# Effect of PD-1 Inhibitor on PI3K/AKT Pathway in Tumor Cell and T Cell Activity in Non-small Cell lung Cancer (NSCLC)

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## Abstract

Lung cancer is one of the most lethal cancers around the world and non-small cell lung cancer takes up about 85% of the cases. PD-1 inhibitor is an antibody used in immunotherapy to treat cancer. Previous studies have reported that the PD-1 inhibitor can block the PD-1/PD-L1 binding and help the immune system recognize and attack tumors. The PI3K/AKT signaling pathway is an important target of the PD-1 downstream pathway. This paper studies the effects of PD-1 inhibitor Pembrolizumab on the activation of the PI3K/AKT pathway in vivo. In this study, A549 cell line will be used as the non-small cell lung cancer model. The immune-deficient mice will be injected with Keytruda, saline (negative control) or Taxol (positive control). Flow cytometry and Western Blot will be used to measure the activation of tumor cells and CD4 T cells. This paper investigates whether or not PD-1 inhibitor would effectively block the PI3K/AKT pathway and increase T cell proliferation. The result of this study will explore the blocking mechanism of Pembrolizumab on PI3K/AKT pathway. Future studies should focus on the acquired resistance caused by mutations and its effects on PD-1 inhibitor treatment with PI3K/AKT pathway activation as the biomarker.

**Keywords:** Non-small cell lung cancer, PI3K/AKT pathway, PD-1 inhibitor, CD4 T cell, Pembrolizumab

## I. Introduction

Lung cancer is the main reason for cancer-related deaths not just in the United States and globally. Its fatality rate is higher than the sum of death rates of the three most common cancers (colon, breast, and pancreatic). Lung cancer has a one-year death rate of over 50% and a five-year survival rate of around 17.8% [1,2]. Non-small cell lung cancer (NSCLC) makes up 85% of all lung cancer cases [1].

When feasible, the most reliable and effective curative method is surgical excision. However, about 70% of lung cancer patients had locally progressed or metastatic disease at the time of diagnosis [1]. Immunotherapy is a ground-breaking cancer treatment that makes use of the body's immune system to fight against cancer. PD-1 inhibitors will block the PD-1/PD-L1 pathway and activate the immune system to attack tumors.

By directing a coordinated immune response, the T cells in the immune system can specifically identify and eliminate infections or unhealthy cells, including cancer cells [3]. There are many checkpoints in order to prevent immune system cells from mistakenly destroying healthy cells during an immune response (known as autoimmune reaction). Immune checkpoint proteins, including PD-1 and PD-L1, can be used by cancer cells to avoid immune identification and destruction.

The PI3K/AKT signaling pathway is an important target of the PD-1 downstream pathway. As PI3K/ AKT signaling pathway regulates the stimulation of cell growth and proliferation, the abnormalities in this pathway are common in cancer [4]. The proliferation, invasion, metastasis, and treatment resistance of tumor cells have all been related to the overactivation of the PI3K/AKT pathway in these cells. Additionally, the tumor angiogenesis process involves the PI3K/AKT signaling pathway [5]. T-cell exhaustion is induced by PD-1 ligation that is persistent [6]. The activation of the PI3K/AKT signaling pathway is tightly controlled via a multistep process. AKT signaling cascade is activated by stimuli and induces phosphoinositide 3-kinase (PI3K) to produce phosphatidylinositol (3,4,5) trisphosphates (PIP3). Then AKT is phosphorylated by its upstream activator PDK1 at T308 and by mTOR at S473 for fully activated [7].

It can be inferred that by the blockade of the PD-1/PD-L1 pathway, PD-1 inhibitor Pembrolizumab can decrease the activation of the PI3K/AKT/mTOR signaling pathway by the dephosphorylation in tumor cells, thus reducing the proliferation of tumor cells. Moreover, as the blocking of PD-1/PD-L1 binding, the activation of T cells may be increased as well.

To test the effect of PD-1 inhibitors on the immune system, an experiment should be carried out. This paper explored the effects of PD-1 inhibitors on the activation of the PI3K/AKT/mTOR signaling pathway and the recovery of T cell activation in NSCLC treatment.

