

## **Mitochondria Determine Response to Anti-PD-1 Therapy: An Evidence-based Hypothesis**

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## Abstract

It has been demonstrated that a decrease in cellular adenosine triphosphate (c-ATP) causes cellular dysfunction. T-cells are not an exception. One of their roles is to properly detect and eliminate cancer cells. These processes occur at the expense of ATP. Therefore, it can be concluded that a decrease in c-ATP can defect T-cell function and promote cancer evolution. In this article, we provide a hypothesis to describe the correlation between the expression of PD-1 protein on T-cells and their c-ATP levels. Moreover, we present the possible predictive factors of Anti-PD(L)-1 therapy which has not yet been determined definitely.

**Keywords:** T-lymphocytes; programmed cell death-1; mitochondria; adenosine triphosphate

## Background

Nowadays, each human cell is exposed to 10,000-20,000 DNA mutations per day. Some of these mutations (known as the driver mutation) can induce normal cell transformation to its cancerous counterpart (1, 2). Therefore, the efficiency of the immune system to detect the newly developed cancer cell is unassailable. Immune checkpoint inhibitors (ICIs) are a novel group of medications that have revolutionized the management of advanced cancers. They eliminate tumors by enhancing the body's own immunity. Tumor microenvironment (TME) contains several supporting cells that protect the cancer cells against the immune system, including myeloid-derived suppressor cells (MDSCs) and cancer-associated fibroblasts (CAFs). The ICIs work through blocking checkpoint proteins from binding to their partner proteins, therefore, prevent the T-cell to be "turned-off" by cancer cells and its supporters. Nowadays, ICIs are the leading choice in advanced and metastatic cancers (3). The ICIs targeting the programmed cell death 1 (PD-1) axis are the most encouraging field of research in this context (4, 5). They markedly augment T-cell responses in several malignancies. However, the objective response rate of Anti-PD(L)-1 therapy is about 20% for unknown reasons (6). So far, the predictive factors determining the subset of patients benefit the most from anti-PD(L)-1 inhibitors have remained unclear. Here we present a hypothesis, originating from the concept of cellular energy, that provides the putative mechanism for response to anti-PD-1 inhibitors.

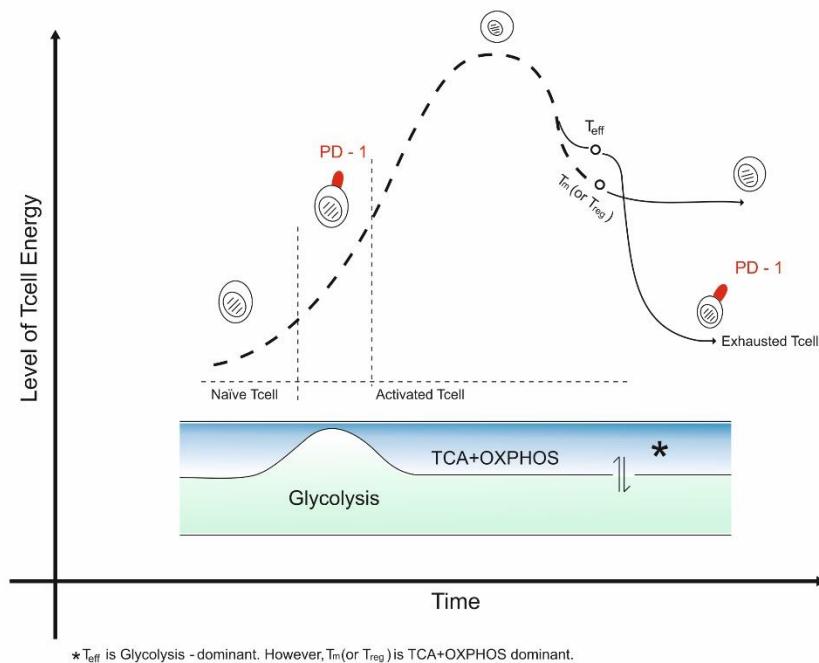
## Methods

In this article we have presented a novel hypothesis to present the key predictors of response to anti-PD-1 monoclonal antibodies (anti-PD-1 mAbs). This hypothesis has originated from the understanding that a decrease in cellular adenosine triphosphate (c-ATP) may induce cellular dysfunction (7, 8). In this article, the level of c-ATP is the representative of cellular energy.

## Results

T-cells play a central role in the prevention and treatment of cancer. When the c-ATP level of T-cells declines to a level that is less than the basal requirements to maintain ion homeostasis for membrane integrity (mediated by the  $\text{Na}^+/\text{K}^+$  ATP-dependent pump), the migration, detection, and destruction of newly developed cancer cells become impaired (9). This defect impairs T-cell function in detecting and eliminating the newly formed cancer cell.

