Immune metabolism in PD-1 blockade-based cancer immunotherapy

Alok Kumar and Kenji Chamoto

Department of Immunology and Genomic Medicine, Graduate School of Medicine, Kyoto University, Yoshida, Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

Correspondence to: K. Chamoto; E-mail: chamoto.kenji.4w@kyoto-u.ac.jp

Received 5 June 2020, editorial decision 30 June 2020; accepted 1 July 2020

Abstract

Energy metabolism plays an important role in proliferating cells. Recent reports indicate that metabolic regulation or metabolic products can control immune cell differentiation, fate and reactions. Cancer immunotherapy based on blockade of programmed cell death protein 1 (PD-1) has been used worldwide, but a significant fraction of patients remain unresponsive. Therefore, clarifying the mechanisms and overcoming the unresponsiveness are urgent issues. Because cancer immunity consists of interactions between the cancer and host immune cells, there has recently been a focus on the metabolic interactions and/or competition between the tumor and the immune system to address these issues. Cancer cells render their microenvironment immunosuppressive, driving T-cell dysfunction or exhaustion, which is advantageous for cancer cell survival. However, accumulating mechanistic evidence of T-cell and cancer cell metabolism has gradually revealed that controlling the metabolic pathways of either type of cell can overcome T-cell dysfunction and reprogram the metabolic balance in the tumor microenvironment. Here, we summarize the role of immune metabolism in T-cell-based immune surveillance and cancer immune escape. This new concept has boosted the development of combination therapy and predictive biomarkers in cancer immunotherapy with immune checkpoint inhibitors.

Keywords: biomarker, combination therapy, energy metabolism, immune checkpoint, mitochondria

Introduction

The immunosuppressive receptor programmed cell death protein 1 (PD-1) is one of the so-called immune checkpoint (IC) molecules and is a master regulator of immune homeostasis. Recently, it has been clarified that the majority of tumors escape from immune surveillance by utilizing the interaction between PD-1 on T cells and one of its ligands (PD-L1) in the tumor microenvironment (TME). Compared with conventional treatment, such as surgery, chemotherapy and radiation therapy, PD-1 blockade-based cancer immunotherapy has led to a paradigm shift in cancer treatment owing to improved survival, fewer side effects and applicability to a wide range of tumors (1–3). However, three main problems remain to be solved: (i) a substantial number of patients show unresponsiveness to PD-1 blockade therapy; (ii) precise predictive biomarkers have not been identified to discriminate responders and non-responders; and (iii) the best combination therapy is not yet known. To address these issues, it is important to understand the complexity of mechanisms that cause unresponsiveness, which include immune escape systems other than the PD-1–PD-L1 interaction.

The TME consists of heterogeneous cell populations and various cell-derived factors. Tumor cells escape immune attack by utilizing a broad range of suppressive factors that are directly derived from the tumor and/or bystander tumor-related factors (Fig. 1). Direct tumor factors that inhibit T-cell activity include (i) negative IC-associated molecules, such as PD-L1, Galectin 9, CD112, CD155, P-selectin glycoprotein ligand-1 (PSG-L1), B7-H3 and B7-H4 expressed on the cell surface (2, 4); (ii) secreted suppressive cytokines such as IL-6, IL-10 and TGF-β (5) and (iii) released small molecules, which are also mentioned among the metabolic products discussed below (6–9).

Bystander factors indirectly suppress tumor-reactive T-cell activity (Fig. 1): (i) by producing particular cytokines and chemokines, tumors induce immunosuppressive cells such as regulatory T cells (Tregs), regulatory B cells (Bregs), suppressive macrophages and some types of innate lymphoid cells (ILCs); (ii) chronic stimulation with tumor antigens induces the expression of IC molecules on T cells, such as PD-1, cytotoxic T lymphocyte antigen 4 (CTLA-4) and T-cell immunoglobulin mucin-domain containing 3 (Tim-3), which inhibit...
antitumor responses; and (iii) nutrient consumption by cancer cells causes nutrient competition with T cells that ultimately dampens T-cell responses. In addition, several metabolic products in the TME, such as lactic acid, kynurenine (KYN) and adenosine, are known as immunosuppressive factors that inhibit the effector functions of immune cells (6–8). Tumor activity can thus create an unfavorable microenvironment for T-cell survival and activation.

Among the immunosuppressive and/or immune escape systems shown in Fig. 1, the metabolic status of the TME is thought to play a critical role in regulating the antitumor immune responses (10–12). We and other groups have demonstrated that T-cell energy and/or mitochondrial metabolism are good biomarkers and targets of combination therapies (9, 13–17). Both the metabolic environment and intracellular metabolism determine the fate and functions of immune cells in the TME. In this review, we will discuss the metabolic profiles and regulation of tumor cells and immune cells, particularly T cells, with further emphasis on how metabolic manipulation improves the immunotherapeutic responses during PD-1 blockade-based cancer immunotherapy.

### Immune suppression by tumor metabolic properties

**Immune suppression by the Warburg effect in tumor cells**

Metabolic reprogramming is a hallmark of cancer progression (18–20). Cancer cells preferentially use glycolytic energy metabolism even under aerobic conditions to meet the increased anabolic and energy demands for rapid proliferation. The aerobic form of glycolysis (known as the Warburg effect) in cancer cells was identified for the first time by Dr Otto Warburg in the 1920s (21, 22). The dominant glycolytic program in cancer cells facilitates the rapid generation of ATP and the accumulation of lactic acid. Skewing the metabolic profile towards glycolysis in vitro up-regulates and down-regulates the expression of oncogenes and tumor suppressor genes, respectively, resulting in enhanced tumor growth in vivo. In contrast, forced up-regulation of oxidative phosphorylation (OXPHOS) in tumor cells slows their growth in vivo (23, 24).