Hypothesis: By the blockade of the PD-1 pathway, PD-1 inhibitor Pembrolizumab will decrease the activation of

cancer cells through dephosphorylation of the PI3K/AKT/mTOR signaling pathway to block the proliferation of cancer cells and increase the activity of T-cells.

## II. Materials and methods

### 1.1. Cell Lines

This experiment will use A549 as a model of non-small cell lung cancer.

### 1.2. PD-1 Inhibitor

This experiment will use the PD-1 inhibitor Pembrolizumab (Keytruda).

### 1.3. Animal experiment

The immune-deficient mice are used in this study. Each mouse will be injected with $10^6$ A549 cell line cells into the lung and housed under specific pathogen-free conditions. After 7 days, the mice will be randomly assigned into 9 groups and 7 mice in each group for experiment:

1. Negative control:
(1) receive saline injection 0.05 mL on day 1, 4, 7, 10, 13
(2) receive saline injection 0.1 mL on day 1, 4, 7, 10, 13
(3) receive saline injection 0.2 mL on day 1, 4, 7, 10, 13
2. Positive control:
(1) receive Taxol injection 50 μg on day 1, 4, 7, 10, 13
(2) receive Taxol injection 100 μg on day 1, 4, 7, 10, 13
(3) receive Taxol injection 200 μg on day 1, 4, 7, 10, 13
3. Inhibitor group:
(1) receive Pembrolizumab injection 50 μg on day 1, 4, 7,

10, 13
(2) receive Pembrolizumab injection 100 μg on day 1, 4, 7, 10, 13
(3) receive Pembrolizumab injection 200 μg on day 1, 4, 7, 10, 13

On day 15, mice will be sacrificed, and the tumor tissues will be removed rapidly and measured the size. Tumor cell samples will be used in the following Western Blotting test.

### 1.4. Flow Cytometry

CD4 T cells in the thymus will be extracted and isolated by flow cytometry for each group 24 hours after the Pembrolizumab, Taxol, or saline injection.

### 1.5. Western Blotting

The activation of the PI3K/AKT pathway in tumor cells will be measured by Western Blotting. Protein was extracted, resolved by Western Blotting, and probed for expression of pAKT (S473 and T308) [8].

Western Blotting will also be used to measure the activation of CD4 T cells isolated and probed for expression of pSrc (Y416) and pErk (T202 and Y204) [9].

### 1.6. Statistical Analysis

The statistical significance of all numerical data gathered in Flow Cytometry and Western Blotting will be analyzed by the student's T-Test at $p < 0.05$.

## III. Possible results

Possible results overview is shown in Table 1.

### Table 1. Possible Results on activation and tumor size

| Activation/ tumor size | Result 1 | Result 2 | Result 3 | Result 4 | Result 5 | Result 6 | Result 7 | Result 8 |
|---|---|---|---|---|---|---|---|---|
| Does PI3K/AKT pathway activation decrease in cancer cell? | + | + | + | - | + | - | - | - |
| Does CD4 T cell activity increase? | + | + | - | + | - | + | - | - |
| Tumor size decreases? | + | - | + | + | - | - | + | - |
| Supports hypothesis? | Fully | Partially | Partially | Partially | Partially | Partially | Partially | Contradict |

*Note.* "+" represents an obvious change compared to control groups, and "-" represents no obvious change in these variables compared to control groups.

**Possible result 1: PD-1 inhibitor Pembrolizumab can decrease the activation of the PI3K/AKT pathway in tumor cells and promote the CD4 T cell activation, and also reduce tumor size.**

PI3K/AKT pathway activity is decreased as the pAKT/AKT expression in the experimental group is lower than control groups. Tumor size is effectively controlled compared to control groups. The CD 4 T cell activity

is also increased by the blockade of the PD-1/PD-L1 pathway.

**Possible result 2: PD-1 inhibitor Pembrolizumab can decrease the activation of the PI3K/AKT pathway in tumor cells and promote the CD4 T cell activation but cannot reduce tumor size.**

PI3K/AKT pathway activity is decreased as the pAKT/AKT expression in the experimental group is lower than

control groups. The CD 4 T cell activity is also increased by the blockade of the PD-1/PD-L1 pathway compared to control groups. However, the inhibitor cannot control and reduce the tumor size.

