prepare and culture xenograft advanced melanoma tumor from the human patient (see Figure 9).

First, extract tumor tissue sample from an advanced melanoma patient, who is HLA-matched with the hematopoietic stem cell donor. Then cultivate it under ideal environment until reach enough amount for transplantation. Mince the proliferated tumor tissue into equal pieces that are sufficient to cause melanoma in the mice in a short amount of time while not lethal immediately. Last, Inject the equivalent minced tissue into the humanized mice by subcutaneous injection.

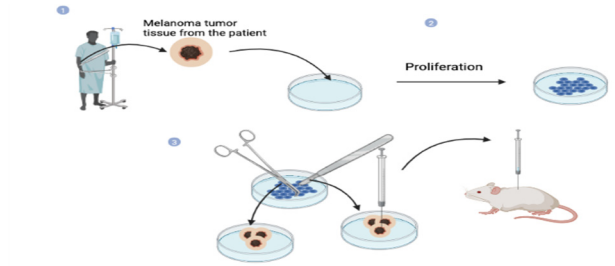

Figure 9: The second step of the experiment is where cultures and transfer of human melanoma tumors are performed.

Step 3 - Inject the corresponding treatment to different humanized Mice groups (see Figure 10)

Wait 4 weeks after tumor injection in order to let the tumor spread in the mice. Then, inject corresponding treatment intravenously to each group of mice.

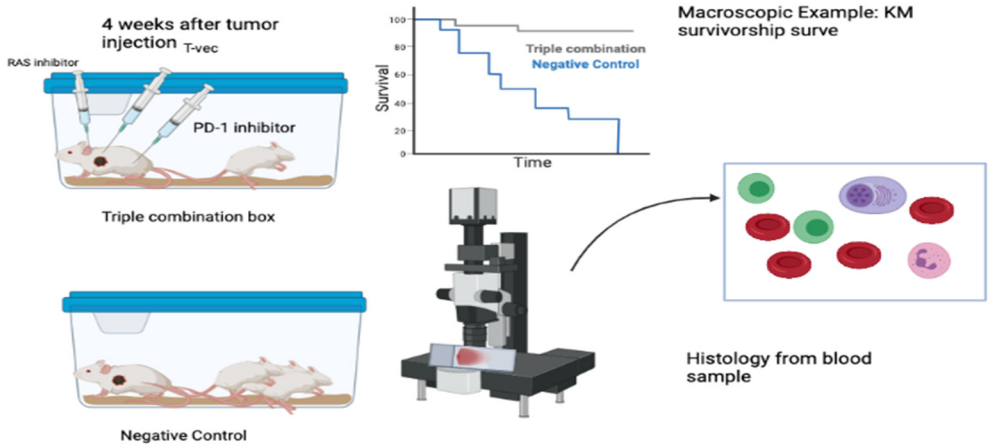

Figure 10: The lab protocol demonstrates the experiment phase where injection of treatments and observations are taking place.

Step 4 - Observation and record

Observe the mice for at least 2 months. The purpose and method to acquire data will be presented in the following section.

3.2.3 Technology and Index for Result Measurement

Both macroscopic observation and data (number of mice dead and clinical symptoms) and microscopic and data (T cell density and activation status) are necessary to support studies about combination therapy. In macroscopic observation, the survival curve and clinical statistics can be obtained (see Figure 11). Kaplan Meier (KM-Curve) is a great estimation of how the model survives during long-time observation.