An increased concentration of lactic acid prevents T-cell infiltration into the tumor mass and inhibits the up-regulation of nuclear factor of activated T cells (NFAT) in T and NK cells, resulting in reduced IFN-γ production in the TME and allowing escape from antitumor immunity (6, 25). Fantin et al. showed that inhibiting lactate dehydrogenase A (LDH-A) prevents the conversion of pyruvate to lactate and reduces tumorigenicity (26). Inhibition of LDH-A activity up-regulates OXPHOS and compromises cancer cell growth in hypoxic TME conditions. High levels of LDH-A in serum before treatment have been considered an independent, negative risk factor for the survival of patients with hematologic or solid neoplasms (27). In 2009, the LDH-A serum level was included in the cancer staging system predefined by the American Joint Committee on Cancer (AJCC) (28, 29). Notably, a high serum LDH-A level is also associated with poor outcomes in PD-1-blockade and/or CTLA-4 blockade therapies in patients with melanoma or non-small cell lung cancer (NSCLC) (30–32).

Glutamine is the only amino acid that can generate all other nonessential amino acids and also acts as a currency to pay for the import of other amino acids into the cells. Glutamine metabolism is important for the production of the anti-oxidative metabolite glutathione and the cellular pool of NADPH to maintain a normal redox state, and provides α-ketoglutarate for entry into the tricarboxylic acid cycle (TCA cycle; also...
known as Krebs cycle) cycle to generate energy (33, 34). Since pyruvate generated by glycolysis is being converted into lactate instead of being used in the TCA cycle in cancer cells, they rely on gluconeogenesis for OXPHOS-mediated energy production. Therefore, glutamine blockade reduces ATP levels in cancer cells, resulting in adenosine monophosphate (AMP)-activated protein kinase (AMPK) up-regulation and glycolysis inhibition, whereas T cells can replenish TCA cycle metabolites from glycolysis (35). Glutamine blockade might be one of the promising strategies of cancer therapy by targeting a weak point of the Warburg effect.

In summary, the glycolytic profile of tumor cells makes them metabolically fit for rapid proliferation under hypoxic conditions and shapes the immunosuppressive environment.

**Metabolic immune suppression in the TME**

Similar to cancer cells, T cells also switch their metabolism to a glycolytic profile upon recognition of tumor antigens to meet the biosynthetic and bioenergetic demand for rapid proliferation, although T cells preferentially use anaerobic glycolysis to produce pyruvic acid but not lactic acid (10, 24, 36). Because of the similar manners by which cancer and T cells proliferate, there is competition for energy sources and metabolic substrates for anabolic pathways. Several reports have shown that excessive glucose consumption by tumors metabolically restricts T-cell glycolytic activity, leading to T-cell dysfunction (Fig. 2) (37, 38).

In addition, particular biochemical programs in cancer cells produce metabolites that dampen the function of T cells in the TME. Apart from amino acid synthesis and consumption, cancer cells catabolize some amino acids and generate catabolites that are suppressive, as one of the immune suppressive mechanisms. Cancer cells avidly consume tryptophan, an essential amino acid for T-cell activation and proliferation, and metabolize it to the downstream catabolites KYNs through two enzymes: indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO) (Fig. 2) (39). Competition for tryptophan is one of the immunosuppressive mechanisms in the TME, as previously mentioned (40–42), and KYNs are immune suppressors in nature and inhibit the effector activity of T cells (43). KYNs are transported in the cells through the system L transporter SLC7A5 binding with aryl hydrocarbon receptor (AhR), a cytosolic transcription factor (44–47) and induce the differentiation of CD4+ T cells into Foxp3+ immunosuppressive T regulatory cells (48, 49). KYN has been also reported to induce reactive oxygen species (ROS), which inhibit IL-2 signaling and impair the generation and function of memory T cells (50).

Following activation, T cells consume L-arginine for their anabolic profile and rapid proliferation. L-Arginine serves as a building block for protein synthesis. Elevating L-arginine levels induces global metabolic change and shifts the metabolic profile from glycolysis to OXPHOS that maintains the T cells as central memory (Tcm)-like cells with higher survival capability (51). In the TME, myeloid-derived suppressor cells (MDSCs) secrete the enzyme arginase that degrades the arginine present in the vicinity and causes T cells to be deprived of arginine (52). Targeting arginase or supplementing L-arginine enhances the efficacy of IC blockade therapies (53–55).

Another metabolic product that suppresses immune responses is adenosine, which is generated by the enzymatic activity of CD38, CD39 and CD73. Adenosine is known to suppress T-cell functions via the A2A receptor, which leads to the synthesis of cyclic AMP (cAMP) (8). cAMP activates multifunctional cAMP kinase (cAMPK)-dependent protein kinase (PKA). One of the major mechanisms for T-cell inhibition is PKA-mediated inhibition of protein kinase B (PKB) and signal transducer and activator of transcription 5 (STAT5) phosphorylation, which are downstream of the T-cell antigen receptor (TCR) and IL-2 receptor (IL-2R), respectively (56, 57). In addition, PKA phosphorylates the transcription factor CREB, which is essential for inducing T regulatory cells (58). Since the cell surface enzymes CD38, CD39 and CD73 are highly expressed on tumors, macrophages and apoptotic T regulatory cells, the TME is rich in adenosine, which maintains a suppressive environment (59).