According to the literature, there may be a correlation between the metabolic reprogramming of T-cell and their antitumor activity (9, 10). **Figure 1** summarizes the current findings regarding the alterations in PD-1 expression upon T-cell activation from the cellular energy aspect (9, 11). It also demonstrates the trend in the c-ATP level of tumor-infiltrating lymphocytes (TILs) following activation. Naïve T-cells are hypometabolic, and their metabolism is based on the tricarboxylic acid cycle and oxidative phosphorylation (TCA + OXPHOS) (PD-1 is not expressed). Following an initial immune stimulus, a short-term switching of metabolism to glycolysis occurs (PD-1 is expressed), but T-cells are energetically inefficient and need to add TCA + OXPHOS to glycolysis to attain acceptable antitumor activity (PD-1 is not expressed). Then, asymmetric cellular division occurs and the activated T-cells are divided into the effector T-cells ( $\text{T}_{\text{eff}}$ ) and memory T-cells ( $\text{T}_m$ )/ regulatory T-cells ( $\text{T}_{\text{reg}}$ ). Upon division,  $\text{T}_{\text{eff}}$  accumulates more c-ATP because it inherits the metabolically active cytoplasm. However, following counteraction with cancer cells, the c-ATP of  $\text{T}_{\text{eff}}$  decreases, and its metabolism is modified into glycolysis when exhaustion occurs (PD-1 is expressed). Exhausted T-cells cannot afford to secrete interferon-gamma that results in further immunosuppression. In contrast to  $\text{T}_{\text{eff}}$ ,  $\text{T}_m$  (or  $\text{T}_{\text{reg}}$ ) inherits considerable lipid sources and young mitochondria and maintains the c-ATP level by its active adenosine monophosphate-activated protein kinase (AMPK) (PD-1 is not expressed) (12). PD-1 expression is regulated epigenetically through promoter methylation or histone modification (13). Being such, we speculate that the mediators of TCA+OXPHOS may epigenetically downregulate the PD-1 expression. In summary, we hypothesize that the extent of PD-1 expression is inversely associated with the c-ATP level of TILs.



**Figure 1.** The correlation between the c-ATP level of tumor-infiltrating lymphocytes, type of metabolism, and PD-1 expression.

## Discussion

Currently, the huge challenge for cancer immunotherapy by PD-1 blockade is varied responses in different individuals. Although many patients with advanced cancer benefit from immunotherapy using anti-PD-1 mAb, there are still no effective predictive biomarkers to guide the clinical precision medicine approach and clinical trial design at present.

**Table 1** summarizes the prognostic factors of PD-1 expression and predictive factors of response to anti-PD-1 therapy from the cellular energy aspect. In summary, the mitochondria depressing factors (e.g. tobacco smoking, obesity, high SDA-diet, and aging) are associated with enhanced expression of PD-1 on TILs. In contrast, mitochondria boosting factors (e.g. exercise or chemicals such as AMPK activators) are associated with down-regulation of PD-1.

**Table 1|** PD-1 expression and response to anti-PD-1 therapy from cellular energy aspect

	<b>Title</b>	<b>Clinical findings</b>	<b>Reference</b>	<b>Cellular findings</b>	<b>Reference</b>	<b>Conclusions from cellular energy aspect</b>
1A	PD-1 expression and exercise	Sedentary lifestyle is associated with higher PD-1 expression on memory CD4 <sup>+</sup> T cells	(14)	Exercise improves lymphocyte mitochondrial functionality	(15)	Augmentation of mitochondria improve anti-PD-1 efficacy through decrease in PD-1 expression
1B	Anti-PD-1 and exercise	Exercise enhances anti-PD-1 efficacy in murine model	(16)			
2A	PD-1 expression and tobacco	Tobacco smokers is associated with higher PD-1 expression	(17)	Tobacco smoke damages mitochondria of lymphocytes	(18)	T-cells with weak mitochondria respond better to anti-PD-1 due to already over-expressed PD-1
2B	Anti-PD-1 and tobacco	anti-PD-1 monotherapy improves the OS of smokers, but not non-smokers	(19)			
3A	PD-1 expression and obesity	Obesity is associated with higher PD-1 expression	(20)	Obesity impairs mitochondrial activity of lymphocytes	(21)	T-cells with weak mitochondria respond better to anti-PD-1 therapy due to already over-expressed PD-1
3B	Anti-PD-1 and obesity	ORR is higher in overweight/obese patients	(22)			
4A	PD-1 expression and aging	Aging is associated with higher PD-1 expression	(23)	Mitochondrial function declines with age	(24)	T-cells with weak mitochondria respond better to anti-PD-1 due to already over-expressed PD-1
4B	Anti-PD-1 and aging	Elderly patients respond better to anti-PD-1	(25)			
5	PD-1 expression and sleep	Obstructive sleep apnea enhances PD-1 expression	(26)	Obstructive sleep apnea damages mitochondria	(27)	T-cells with weak mitochondria more express PD-1
6	PD-1 expression and diet	Western diet (high SDA) increases PD-1 expression in murine model	(28)	<ul style="list-style-type: none"> <li>- Mediterranean diet (low SDA) enhance the immune system against cancer</li> <li>- Mediterranean diet improves mitochondrial function</li> </ul>	(29) (30)	Increase in c-ATP decreases PD-1 expression
7	Anti-PD-1 and mitochondria boosting chemicals	ROSs enhances the therapeutic efficiency of anti-PD-1 therapy through AMPK expression	(31)	AMPK increases the c-ATP	(32)	Increase in c-ATP is associated with anti-PD-1 efficacy