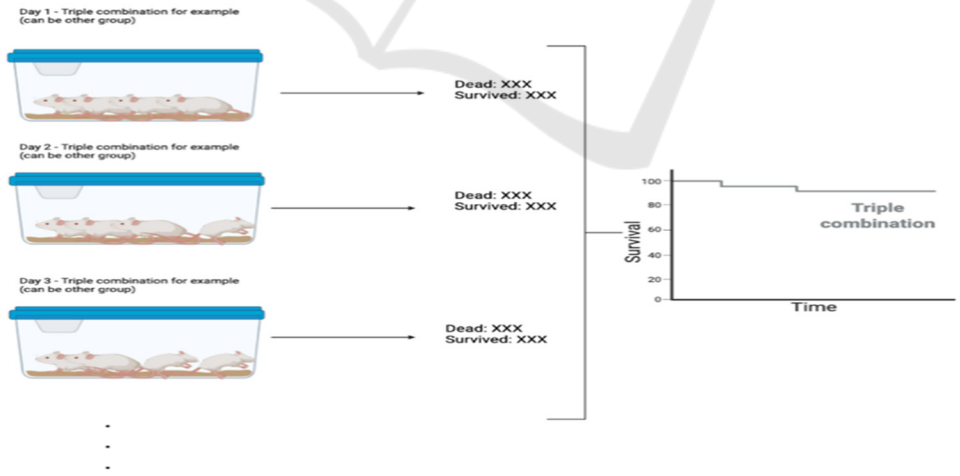

Figure 11: The figure shows how the Kaplan Meier graph is obtained by counting the number of survived and dead mice models in each group every day.

3.2.4 Anticipated Results

Survival rates of mice, average tumor size and T cell density are main index being expected to be measured, to evaluate the efficacy of each treatment through macro and micro approaches.

Negative control (no treatment):

Negative control group is expected to have Poorest Kaplan-Meier (K-M) curve that has the steepest slope (which means many mice dead in a short amount of time). It also expected to have largest average tumor size and most tumors with least number of lymphocytes and myeloid cells. Also, it is expected to have least amount of fluorescence (least amount of activated T cell against tumor cell)

ICI (PD-1) only:

With only ICI treatment only, the mice expected to have a better and flatter K-M curve compared to negative control. The tumor size and density should have smaller average tumor size and reduced number of tumor (despite pseudo-progression). It should have more active (higher fluorescence density) lymphocytes compared to negative control.

ICI with MAPK (RAS inhibitor):

As expected, double treatment should result in better K-M curve, smaller average tumor size and density, and more and active lymphocytes compare to ICI treatment only and negative control.

ICI with the oncolytic virus (T-VEC):

The result obtained with ICI and T-VEC double treatment expected to similar with ICI and MAPK double treatment without significance differences ($p>0.05$).

Triple combination (ICI + T-VEC + kinase inhibitors):

The triple combination will be expected to have best K-M curve, least tumor size and density, and most numerous and active lymphocytes (with tumor).

Positive control (no tumor): normal lifespan without injection

The K-M curve should follow natural rate of death, and with no tumor observed. Also, there should be normal number of lymphocytes and normal activation status since no infection or cancer takes place.

ICI treatment can initially increase tumor size, then suppress the tumor size at later date, as expected with pseudo-progression (see Figure 12). The negative control group is a mice model that grafted with a tumor but does not receive any kind of treatment. The positive control group is a mice model that is normal (no tumor graft).