Prostaglandin E2 (PGE2), a small molecule lipid mediator synthesized from arachidonic acid by the sequential actions of cyclooxygenase 2 (COX-2) and microsomal prostaglandin
E synthesis-1 (mPGES-1), has been demonstrated to suppress T cell differentiation, B-cell functions, T-cell activation and allergic reactions (60–62). PGE2 exerts its action through G-protein-coupled receptors (GPCRs), which are ubiquitously expressed in response to various stimuli under pathophysiological conditions (63). PGE2 inhibits T cell proliferation at two distinct levels: inhibition of IL-2 production and down-regulation of the transferrin receptor via a cAMP-dependent signal transduction pathway (64, 65). Research and development of drugs targeting IDO, adenosine and PGE2 are progressing, and the combined effect of anti-PD-1 and/or anti-PD-L1 antibodies has been verified in clinical practices (66–70).

Recently, our group showed that some unresponsive tumors release small molecules that directly inhibit the mitochondria of T cells (9). To understand whether the tumor is unresponsive because of local immune evasion (a tumor intrinsic property that allows the tumor to be undetected by immune cells) or the induction of systemic immune suppression, we devised a novel bilateral tumor model, in which responsive and unresponsive tumors were injected bilaterally and the growth of the responsive tumor was evaluated. In this model, if responsive tumors became unresponsive under PD-1 blockade conditions, the unresponsive tumor must at least use systemic immune suppression. With this model, we successfully classified unresponsive tumors into two groups—those with or without a systemic immunosuppressive property (SIP). Furthermore, we found that SIP-positive tumors (for example, LLC and CT26) release uncharacterized non-proteinaceous small molecules that directly inhibit mitochondrial activation and proliferation of T cells. We ruled out the possibility of previously reported small molecules such as adenosine, KYNs and PGE2. Therefore, there are still unknown suppressive tumor-derived small molecules (metabolites) in the TME (Fig. 1).

**The metabolic program in T-cell differentiation**

**Impact of energy metabolism on T-cell function**

The final effector immune cells in IC blockade cancer immunotherapy (ICI) are T cells. Indeed, the number of T cells that infiltrate the tumor site is one of the predictive and/or prognostic factors in ICI (71–74). Therefore, it is necessary to decipher the mechanism by which energy metabolism regulates T-cell activation, function and differentiation. Glycolysis provides not only fast energy production but also pentose phosphate pathway-mediated NADPH generation for subsequent redox control in T cells (75). In addition, glycolysis produces metabolic intermediates that are used in the subsequent mitochondrial TCA cycle to produce large amounts of ATP in mitochondria. Therefore, both robust glycolysis and mitochondrial metabolism are essential for the rapid proliferation of T cells (Fig. 3).

Accumulating evidence over the last two decades suggests a critical role of mitochondria in T-cell activation and proliferation. Mitochondrial ROS (mROS) generated at complex III during OXPHOS are converted into H2O2 by manganese superoxide dismutase (MnSOD) and ultimately activate NFAT to regulate T-cell activation (76, 77). Sena et al. showed that in Uqcrfs1−/− mice (lacking the gene encoding Rieske iron-sulfur protein, a component of complex III in mice), T cells had greatly reduced mROS production, leading to a reduction in NFAT activity, as well as reduced IL-2 production, and the T cells could not proliferate upon antigen stimulation in vivo (78, 79). On the other hand, Fisicaro et al. reported that mROS induces mitochondrial dysfunction in T cells, and antioxidant reagents attenuate the induction of T-cell exhaustion (80).

These apparently contradictory reports suggest that a low amount of mROS is critical for the early phase of T-cell differentiation, whereas accumulation of mROS damages the mitochondria in the later phase and induces an exhausted (unresponsive) state. Therefore, mitochondrial activation, as well as glycolysis, controls T-cell functionality.

**Balanced energy sensors are involved in T-cell differentiation**

Immunological memory is a hallmark of adaptive immunity. During the activation of immune responses against foreign antigens or against neoantigens on cancer cells, T cells differentiate from metabolically dormant naive cells to active, highly proliferative effector cells. After antigen clearance, some of these cells become long-lasting memory T cells with a dormant metabolism, but these memory T cells have a rapid and robust recall ability upon antigen recognition (81, 82). To meet the high demands for ATP during anabolic processes, effector T cells utilize both glycolysis and OXPHOS pathways more than naive and memory T cells do, although effector T cells depend more on glycolysis than OXPHOS. In contrast, memory T cells depend more on OXPHOS-mediated catabolic pathways than on glycolysis because they do not need to proliferate (Fig. 3) (83, 84).

Dependence on anabolic and catabolic pathways is mainly regulated by two opposing key energy sensors—mechanistic target of rapamycin (mTOR) and AMPK, respectively (85–87). The mTOR complex 1 (mTORC1) pathway preferentially activates anabolic glycolytic metabolism under nutrient and energy-rich conditions and the AMPK pathway regulates...
have reduced mitochondrial depolarization with fewer
ganization of mitochondrial cristae. PD-1-engaged T cells
and Mic14, which are two important proteins for the or-
chondrial ultrastructure, reducing the expression of Mic19
101). PD-1 engagement on T cells attenuates glycolysis,
CD28, which is a positive costimulatory IC molecule (100,
dephosphorylates downstream signals from the TCR and
PD-1 signaling recruits the phosphatase SHP-2 and
PD-1 signaling and metabolic regulation
energy sensor balance and mitochondrial morphology.

Both mTOR and AMPK signaling are associated with the
ultrastructure and cristae organization in mitochondria (93–
Buck et al. showed that effector T cells show punctate
mitochondria, whereas in memory T cells, the mitochondria
have a fused network (13). Opa1, a protein required for
mitochondrial fusion, is expressed in memory T cells but not ef-
fector T cells during adaptive immune responses. Altering the
morphology of cristae by fusion configures the associations
of electron transport chain (ETC) complexes, and they be-
come condensed (tight), which favorsOXPHOS and FAO (98,
99). On the other hand, mitochondrial fission preferentially
used in effector T cells, expands cristae (i.e. loosens them)
and reduces ETC efficiency but is required for mitophagy-
based anabolism (96).