PD-1, Programmed cell death-1; OS, overall survival; ORR, overall response rate; SDA, specific dynamic action; c-ATP, cellular adenosine triphosphate

There are a bunch of TILs in the TME with various c-ATP levels due to different access to nutrients and different levels of encounter with cancer cells. Based on our hypothesis, this difference may result in the varied expression of PD-1 on TILs. If we augment the mitochondrial function of exhausted T-cells, the rise in c-ATP causes down-regulation of PD-1, thus, enhances the anti-tumor efficacy of TILs. Two recently published papers, showing that enhanced mitochondrial activity has an adjuvant effect for anti-PD-1 immunotherapy, strongly support our hypothesis (16, 31).

Reviewing the literature from the cellular energy aspect provides interesting findings. Chamoto *et al.* demonstrated that mitochondria boosting chemicals (such as ROS, AMPK activators, mTOR activators, and PGC-1 $\alpha$  activators) enhance the antitumor activity of anti-PD-1 mAbs (31). However, the underlying mechanism was not delineated. Based on our hypothesis, the augmentation of mitochondria activity down-regulates the expression of PD-1 on already exhausted TILs. Moreover, an increase in c-ATP enhances their ability to counteract with cancer

cells and their supporters (33). Therefore, it improves their anti-tumoral activity by either improvement of diagnostic or killing ability.

Obviously, high expression of PD-1 on TILs and PD-L1 on cancer cells, MDSCs, and CAFs predict the better response to anti-PD-1 mAbs (34). However, based on our hypothesis, this is only part of the story. Upon PD-1/PD-L1 interaction, cancer cells and their supporters cause damage to the mitochondria of TILs and switch them to the exhausted form (35). The exhausted TILs cannot afford to efficiently eliminate the cancer cells, even after their recognition through PD-1/PD-L1 axis blockade. Therefore, short-term or limited efficacy of anti-PD-1 mAbs is expected in individuals with already low-energy TILs. In this case, mitochondria boosting approaches may be considered as an adjuvant for anti-PD-1 mAbs by augmenting the energy level of TILs and down-regulation of PD-1 protein. This notion explains the Chamoto *et al.* question that how mitochondria enhancing chemicals improve the response to anti-PD-1 mAbs (31). In contrast, patients with strong mitochondria are expected to have a lower response rate to anti-PD-1 mAbs due to lower expression of PD-1 protein. However, the high-energy group has a potentially better prognosis because of a more efficient diagnosis and elimination of cancer cells due to less expression of PD-1 protein and less interaction with PD-L1 protein, respectively.

Our hypothesis answers the following questions, how exercise enhances the response to anti-PD-1 mAb? Why cigarette smokers respond better to anti-PD-1 therapy? Why overweight/obese patients have more response rates to anti-PD-1 mAb? And how aging is a good predictive factor for anti-PD-1 therapy?

There are several approaches to improve c-ATP. Most of them are accessible through a change in lifestyle. First, regular exercise improves mitochondrial respiratory capacity through an increase in PGC-1 $\alpha$  (36). Smoking cessation is the second approach to improve mitochondrial capacity and improvement in c-ATP (37). Third, consuming foods with low specific dynamic action (SDA), as the energetic budget for consuming food, can potentially boost the immune system through improving the c-ATP. Recent studies have demonstrated the effect of low-SDA meals on the up-regulation of the innate immune system in corn snakes (38). Fourth, improvement in sleep quality can potentially improve the mitochondrial bioenergetic capacity (39). As demonstrated by Chamoto *et al.* several chemicals can also improve the mitochondrial activity, including ROS, uncouplers, AMPK activators, mTOR activators, and PGC-1 $\alpha$  activators (31).

Further clinical trials can use these approaches to improve the efficacy of anti-PD-1 therapy. Moreover, future studies may consider this hypothesis to better determine the predictive factors of response to anti-PD-1 therapy.

## Conclusion

Our hypothesis demonstrated the correlation between the c-ATP level of TILs and their antitumor activity. It also demonstrated a correlation between PD-1 expression on T-cells and the level of c-ATP. These correlations may simply occur coincidentally or may have a causal relationship. Further studies are needed to clarify the possible link.

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### List of abbreviations

AMPK	Adenosine monophosphate-activated protein kinase
ATP	Adenosine triphosphate
CAF	Cancer-associated fibroblast
ICI	Immune checkpoint inhibitor
MDSC	Myeloid-derived suppressor cell
mTOR	Mammalian target of rapamycin
PGC-1 $\alpha$	Proliferator-activated receptor gamma coactivator-1 $\alpha$
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1
SDA	Specific dynamic action
T <sub>eff</sub>	Effector T-cell
T <sub>m</sub>	Memory T-cell
TME	Tumor microenvironment
T <sub>reg</sub>	Regulatory T-cell