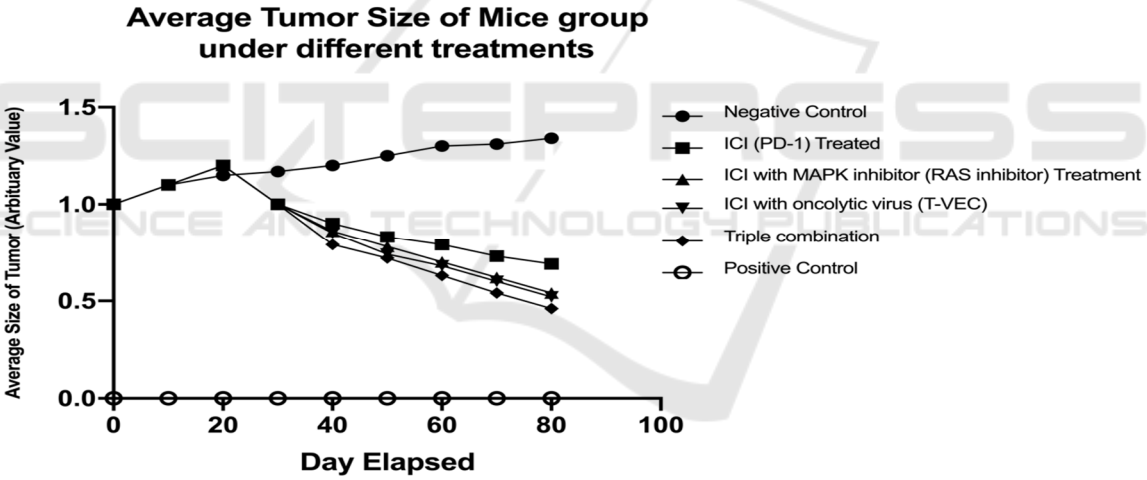

Figure 12: The average tumor size of each experimental group.

3.3 The Experiment of Management of Side Effects

3.3.1 Positive and Negative Control

The positive control in this experiment is healthy mice without xenograft tumors. The negative control is tumor xenograft humanized mice with the triple combination but does not have any medicine to manage side effects.

3.3.2 Anticipated Results

In the ideal state, the same number of the humanized mice in the four groups have both symptoms cause by ICIs. Here we use DCR (disease control rate) to describe the anti-irAE efficacy through each treatment. Overall survival rate is expected to be measured to characterize disease control and is demonstrated in Figure 13.

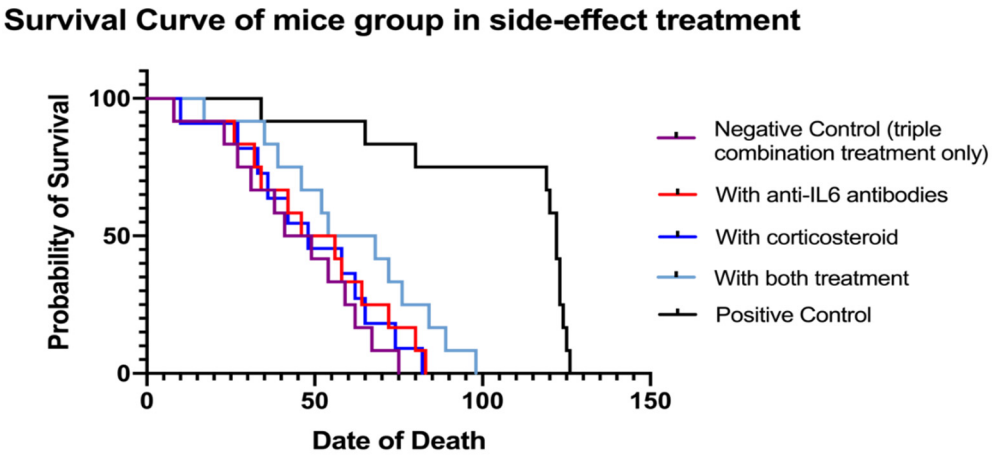

Figure 13: The overall survival (OS) curve of experimental groups. OSR stands for Overall Survival Rate.

The negative control is a mice model with melanoma xenograft tumor that only treated with combinational therapy (without any anti-side effect treatment). The positive control is normal mice with a natural rate of death. The triple combination with anti-IL6 antibodies performs worse than corticosteroids because anti-IL6 antibodies reduce the therapeutic effect of the triple combination.

4 DISCUSSION

4.1 Advantages and Limitations for Melanoma

Combination with a variety of drugs can be induced by a different mechanism and thus increase antitumor immune response, leading to improvements in the overall curative effect. Despite the humanized mice more closely mimicking diverse populations reconstruct the human immune system, discrepancies still exist. Triple therapy might be incompatible with anti-cytokine inhibitors such as by reducing or even abolishing the effects.