However, the mechanism by which fusion and fission are
regulated has not been established. Morita et al. reported that mTORC1 regulates translation of mitochondrial fission
process 1 (MFFP1) and mitochondrial recruitment of the fission
GTPase dynamin-related protein 1 (DRP1) via signaling
downstream of mTORC1 (95). Other studies have demonstr-
ated that AMPK directly induces mitochondrial fission by
activating mitochondrial fission factor (MFF) and armadillo-
containing protein 10 (ARMC10) (96). Further studies are
necessary to clarify the interaction between T-cell function,
energy sensor balance and mitochondrial morphology.

**PD-1 signaling and metabolic regulation**

PD-1 signaling recruits the phosphatase SHP-2 and
dephosphorylates downstream signals from the TCR and
CD28, which is a positive costimulatory IC molecule (100,
101). PD-1 engagement on T cells attenuates glycolysis,
glutaminolysis and metabolism associated with branched-
chain amino acids (102). PD-1 signaling also affects mito-
ochondrial ultrastructure, reducing the expression of Mic19
and Mic14, which are two important proteins for the or-
organization of mitochondrial cristae. PD-1-engaged T cells
have reduced mitochondrial depolarization with fewer
mitochondrial cristae that lead to mitochondrial dysfunction
in T cells (103). In contrast, PD-1 engagement reprograms
T-cell metabolism towards FAO of endogenous lipids by
enhancing the expression of CPT1A, an FAO rate-limiting
enzyme (102). Therefore, PD-1 engagement attenuates the
overall metabolic activity of T cells while it rescues effector T
cells from terminal differentiation and a quick death induced
by boosted glycolysis by skewing the metabolic balance to-
wards a fat-based metabolic profile, resulting in longevity
(Fig. 4) (102).

Accordingly, blocking PD-1 signaling triggers glycolysis-
based terminal differentiation of effector T cells, ultimately
leading to clonal deletion by apoptosis (15). It is likely that
during PD-1 blockade monotherapy, some patients ini-
itially show a response but later become unresponsive; the
possible explanation may be the reduced availability of ef-
fector T cells, as PD-1 blockade induces cell death through
overactivation of effector T cells.

**Enhancement of antitumor immunity by controlling
immune metabolic regulation**

As mentioned in the previous sections, the balance between
AMPK and mTOR regulates T-cell fate. Effector T cells rely on
the mTOR pathway, whereas memory T cells show depend-
ence on AMPK. Metformin (dimethylbiguanide), a drug pre-
scribed for patients with type 2 diabetes, has been reported
to have an anticancer effect. Metformin protects CD8+ tumor-
filtrating lymphocytes (TILs) from apoptosis by decreasing
caspase-3 expression, leading to an increase in TIL number
and function (104). The levels of phosphorylated AMPK
(pAMPK) are increased, but the levels of a downstream
target of mTOR, pS6 are decreased by metformin. Therefore,
metformin reprograms T cells towards AMPK and away from
the mTOR pathway, which endows T cells with memory-like

---

**Fig. 4.** Combination therapy with metabolic regulators enhances the function and longevity of T cells by up-regulating FAO and OXPHOS during PD-1 blockade. (I) CD8+ T cells with PD-1 engagement rely on less glycolysis and more FAO/OXPHOS. (II) PD-1 blockade monotherapy causes a shift towards a glycolytic profile, leading to ter-

---

**I. Before treatment**

- Glycolysis
- OXPHOS

**II. PD-1 blockade**

- Dysfunctional
- Longevity

**III. PD-1 blockade + metabolic regulator**

- Functional
- Short-lived effector T cells
- Quick apoptosis
- Long-lived (Bcl2†)

---

**Importance of metabolism in cancer immunotherapy**

21
longevity, although the precise mechanism of the enhancement effect of metformin is still unknown.

Fatty acid metabolism, which mainly includes fatty acid biosynthesis and FAO, is tightly associated with T-cell differentiation. Fatty acids serve as a component of cells and an energy source. Lipid biosynthesis is, therefore, increased in activated T cells and reduced in memory T cells, which preferentially use FAO (105, 106). Endo et al. showed that the inhibition of fatty acid biosynthesis by an inhibitor or deletion of acetyl coenzyme A carboxylase 1 (ACC1), which is a master regulator of fatty acid biosynthesis, increased memory CD4+ T-cell formation by shifting the intrinsic metabolic profiles from fatty acid synthesis to FAO (107).

We reported that combinations of small molecules which improve mitochondrial metabolic pathway (OXPHOS and FAO) of CD8+ T cells could improve PD-1 blockade treatment (14, 15). As previously discussed, PD-1 blockade recovers T-cell function and boosts the terminal differentiation of T cells via up-regulation of the glycolysis pathway (15, 102). We hypothesized that this terminal differentiation resulted in the apoptosis of tumor-reactive T cells after PD-1 blockade and is one of the mechanisms associated with unresponsiveness (Fig. 4). Even under glycolysis-dependent metabolism during PD-1 blockade, we showed that enhancement of mitochondria-mediated FAO by metabolic drugs increases antiapoptotic gene expression, increases T-cell longevity and boosts their function. For example, skewing the metabolism of T cells towards FAO by enhancing the peroxisome proliferator-activated receptor (PPAR)/PPAR-γ gamma coactivator-1α (PGC-1α) axis using bezafibrate (a pan-PPAR agonist) rescues T cells from apoptosis, and they become long-lived under PD-1 blockade. This combination therapy ameliorated the tumor rejection rate and the survival of tumor-bearing hosts (15, 17). Therefore, mitochondria-mediated FAO activation in T cells during PD-1 blockade therapy is a promising strategy to maintain functional effector T cells.