**Possible result 3: PD-1 inhibitor Pembrolizumab can decrease the activation of the PI3K/AKT pathway by inhibiting phosphorylation of AKT and reducing tumor size, but it cannot promote the CD4 T cell activation.**

PI3K/AKT pathway activity decreases as the pAKT/AKT expression in the experimental group is lower than in the control groups. PD-1 inhibitor can help with control and reduce tumor size as the tumor size is also smaller compared to control groups. However, the activity of CD4 T cells in the experimental group is not increased and Pembrolizumab cannot promote the CD4 T cell activation by inhibiting the tumor cell proliferation.

**Possible result 4: PD-1 inhibitor Pembrolizumab can reduce tumor size and promote the CD4 T cell activation but cannot decrease the activation of the PI3K/AKT pathway by inhibiting the phosphorylation of AKT.**

Pembrolizumab is effective in reducing tumor size compared to the control groups, which receive saline or Taxol injections. The inhibitor can also increase CD4 T cell activation. However, Pembrolizumab cannot inhibit the PI3K/AKT signaling pathway as the pAKT/AKT expression has no obvious decrease compared to control groups.

**Possible result 5: PD-1 inhibitor Pembrolizumab can only decrease the activation of the PI3K/AKT pathway by inhibiting phosphorylation of AKT but cannot promote the CD4 T cell activation or reduce the tumor size.**

The PI3K/AKT pathway activity decreases as the pAKT/AKT expression in the experimental group is lower than in the control groups. However, tumor size has no obvious reduction after the inhibitor treatment compared to control groups. The inhibitor cannot promote the CD4 T cell activation by inhibiting the tumor cell proliferation.

**Possible result 6: PD-1 inhibitor Pembrolizumab can only promote the CD 4 T cell but cannot decrease the activation of the PI3K/AKT pathway by inhibiting phosphorylation of AKT or reducing the tumor size.**

The experimental group has a higher level of CD4 T cell activation than the control groups, so Pembrolizumab can promote T cell activation. However, the inhibitor cannot control the cancer development as the PI3K/AKT pathway is not blocked and the tumor size is not reduced compared to control groups.

**Possible result 7: PD-1 inhibitor Pembrolizumab can only reduce tumor size but cannot decrease the activation of the PI3K/AKT pathway or promote the CD 4 T cell.**

Pembrolizumab is effective in inhibiting tumor size as the size of the tumor decreases compared to control groups. However, the inhibitor cannot block the PI3K/AKT pathway activation and does not affect promoting T cell activity.

**Possible result 8: PD-1 inhibitor Pembrolizumab does not affect the activation of the PI3K/AKT pathway, CD4 T cell activation, or tumor size.**

The expression of pAKT/AKT is still high, so Pembrolizumab cannot block the PI3K/AKT pathway. The inhibitor does not affect CD4 T cell activation or tumor size as well.

## IV. Discussion

The experiment uses 3 different drug amounts (50μg, 100μg, and 200μg) to test and ensure sufficient dosage. If there is no difference between experimental results of different drug amounts, the drug amount does not affect the effect of inhibitor treatment. The other possible result is that the experimental results become more obvious with the increase in drug amount.

Possible result 1 fully supports the hypothesis. By blocking the PD-1 pathway, PD-1 inhibitor Pembrolizumab can decrease the activation of the downstream signaling pathway PI3K/AKT in NSCLC. The decrease of phosphorylation on the active site of AKT indicates the decrease of PI3K/AKT pathway activation. As Pembrolizumab inhibits the proliferation of tumor cells, tumor size can be reduced due to the blockade of tumor cell proliferation, invasion, metastasis, and treatment resistance stimulated by the PI3K/AKT pathway. For future experiments, different PD-1 inhibitors can be used to explore the universality of the hypothesis and whether it applies to all types of PD-1 inhibitors.