4.2 Possibilities of Triple Combination Therapy in Other Cancers

As hundreds of clinical trials have been conducted on malignancies that have a relatively dramatic response to PD-1 and PD-L1 blockade, nine cancer types have been approved for the PD-1/PD-L1 treatment (Gong, 2018). Among them, MAPK inhibitors and oncolytic viruses were seen significant activity in non-small cell lung cancer (NSCLC), Renal cell carcinoma (RCC), and Hepatocellular carcinoma (HCC) in previous studies (Baines, 2011) Therefore, we can presume the possibilities of such a triple therapeutic modality applying for these kinds of cancers. Xing F et al have made efforts on the combination of anti-PD-1 treatment, kinase inhibitor (PI3K), and OV and testified that its efficacy was synergistically and safely restored in PTEN-deficient GBM models (Matsuoka, 2020). Under the support that ICIs, kinase inhibitors (MAPK), and OV each have positive responses in these cancer types, meanwhile, dual combinations among these therapies have enhanced the effects, we can similarly expect higher effectiveness of therapeutic responses by utilizing our triple combination therapy. Facing the challenge of irAEs caused by ICI therapies, we launched our solutions that using inflammatory cytokines inhibitors or corticosteroids collaboratively to reduce the irAE grade. For cancers of NSCLC, RCC, and HCC, the same levels of irAEs compared with melanoma were detected (Shankar, 2020); Baines, 2011; Ornstein, 2017). After triple combination therapy is used to raise efficacy, anti-irAE strategies (anti-cytokine and corticosteroids) may also be able to be applied.

ACKNOWLEDGMENT

All authors contributed equally to this work and should be considered co-first authors.

REFERENCES

Bai, Y., et al. (2019). "Updates to the antitumor mechanism of oncolytic virus." Thoracic cancer 10(5): 1031-1035.

Baines, A. T., et al. (2011). "Inhibition of Ras for cancer treatment: the search continues." Future medicinal chemistry 3(14): 1787-1808.

Braicu, C., et al. (2019). "A comprehensive review on MAPK: a promising therapeutic target in cancer." Cancers 11(10): 1618.

Brehm, M. A., et al. (2010). "Parameters for establishing humanized mouse models to study human immunity: analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rgamma(null) mutation." Clin Immunol 135(1): 84-98.

Cui, T.-m., et al. (2020). "Adverse Effects of Immune-Checkpoint Inhibitors in Hepatocellular Carcinoma." OncoTargets & Therapy 13.

Darvin, P., et al. (2018). "Immune checkpoint inhibitors: recent progress and potential biomarkers." Experimental & molecular medicine 50(12): 1-11.

Domingues, B., et al. (2018). "Melanoma treatment in review." ImmunoTargets and therapy 7: 35.

Durrechou, Q., et al. (2020). "Management of Immune Checkpoint Inhibitor Toxicities." Cancer Manag Res 12: 9139-9158.

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

Germann, U. A., et al. (2017). "Targeting the MAPK signaling pathway in cancer: promising preclinical activity with the novel selective ERK1/2 inhibitor BVD-523 (ulixertinib)." Molecular cancer therapeutics 16(11): 2351-2363.

Gonzalez, L., et al. (2013). "Humanized mice: novel model for studying mechanisms of human immune-based therapies." Immunol Res 57(1-3): 326-334.

Gong, J., et al. (2018). "Development of PD-1 and PD-L1 inhibitors as a form of cancer immunotherapy: a comprehensive review of registration trials and future considerations." Journal for immunotherapy of cancer 6(1): 1-18.

Grossi, V., et al. (2014). "p38Œ± MAPK pathway: A key factor in colorectal cancer therapy and chemoresistance." World journal of gastroenterology: WJG 20(29): 9744.

Kuzu, O. F., et al. (2015). "Current state of animal (mouse) modeling in melanoma research." Cancer growth and metastasis 8: CGM. S21214.

Lee, J. Y., et al. (2016). "Structural basis of checkpoint blockade by monoclonal antibodies in cancer immunotherapy." Nature communications 7(1): 1-10.

“Melanoma staging”, by Biorender.com (2021). Retrieved from https://app.biorender.com/biorender-templates

Mueller, K. L. (2015). "Cancer immunology and immunotherapy. Realizing the promise. Introduction." Science 348(6230): 54-55.