Together, metabolic regulation in tumors or immune cells has received attention, and several trials of combination therapies with ICI therapy are being tested. We have summarized the metabolic modulators tested in combination therapy with PD-1 blockade to improve treatment efficacy in Table 1.

**Metabolic biomarkers of PD-1 blockade cancer immunotherapy**

Although few studies have been reported on the efficacy-predictive biomarkers related to immune metabolism in ICI therapy, the concept of immune metabolism would be very useful for biomarker development. We reported that PD-1 blockade activates the mitochondria in T cells (14). Accordingly, a mitochondrial surge was observed in T cells from draining lymph nodes of hosts bearing responsive tumors but not unresponsive tumors during PD-1 blockade (9). Miyajima et al. reported that the levels of serum metabolites associated with energy metabolism, including amino acids, are reduced in PD-1−/− mice because of consumption by constitutively activated T cells (108). It has also been shown that blood metabolite levels related to the TCA cycle was reduced in models of PD-1 blockade cancer immunotherapy (14). T-cell activation and the blood levels of metabolites are tightly co-regulated. The metabolic profile of responsive patients should therefore be altered and contain effective biomarkers.

We investigated plasma metabolites in patients with NSCLC before and after treatment with the PD-1 blocking antibody nivolumab (16). Responsive patients reduced levels of the TCA cycle (α-ketoglutaric acid) and FAO-related metabolites (acylcarnitine families). Unexpectedly, responders showed higher levels of microbiome-related metabolites (hippuric acid and 4-cresol) and redox-related metabolites (glutathione disulfide and cysteine) before and/or after PD-1 blockade therapy. These results suggest that flora, redox and energy metabolism interact in antitumor immune responses in cancer patients.

Additionally, we extracted peripheral blood mononuclear cells from the same blood samples and analyzed cellular markers, including those related to T-cell mitochondria, by flow cytometry. The combination of four T-cell markers—(i) markers associated with mitochondrial activity (PGC-1α/β expression in CD8+ T cells), (ii) markers associated with the oxidative state (ROS expression in CD8+ T cells), (iii) the frequency of PD-1high CD8+ T cells and (iv) the frequency of CD4+ T cells—was highly predictive for responders (mean AUC = 0.956 by machine learning cross-validation). Among all plasma metabolites and cellular markers tested, these four cellular markers were exclusively selected as the best combination for predicting the responding patients.

Subsequent correlation analysis revealed that metabolite markers related to microbiota, FAO and redox reactions are strongly linked to the cellular markers of PGC-1α/β expression, the frequency of PD-1high cells and ROS expression in CD8+ killer T cells, respectively. We concluded that this strong linkage excludes metabolite markers, which suggest that the metabolite markers could reflect T cell immune responses against tumors. Therefore, a combination of markers including metabolites and/or T-cell mitochondria-associated molecules is quite valuable. It should also be noted that an examination of immune biomarkers using blood cells would be less traumatic for patients than a tumor tissue biopsy, which is clinically used as ICI biomarkers (109, 110).

**Conclusions**

Energy metabolism is a fundamental physiological activity in cells and individuals. As shown in Table 1, various clinical trials and animal models of combination therapies using metabolic modulators have been reported. Encouraged by the increased knowledge of tumor and T-cell metabolism, efforts should be made to manipulate anti-tumor immune responses for clinical benefit as a next step.

However, the metabolic system is still complicated, especially the cross-talk between cancer cells and immune cells. We focused on conventional T-cell metabolism in this review, but there are various types of immune cells involved in antitumor activity, such as Treg, macrophages, dendritic cells and B cells, that use different pathways of metabolic regulation (111–114). Suppressive immune cells such as Treg and macrophages (M2) depend more on FAO/OXPHOS for their
Importance of metabolism in cancer immunotherapy

Table 1. List of metabolic drugs showing synergistic effects with PD-1 blockade therapy