Possible result 2 partially supports the hypothesis as the tumor size is not well reduced compared to control groups. The acquired resistance may act as an escape mechanism. The evolution of neoantigen loss will increase the medication resistance of tumors after PD-1/PD-L1 pathway blockade therapy, according to several recent studies [10]. Future experiments can be focused on the drug resistance mechanism to inhibitors. The reaction of other tumor signaling pathways to Pembrolizumab can also be investigated.

Possible result 3 partially supports the hypothesis as the inhibitor cannot promote CD4 T cell proliferation. Even if the tumor size is controlled and reduced after the blockade of the PI3K/AKT signaling pathway in tumor cells, the exhausted CD4 T cells due to PD-1/PD-L1 binding still

cannot recover the activity and the activation of CD4 T cells is not significantly increased. Future experiments can be focused on exploring the effect of PD-1 inhibitors on the regulation of CD4 T cell activity.

Possible result 4 partially supports the hypothesis. It indicates that Pembrolizumab can control tumor size and activate CD4 T cells, but it is not related to PI3K/AKT pathway. This may be due to the other signal pathways in cancer cells. Multiple signals, such as those from the PI3K/AKT pathway, MAPK pathway, JAK/STAT pathway, WNT pathway, and others, can influence the PD-1/PD-L1 axis in cancer cells [11]. Pembrolizumab may have blocked the other signals, thereby inhibiting tumor growth. Future experiments can be designed to explore the effects of Pembrolizumab treatment on other signaling pathways of NSCLC.

Possible result 5 partially supports the hypothesis as the inhibitor can only decrease the PI3K/AKT pathway activity in tumor cells but cannot promote the CD4 T cell activation or reduce the tumor size. This may also be due to the acquired resistance increased by the evolution of neoantigen loss after PD-1/PD-L1 pathway blockade treatment [11]. Future experiments can be focused on the drug resistance mechanism to inhibitors. Experiments can also be focused on how PD-1 inhibitor regulates the activity of CD4 T cells.

Possible result 6 partially supports the hypothesis as Pembrolizumab can only promote the CD4 T cell activation, but it is not able to block PI3K/AKT pathway in cancer cells or control the tumor size. The failure of inhibiting the PI3K/AKT pathway may be due to the complex regulation pathway of PI3K/AKT [7]. Future experiments can be focused on the acquired drug resistance to Pembrolizumab and other signaling pathways that promotes tumor proliferation.

Possible result 7 partially supports the hypothesis as Pembrolizumab can only reduce tumor size but cannot decrease the PI3K/AKT pathway activity in tumor cells or promote the CD 4 T cell. Future experiments can be focused on the effect of PD-1 inhibitor blockade on other signaling pathways to prevent tumor growth.

Possible result 8 fully contradicts the hypothesis as Pembrolizumab PD-1 inhibitor Pembrolizumab does not affect the PI3K/AKT pathway activity in tumor cells, CD4 T cell activation, or tumor size. Under the condition of ensuring that all experimental operations are correct and standardized, this may be due to the improper design of the experiment. For future animal experiments, change PD-1 inhibitors, experimental groups, drug injection methods, or mouse samples and repeat the experiment. Future experiments can also be focused on exploring the effect of PD-1 inhibitors on other signaling pathways in tumor cells. This result may also be likely to happen in future clinical treatment, as genetic mutations can be very different for every patient. In future clinical experiments, the treatment should be customized for each specific patient.

# V. Conclusion

Generally, this study explores the treatment effect of PD-1 inhibitor Pembrolizumab on non-small cell lung cancer in mouse models. The results of this study will indicate whether Pembrolizumab can promote the CD4 T cells and reduce the tumor size by decreasing the activation of the PI3K/AKT signaling pathway in tumor cells. The result of this study will explore the blocking mechanism of Pembrolizumab on PI3K/AKT pathway. The possible controversial results will also indicate the potential causes and relationship between PD-1 blockade treatment and NSCLC tumors. The results are also helpful to study the drug resistance of PD-1 inhibitors.

Future studies should be focused on the acquired resistance caused by mutations and its effects on PD-1 inhibitor treatment with PI3K/AKT pathway activation as the biomarker.

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