Moore, A. R., et al. (2020). "RAS-targeted therapies: is the undruggable drugged?" Nature Reviews Drug Discovery 19(8): 533-552.

Marelli, G., et al. (2018). "Oncolytic viral therapy and the immune system: a double-edged sword against cancer." Frontiers in immunology 9: 866.

Martins, F., et al. (2019). "Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance." Nature Reviews Clinical Oncology 16(9): 563-580.

Meager, A. and M. Wadhwa (2014). "Detection of anti-cytokine antibodies and their clinical relevance." Expert Rev Clin Immunol 10(8): 1029-1047.

Matsuoka, H., et al. (2020). "Correlation between immune-related adverse events and prognosis in patients with various cancers treated with anti PD-1 antibody." BMC Cancer 20(1): 656.

Morton, J. J., et al. (2016). "Humanized mouse xenograft models: narrowing the tumor, Äìmicroenvironment gap." Cancer research 76(21): 6153-6158.

Nishino, M., et al. (2017). "Monitoring immune-checkpoint blockade: response evaluation and biomarker development." Nat Rev Clin Oncol 14(11): 655-668.

Ornstein, M. C. and J. A. Garcia (2017). "Toxicity of checkpoint inhibition in advanced RCC: A systematic review." Kidney Cancer 1(2): 133-141.

Pfirschke, C., et al. (2016). "Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy." Immunity 44(2): 343-354.

Pol, J., et al. (2016). "First oncolytic virus approved for melanoma immunotherapy." Oncoimmunology 5(1): e1115641.

Reprinted from “T cell Activation in Cancer”, by Biorender.com (2021). Retrieved from https://app.biorender.com/biorender-templates

Reprinted from “PI3K/Akt, RAS/MAPK, JAK/STAT Signaling”, by Biorender.com (2021). Retrieved from https://app.biorender.com/biorender-templates

Reprinted from “Properties of Oncolytic Virus”, by Biorender.com (2021). Retrieved from https://app.biorender.com/biorender-templates.

Reprinted from “T-VEC mode of action”, by Biorender.com (2021). Retrieved from https://app.biorender.com/biorender-templates

Richmond, A. and Y. Su (2008). Mouse xenograft models vs GEM models for human cancer therapeutics, The Company of Biologists Limited.

Russell, L. and K.-W. Peng (2018). "The emerging role of oncolytic virus therapy against cancer." Chinese clinical oncology 7(2): 16.

Sharpe, A. H. and K. E. Pauken (2018). "The diverse functions of the PD1 inhibitory pathway." Nature Reviews Immunology 18(3): 153-167.

Smith, M. P., et al. (2014). "The immune microenvironment confers resistance to MAPK pathway inhibitors through macrophage-derived TNFŒ±." Cancer discovery 4(10): 1214-1229.

Simonaggio, A., et al. (2019). "Evaluation of Readministration of Immune Checkpoint Inhibitors After Immune-Related Adverse Events in Patients with Cancer." JAMA Oncol 5(9): 1310-1317.

Shankar, B., et al. (2020). "Multisystem Immune-Related Adverse Events Associated with Immune Checkpoint Inhibitors for Treatment of Non,ÄìSmall Cell Lung Cancer." JAMA Oncology 6(12): 1952-1956.

Twumasi-Boateng, K., et al. (2018). "Oncolytic viruses as engineering platforms for combination immunotherapy." Nature Reviews Cancer 18(7): 419-432.

Wang, G., et al. (2020). "An engineered oncolytic virus expressing PD-L1 inhibitors activates tumor neoantigen-specific T cell responses." Nature communications 11(1): 1-14.

Xing, F., et al. (2021). "Modulating the tumor microenvironment via oncolytic virus and PI3K inhibition synergistically restores immune checkpoint therapy response in PTEN-deficient glioblastoma." Signal Transduction and Targeted Therapy 6(1): 275.