| Drug name     | Target                        | Action                                                                                                                                                                                                 | Reference |
|---------------|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| BGB-5777      | IDO1 inhibitor                | BGB-5777 antagonizes IDO1 that results in reduced tryptophan catabolism and less kynurenine (immunosuppressive) production. It results in enhanced T-cell infiltration and effector function of T cells. | (118)     |
| PEGylated     | Degradation of kynurenines   | PEG-KYNase degrades the kynurenines (immunosuppressive) which are the catabolic products of tryptophan metabolism and thereby enhances immune responses.                                                                 | (119)     |
| kynureninase  | (PEG-KYNase)                   | CB-1158 inhibits arginase (secreted by MDSCs and polymorphonuclear cells in the TME) that breaks down arginine which is essential for T-cell activation and proliferation.                                      | (53) (54) |
| CB-1158       | Arginase inhibitor            | CB-1158 inhibits arginase (secreted by MDSCs and polymorphonuclear cells in the TME) that breaks down arginine which is essential for T-cell activation and proliferation.                                      | [NCT02903914] |
| L-Arginine    | L-Arginine as supplement      | L-arginine supplementation improves T-cell proliferation, effector function (IFN-γ production), differentiation and survival and prolongs the survival of mice in vivo.                                                                        | (55)      |
| IPH5201       | Anti-CD39 mAb                 | Blocking CD39 causes inhibited adenosine production that results in restored T-cell activation and immune response.                                                                                   | (68)      |
| IPH5301       | Anti-CD73 mAb                 | Blocking CD73 causes inhibited adenosine production that leads to enhanced activation of effector T cells and immune responses.                                                                       | (68)      |
| CPI-444       | A2AR antagonist               | Blocking A2AR with CPI-444 restores T-cell signaling, IL-2 and IFN-γ production.                                                                                                                  | (120) [NCT03337698] |
| PBF-509       | A2AR antagonist               | Blocking A2AR with PBF-509 enhances T-cell responsiveness.                                                                                                                                            | (121) [NCT02403193] |
| Aspirin       | COX inhibitor                 | Aspirin inhibits PGE2 (immunosuppressive) synthesis that results in enhanced T-cell activation and immune responses.                                                                                   | (70)      |
| Melafolone    | Dual inhibitor of COX-2       | The flavonoid, melafolone, a dual inhibitor of COX-2 and EGFR, improves immunotherapy through normalizing tumor vasculature and PD-L1 down-regulation.                                                      | (69)      |
| 2-Deoxyglucose| Glycolysis inhibitor          | 2-Deoxyglucose, by inhibiting glycolytic pathways, skews metabolism towards OXPHOS that results in the formation of long-lived memory CD8+ T cells.                                                          | (84)      |
| JHU083        | Glutamine antagonist          | JHU083 treatment along with PD-1 blockade suppresses oxidative and glycolytic metabolism in cancer cells, leading to decreased hypoxia, acidosis and nutrient depletion while effector T cells up-regulate oxidative metabolism and adopt a long-lived phenotype. | (35)      |
| Hydroxocitrate| Inhibitor of ATP citrate      | Hydroxocitrate mimics the biochemical effects of nutrient deprivation by reducing lysine acetylation of cellular proteins, thus triggering autophagy-mediated immune enhancement.                        | (122)     |
| Lyase          | (calorie restriction mimetics)|                                                                                                           |           |
| FCCP           | Mitochondrial uncoupler       | FCCP uncouples the proton gradient of mitochondria, resulting in reduced membrane potential and enhanced ROS generation in T cells that causes T-cell activation and enhanced mitochondrial function via PGC-1α downstream pathways induced by ROS. | (14)      |
| Luperox       | ROS generator / H₂O₂ precursor| ROS generation by liperox causes up-regulation of the PGC-1α pathway for mitochondrial activation and effector functions of T cells.                                                                   | (14)      |
| Bezafibrate   | PGC-1α/pan-PPAR complex activator | Bezafibrate enhances OXPHOS, FAO and anti-apoptotic genes (Bcl2) expression, resulting in longevity of immune effector T cells.                                                                         | (15) (17) |
| Metformin     | AMPK activator                | Metformin activates the AMPK pathway, leading to enhanced memory formation in T cells. It enhances TIL infiltration and protects them from apoptosis and exhaustion.                                            | (104)     |
| GW501516      | PGC-1α/PARPα and δ activator | GW501516 boosts FAO in T cells that results in enhanced persistence of effector T cells.                                                                                                             | (123)     |
| Rapamycin     | mTORC1 inhibitor              | Rapamycin expands memory T cells and enhances IFN-γ production. mTOR inhibition reduces the exhaustion phenotype of TILs and improves the life span of effector immune T cells.                           | (124) (125) |
| Vistusertib   | mTORC1/2 dual kinase inhibitor | Blocking mTOR with everolimus in combination with anti-PD-L1 treatment increases TILs and the ratio of cytotoxic CD8+ T cells to TILs.                                                              | (126)     |
| Everolimus    | mTOR inhibitor                | TWS119 mimics GSK-3βi and up-regulates Wnt-β-catenin signaling which down-regulates mTOR signaling. Reduced mTOR signaling causes induction of a stem memory T cell (TSCM) population.                                      | (127)     |

Survival (111–114). Although we discussed the benefits of FAO/OXPHOS introduction in T cells for antitumor efficacy, we need to consider the balance of these other suppressive immune cells.

It has also been reported that the microbiota and aging affect the metabolic state not only in the whole body but also at the level of single cells (115–117). Even at the single-cell level, the cross-talk between mTOR and AMPK signaling is controversial, which is exemplified by the fact that both mTOR and AMPK activators enhance the efficacy of IC inhibitors (14, 87, 104). Therefore, more mechanistic analysis of immune metabolism is required to precisely control immune reactions in the future.

**Funding**

This work was supported by the Japanese Agency for Medical Research and Development (AMED) (grant number 18dk140306h0002, K.C.), the Japan Society for the Promotion of Science (JSPS) KAKENHI.
Conflicts of interest statement: the authors declared no conflicts of interest.

References

1. Chowdhury, P. S., Chamoto, K. and Honjo, T. 2018. Combination therapy strategies for improving PD-1 blockade efficacy: a new era in cancer immunotherapy. *J. Intern. Med.* 283:110.

2. Iwai, Y., Hamanishi, J., Chamoto, K. and Honjo, T. 2017. Cancer immunotherapies targeting the PD-1 signaling pathway. *J. Biomed. Sci.* 24:26.

3. Chamoto, K., Hatae, R. and Honjo, T. 2020. Current issues and perspectives in PD-1 blockade cancer immunotherapy. *Int. J. Clin. Oncol.* 25:790.

4. Andrews, L. P., Yano, H. and Vignali, D. A. A. 2019. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. *Nat. Immuno.* 20:1425.

5. Kusmartsev, S. and Gabrilovich, D. I. 2006. Effect of tumor-induced fatty acid oxidation in tumor-infiltrating CTLs enhances activation-α and γδ T-cell responses. *Immunity* 21:250.

6. Brand, A., Singer, K., Koehl, G. E. 2015. System L amino acid transport in T cells. *Nat. Commun.* 9:1981.

7. Yaqoob, P., and Calder, P. C. 1997. Glutamine requirement of prostate cancer cells: tolerance and tryptophan catabolism. *Front. Immunol.* 7:109.

8. Zhang, Z., Li, Y., Zou, M., Atwood, J. T. et al. 1998. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Immunol. Rev.* 162:1229.

9. Sinclair, L. V., Neyens, D., Ramsay, G., Taylor, P. M. and Cantrell, D. A. 2018. Single cell analysis of kynurenine and System L amino acid transport in T cells. *Nat. Commun.* 9:1981.
Importance of metabolism in cancer immunotherapy

pathway unleash immune responses in combination cancer therapeutics. Cell Rep. 27:2411

69 Tang, H., Liu, Y., Wang, C. et al. 2019. Inhibition of COX-2 and EGFR by melafonole improves Anti-PD-1 therapy through vascular normalization and PD-L1 downregulation in lung cancer. J. Pharmacol. Exp. Ther. 368:401.

70 Zelenay, S., van der Veen, A. G., Böttcher, J. P. et al. 2015. Cyclooxygenase-dependent tumor growth through evasion of immunity. Cell 162:1257.

71 Galon, J., Costes, A., Sanchez-Cabo, F. et al. 2006. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313:1960.

72 Turmeh, P. C., Harvieu, C. L., Yearley, J. H. et al. 2014. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515:568.

73 van der Woude, L. L., Gorris, M. A. J., HalliVio, A., Figdor, C. G., de Vries, I. J. M. 2017. Migrating into the tumor: a roadmap for T cell. Trends Cancer 3:797.

74 Bonaventura, P., Shekarian, T., Alcazer, V. et al. 2019. Cold tumors: a therapeutic challenge for immunotherapy. Front. Immunol. 10:168.

75 Patsoukis, N., Bardhan, K., Weaver, J. et al. 2016. The role of metabolic reprogramming in T cell fate and function. Curr. Trends Immunol. 17:1.

76 Turrens, J. F. 2003. Mitochondrial formation of reactive oxygen species. J. Physiol. 552(Pt 2):335.

77 Weinberg, S. E., Sena, L. A. and Chandel, N. S. 2015. Mitochondria in the regulation of innate and adaptive immunity. Immunology 42:406.

78 Sena, L. A., Li, S., Jailaman, A. et al. 2013. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. J. Immunol. 38:225.

79 Murphy, M. P. and SiegeI, R. M. 2013. Mitochondrial ROS fire up T cell activation. Immunity 38:201.

80 Patsoukis, N., Bardhan, K., Weaver, J. et al. 2016. The role of metabolic reprogramming in T cell fate and function. Curr. Trends Immunol. 17:1.

81 van der Windt, G. J., O'Sullivan, D., Everts, B. et al. 2013. CD8+ memory T cells have a bioenergetic advantage that underlies their rapid recall ability. Proc. Natl Acad. Sci. USA 110:14336.

82 Martin, M. D. and Badovinac, V. P. 2018. Defining memory CD8 T cell. Front. Immunol. 9:2692.

83 Pearce, E. L., Walsh, M. C., Cejas, P. J. et al. 2009. Enhancing CD8 T cell memory by modulating fatty acid metabolism. Nature 460:103.

84 Sena, L. A., Li, S., Jailaman, A. et al. 2013. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. J. Immunol. 38:225.

85 Pearce, E. L., Walsh, M. C., Cejas, P. J. et al. 2009. Enhancing CD8 T cell memory by modulating fatty acid metabolism. Nature 460:103.

86 Xu, J., Ji, J. and Yan, X. H. 2012. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. J. Immunol. 188:21.

87 Vadlakonda, L., Dash, A., Pasupuleti, M., Anil Kumar, K. and Reddanna, P. 2013. The paradox of Akt-mTOR interactions. Front. Oncol. 3:165.

88 Buck, M. D., O’Sullivan, D. and Pearce, E. L. 2015. T cell metabolism drives immunity. J. Exp. Med. 212:1345.

89 Buck, M. D., O’Sullivan, D. and Pearce, E. L. 2015. T cell metabolism drives immunity. J. Exp. Med. 212:1345.

90 Buck, M. D., O’Sullivan, D. and Pearce, E. L. 2015. T cell metabolism drives immunity. J. Exp. Med. 212:1345.
Importance of metabolism in cancer immunotherapy

93 Parra, V., Verdejo, H. E., Iglewski, M. et al. 2014. Insulin stimulates mitochondrial fusion and function in cardiomyocytes via the Akt-mTOR-NFkB-Opa-1 signaling pathway. Diabetes 63:75.

94 Toyama, E. Q., Herzig, S., Courchel, J. et al. 2016. Metabolism, AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. Science 351:275.

95 Morita, M., Prudent, J., Basu, K. et al. 2017. mTOR controls mitochondrial dynamics and cell survival via MTFP1. Mol. Cell 67:922.

96 Chen, Z., Lei, C., Wang, C. et al. 2019. Global phosphoproteomic analysis reveals ARMC10 as an AMPK substrate that regulates mitochondrial dynamics. Nat. Commun. 10:104.

97 Xue, R. Q., Zhao, M., Wu, Q. et al. 2019. Regulation of mitochondrial cristae remodelling by acetylohine alleviates palmitate-induced cardiomyocyte hypertrophy. Free Radic. Biol. Med. 145:103.

98 Cogliati, S., Frezza, C., Soriano, M. E. 2013. Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. Cell 155:160.

99 Civiletto, G., Varanita, T., Cerutti, R. et al. 2015. Opa1 overexpression ameliorates the phenotype of two mitochondrial disease mouse models. Cell Metab. 21:845.

100 Okazaki, T., Maeda, A., Nishimura, H., Kurosaki, T. and Honjo, T. 2001. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine-phosphatase 2 to phosphotyrosine. Proc. Natl Acad. Sci. USA 98:13866.

101 Hui, E., Cheung, J., Zhu, J. et al. 2017. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science 355:1428.

102 Patsoukis, N., Bardhan, K., Chatterjee, P. et al. 2015. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat. Commun. 6:6692.

103 Ogando, J., Sáez, M. E., Santos, J. et al. 2019. PD-1 signaling affects cristae morphology and leads to mitochondrial dysfunction in human CDB+ T lymphocytes. J. Immunother. Cancer 7:151.

104 Eikawa, S., Nishida, M., Mizukami, S., Yamazaki, C., Nakayama, E. and Udono, H. 2015. Immune-mediated antitumor effect by type 2 diabetes drug, metformin. Proc. Natl Acad. Sci. USA 112:1809.

105 Kidani, Y., Elsaesser, H., Hock, M. B. et al. 2013. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. Nat. Immunol. 14:489.

106 O’Sullivan, D., van der Windt, G. J., Huang, S. C. et al. 2014. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. Immunity 41:75.

107 Endo, Y., Onodera, A., Obata-Ninomiya, K. et al. 2019. ACC1 determines memory potential of individual CD4+ T cells by regulating de novo fatty acid biosynthesis. Nat. Metabol. 1:261.

108 Miyajima, M., Zhang, B., Sugiuira, Y. et al. 2017. Metabolic shift induced by systemic activation of T cells in PD-1-deficient mice perturbs brain monoamines and emotional behavior. Nat. Immunol. 18:1342.

109 Nixon, A. B., Schalper, K. A., Jacobs, I., Potluri, S., Wang, I. M. and Fleener, C. 2019. Peripheral immune-based biomarkers in cancer immunotherapy: can we realize their predictive potential? J. Immunother. Cancer 7:325.

110 Kim, K. H., Kim, C. G. and Shin, E. C. 2020. Peripheral blood immune cell-based biomarkers in anti-PD-1/PD-L1 therapy. Immune Netw. 20:e8.

111 Michalek, R. D., Gerriets, V. A., Jacobs, S. R. et al. 2011. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. J. Immunol. 186:3299.

112 Galgani, M., De Rosa, V., La Cava, A. and Matese, G. 2016. Role of metabolism in the immunobiology of regulatory T cells. J. Immunol. 197:2567.

113 Shi, H. and Chi, H. 2019. Metabolic control of T reg cell stability, plasticity, and tissue-specific heterogeneity. Front. Immunol. 10:2716.

114 Viola, A., Munari, F., Sánchez-Rodríguez, R., Scolaro, T. and Castegna, A. 2019. The metabolic signature of macrophage responses. Front. Immunol. 10:1462.

115 Barzilai, N., Huffman, D. M., Muzumdar, R. H. and Bartke, A. 2012. The critical role of metabolic pathways in aging. Diabetes 61:1315.

116 Levy, M., Thaiss, C. A. and Elinav, E. 2016. Metabolites: messengers between the microbiota and the immune system. Genes Dev. 30:1589.

117 Quinn, K. M., Palchaudhuri, R., Palmer, C. S. and La Gruta, N. L. 2019. The clock is ticking: the impact of ageing on T cell metabolism. Clin. Transl. Immunol. 8:e01091.

118 Ladomerysky, E., Zhai, L., Lenzan, A. et al. 2018. IDO1 inhibition synergizes with radiation and PD-1 blockade to durably increase survival against advanced glioblastoma. Clin. Cancer Res. 24:2559.

119 Triplette, T. A., Garrison, K. C., Marshall, N. et al. 2018. Reversal of indoleamine 2,3-dioxygenase-mediated cancer immune suppression by systemic kynurenine depletion with a therapeutic enzyme. Nat. Biotechnol. 36:759.

120 Willingham, S. B., Ho, P. Y., Hotson, A. et al. 2018. A2AR antagonism with CPI-444 induces antitumor responses and augments efficacy to anti-PD-(L)1 and anti-CTLA-4 in preclinical models. Cancer Immunol. Res. 6:1136.

121 Mediavilla-Varela, M., Castro, J., Chiappori, A. et al. 2017. A novel antagonist of the immune checkpoint protein adenosine A2a receptor restores tumor-infiltrating lymphocyte activity in the context of the tumor microenvironment. Neoplasia 19:530.

122 Pietrocolala, F., Poli, J., Vacchelli, E. et al. 2016. Caloric restriction mimetics enhance anticancer immunosurveillance. Cancer Cell 30:147.

123 Saibil, S. D., St Paul, M., Laister, R. C. et al. 2019. Activation of peroxisome proliferator-activated receptors α and δ synergizes with inflammatory signals to enhance adoptive cell therapy. Cancer Res. 79:445.

124 Moore, E. C., Cash, H. A., Caruso, A. M. et al. 2016. Enhanced tumor control with combination mTOR and PD-L1 inhibition in syngeneic oral cavity cancers. Cancer Immunol. Res. 4:611.

125 Langdon, S., Hughes, A., Taylor, M. A. et al. 2018. Combination of dual mTORC1/2 inhibition and immune-checkpoint blockade potentiates anti-tumour immunity. Oncotarget 30:1589.

126 Hirayama, Y., Gi, M., Yamano, S. et al. 2016. Anti-PD-L1 treatment enhances antitumor effect of everolimus in a mouse model of renal cell carcinoma. Cancer Sci. 107:1736.

127 Schoiz, G., Jandus, C., Zhang, L. et al. 2016. Modulation of mTOR signalling triggers the formation of stem cell-like memory T cells. EBioMedicine 4:50.